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# Antilipidemic Drug Therapy Today and in the Future

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## Contents

1	Introduction .....	376
2	Dyslipidemia and Cardiovascular Disease .....	376
2.1	Low-Density Lipoproteins and CVD Risk .....	376
2.2	High-Density Lipoproteins and CVD .....	379
2.3	Plasma Triglycerides and CVD .....	380
3	Current Treatment of Dyslipidemia .....	381
3.1	HMG-Co-Reductase Inhibitors (Statins) .....	381
3.2	Bile Acid Sequestrants .....	384
3.3	Ezetimibe .....	386
3.4	Fibrates .....	388
3.5	Nicotinic Acid .....	389
4	Novel Approaches for the Treatment of Dyslipidemias .....	392
4.1	LDL- and Apolipoprotein B-Lowering Approaches .....	397
4.2	Novel Approaches to Increase HDL .....	413
4.3	Therapeutic Proteins .....	420
5	Conclusions .....	421
	References .....	421

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## Abstract

The armamentarium for the treatment of dyslipidemia today comprises six different modes of action with overall around 24 different drugs. The treatment of lipid disorders was revolutionized with the introduction of statins which have become the most important therapeutic option available today to reduce and prevent atherosclerosis and its detrimental consequences like cardiovascular

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diseases and stroke. With and optimized reduction of elevated LDL levels with statins, the risk for cardiovascular diseases (CVD) can be reduced by 30%, indicating a residual remaining risk of 70% for the development and progression of CVD notifying still a high medical need for more effective antilipidemic drugs. Consequently, the search for novel lipid-modifying drugs is still one of the most active areas in research and development in the pharmaceutical industry. Major focus lies on approaches to LDL-lowering drugs superior to statins with regard to efficacy, safety, and patient compliance and on approaches modifying plasma levels and functionality of HDL particles based on the clinically validated inverse relationship between high-plasma HDL levels and the risk for CVD. The available drugs today for the treatment of dyslipidemia are small organic molecules or nonabsorbable polymers for binding of bile acids to be applied orally. Besides small molecules for novel targets, biological drugs such as monoclonal antibodies, antisense or gene-silencing oligonucleotides, peptidomimetics, reconstituted synthetic HDL particles and therapeutic proteins are novel approaches in clinical development are which have to be applied by injection or infusion. The promising clinical results of several novel drug candidates, particularly for LDL cholesterol lowering with monoclonal antibodies raised against PCSK9, may indicate more than a decade after the statins, the entrance of new breakthrough therapies to treat lipid disorders.

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**Keywords**

Drug development pipeline · Dyslipidemia · Novel lipid-modifying drugs

## Abbreviations

ABCA1	ABC Transporter A1
ACC2	Acetyl-CoA carboxylase 2
ACS	Acute coronary syndrome
AMPK	AMP-activated protein kinase
ANPTL3	Angiopietin-like 3
ApoA-I	Apolipoprotein A-I
ApoA-II	Apolipoprotein A-II
ApoB	Apolipoprotein B
ApoC-III	Apolipoprotein C-III
ApoE	Apolipoprotein E
ASO	Antisense oligonucleotides
BET-protein	Bromodomain and extracellular domain protein
CABG	Coronary artery bypass graft
CAD	Coronary artery disease
CE	Cholesterol ester

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CETP	Cholesterol ester transfer protein
CHD	Coronary heart disease
CRP	C-reactive protein
CVD	Cardiovascular disease
DGAT	Diacylglycerol acyl transferase
EL	Endothelial lipase
FGF 21	Fibroblast growth factor 21
FH	Familial hypercholesterolemia
FXR	Farnesoid X receptor
GI	Gastrointestinal
GLP-1	Glucagon-like peptide-1
GWAS	Genome-wide association study
heFH	Heterozygous familial hypercholesterolemia
HDL	High-density lipoprotein
HDL-C	High-density lipoprotein cholesterol
HL	Hepatic lipase
HMG-CoA	Hydroxymethylglutaryl coenzyme A
hoFH	Homozygous familial hypercholesterolemia
IBAT	Ileal bile acid transporter
IDL	Intermediate-density lipoprotein
LCAT	Lecithin-cholesterol acyl transferase
LDL	Low-density lipoprotein
LDL-C	Low-density lipoprotein cholesterol
Lp(a)	Lipoprotein (a)
LPL	Lipoprotein lipase
Lrh-1	Liver receptor homolog-1
moAb	Monoclonal antibody
MTP	Microsomal lipid transfer protein
NASH	Nonalcoholic steatohepatitis
NPC1L1	Niemann-Pick C1-like protein 1
OATP1B1	Organic anion transporting peptide 1B1
PAI-1	Plasminogen activator inhibitor-1
PBC	Primary biliary cirrhosis
PCSK9	Proprotein convertase subtilisin/kexin type 9
PEG	Polyethylene glycol
PGD2R	Prostaglandin D2 receptor
PK	Pharmacokinetic
PPAR	Peroxisome proliferator-activated receptor
PSC	Primary sclerosing cholangitis
RCT	Reverse cholesterol transport
RNAi	RNA interference
RXR	Retinoid X receptor
S1P	Site-1 protease
S2P	Site-2 protease
SARS	Severe acute respiratory syndrome

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SCD-1	Stearoyl-coenzyme A desaturase 1
SHP	Small heterodimer partner
SR-BI	Scavenger receptor BI
Srebp-1	Sterol regulatory element-binding protein 1
TG	Triglyceride
VLDL	Very-low density lipoprotein

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## 1 Introduction

Worldwide cardiovascular diseases (CVD including coronary heart disease (CHD) and stroke) are the leading causes of mortality with an increasing prevalence (National Clinical Guideline 2014); atherosclerotic cardiovascular disease accounts for 17.3 million deaths/year – more than 4.3 million in Europe (Stone et al. 2013) and more than 30% of all deaths in the United States (Graham et al. 2007a; Lloyd-Jones et al. 2010) – and is projected to increase to 23.6 death cases in 2030 (Mendis et al. 2011). The overall risk for CHD is determined by multiple parameters such as dyslipidemia, hypertension, smoking, diabetes mellitus, obesity, coagulopathies, diet, and sedentary lifestyle as well as hereditary genetic variations (Roger et al. 2012; Prospective Studies Collaboration 1995). These risk factors account for more than 90% of the population-attributable risk for CVD (Yusuf et al. 2004), and up to 80% of CVD could be prevented by prevention or treatment of these risk modalities (Mendis et al. 2011). Dyslipidemia, mainly hypercholesterolemia, plays a causal role in the pathogenesis of atherosclerosis being responsible for the development and progression of CHD, stroke, and peripheral vascular disease (Roger et al. 2012). Out of nine independent risk factors for CVD, dyslipidemia is associated with the highest population-attributable risk, and 55% of CVD cases can directly be assigned to lipids (Yusuf et al. 2004). Consequently, lowering or modifying plasma lipid levels has become one of the most important options for treatment and prevention to combat the leading “killing disease no. 1” in the world (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults 2001), taking 17.1 million lives a year (Rozman and Monostory 2010).

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## 2 Dyslipidemia and Cardiovascular Disease

### 2.1 Low-Density Lipoproteins and CVD Risk

Dyslipidemia is defined as a spectrum of deviations from lipid homeostasis characterized by elevations in plasma concentrations of low-density lipoproteins (LDL), lipoprotein a (Lp(a)), apolipoproteins B and triglycerides (TG), as well as decreased plasma levels of high-density lipoproteins (HDL) or apolipoprotein A1 (ApoA-I). Primary dyslipidemias result from mutations in proteins involved in critical pathways involved in lipid and lipoprotein metabolism such as the LDL

receptor (familial hypercholesterolemia FH), apolipoprotein B (familial defective ApoB-100), or PCSK9 (variant FH). Most cases of dyslipidemia are of secondary cause, resulting from other diseases or health conditions such as diabetes, hypothyroidism, or a sedentary lifestyle with overcaloric nutrition, decreased physical exercise, or excessive alcohol consumption. Aging of the general population also contributes to the growing incidence of hypercholesterolemia because plasma levels of LDL-C exert a natural rise with increasing lifetime (Schaefer et al. 1994). Plasma cholesterol levels are lowest immediately after birth with 50 mg/dL total cholesterol and 30 mg/dL LDL-C (Parker et al. 1983; Dietschy and Turley 2004). With onset of breast feeding, total cholesterol levels rapidly increase to 180 mg/dL due to the high cholesterol content of breast milk.

Epidemiological studies [NHANES III] show that mean total cholesterol levels in adolescents aged 4–19 is 165 mg/dL; during lifetime cholesterol levels gradually increase, and in healthy subjects consuming typical Western diets, mean total cholesterol levels rise to around 200 mg/dL (Cohen et al. 2010; Martin et al. 1986). Compared to “wild-forging,” non-primates with LDL-C levels of 30–50 mg/dL and total cholesterol of 70–100 mg/dL humans have much higher plasma cholesterol concentrations. This is probably the result from changes in the eating habits from a low-carbohydrate/high protein rich diet to a greater consumption of grain and animal meat rich in saturated fat (Mann 2000).

From pathological examination, we know that the earliest stages of the atherogenic process can be detected in coronary arteries already in adolescence and early adulthood (Strong and McGill 1969; Newman et al. 1986); 75% of young men (mean age 22 years) killed in the Korean and Vietnam war had already detectable fibrous plaques (McNamara et al. 1971). Pathological examination of 2,876 individuals in the age range of 15–34 years died from noncardiovascular reasons revealed an increase of fatty streaks and lesions with increasing age and a positive correlation of the lesions with plasma cholesterol levels (McGill et al. 2000).

Epidemiological studies have shown a continuous relationship between total plasma cholesterol levels and the risk of coronary heart disease (National Heart Foundation of Australia and New Zealand 2001). 1% reduction in plasma LDL-C/non-HDL-C correlates to a 1% reduction in CVD events, whereas the increase of HDL-C by 1% decreases CVD events by 3% (O’Keefe et al. 2004; Sacks and Expert group on HDL Cholesterol 2002). The major causative role of elevated levels of LDL cholesterol in atherosclerotic vascular disease has been unequivocally proven by clinical landmark studies where reduction of plasma LDL cholesterol levels by statins – inhibitors of cholesterol biosynthesis (Scandinavian Simvastatin Survival Study Group 1994; Downs et al. 1998; Shepherd et al. 1995) – or surgical interruption of the enterohepatic circulation of bile acids (Buchwald et al. 1990) reduced cardiovascular morbidity and death. A meta-

analysis of 27 clinical trials with 170,000 participants has revealed significant risk reduction by statin therapy (Cholesterol Treatment Trialists' (CTT) Collaborators et al. 2012):

1. -21% for CVD events
2. -27% for coronary events (Ridker 2014)
3. -15% for stroke (Ridker 2014)
4. +24% for coronary revascularization (Ridker 2014)

for a reduction of plasma LDL-C levels by 1 mmol/L (38.7 mg/dL).

In the Cochrane review from 18 randomized clinical trials with 56,934 participants, statin therapy resulted in a reduction of 14% in total mortality and 25% of combined fatal and nonfatal CVD events (Taylor et al. 2013), i.e., primary prevention of dyslipidemia is working. In the year 2000, 341,745 fewer death cases from coronary heart disease compared to 1980 were registered in the United States after introduction of statin therapy into clinical practice, whereby a percentage of 24% could directly be aligned to a decrease of total plasma cholesterol levels (Ford et al. 2007). Similarly, a 35% decrease in CHD mortality was reported in Canada between 1994 and 2005, 48% of this reduction being associated with improved control of lipids and blood pressure (Wijeysundera et al. 2010). The mean age of participants in these statin trials was 63 years (Wijeysundera et al. 2010), raising the question whether the efficacy of LDL-C lowering therapy in reduction of CHD events would be higher if initiated earlier in life based on the observational studies with a correlation of atherosclerotic lesions to plasma cholesterol levels with increasing lifetime (Strong and McGill 1969; Newman et al. 1986; McNamara et al. 1971; McGill et al. 2000), and the findings that elevated cholesterol levels in early adulthood are associated with CVD in later life (Pearson et al. 1990). The observation that lowering of LDL-C levels can stop the progression of coronary atherosclerotic lesions is obviously linearly correlated with plasma LDL-C levels achieved, indicating that more intensive lowering of LDL-C leads to less plaque progression which suggests that lowering of elevated LDL-C levels early in life should be more effective in the prevention/progression of CHD/CVD than onset of therapy in later life (FERENCE and Mahajan 2013). Despite the quantum leap in the treatment of dyslipidemias by the introduction of statins into clinical practice, major gaps in the management of lipid disorders persist. Statins are undoubtedly the 1st-line therapy for the prevention of CVD in almost all population groups except those with severe renal or cardiac failure (Saydah et al. 2004) but do not address the entire spectrum of CVD risk, and the residual risk of major vascular events remains high with more than 20% over 5 years despite optimal management of LDL-C levels with statins (Cholesterol Treatment Trialists' (CTT) Collaboration et al. 2010). However, only 30–70% of high-risk patients will attain standard LDL-C targets recommended in the guidelines; for example, patients with FH and those with mixed dyslipidemias secondary to the metabolic syndrome are at an increased risk due to other risk factors such as low HDL, high TGs, or non-lipid

risk factors (Barnett et al. 2013). Particularly, in patients with complex metabolic diseases like diabetes mellitus, significant improvements in the control of risk factors are necessary; only 7.3% of adults with diabetes in the NHANES 1999–2000 survey attained the recommended targets for treatment of hyperglycemia, dyslipidemia, and hypertension (Saydah et al. 2004).

## 2.2 High-Density Lipoproteins and CVD

The relationship between total plasma cholesterol and CHD risk is continuous but not linear; the risk for CHD rises more strongly at higher LDL-C levels (Schaefer et al. 1994), indicating the involvement of further risk factors. However, unequivocal evidence from randomized clinical trials that reduction of other risk factors like low HDL or HDL-C, elevated TG, or non-lipid influences like hypertension, inflammation, and diabetes reduces the risk of CHD and therefore the number of patients with CVD is only available for hypertension (Sever et al. 2003). In a recent meta-analysis of 46 lipid GWAS-studies with more than 100,000 individuals of European descent, 95 genetic loci associated with serum lipid traits were identified, many of them harboring genes involved in lipid metabolism like HMG-CoA reductase, NPC1L1, cholesterol-7 $\alpha$  hydroxylase, or ANPTL3/4 proteins, all of them being targets of available lipid-lowering drugs, but many other newly identified genes representing possible new risk factors or pharmacological targets to monitor or treat CAD (Teslovich et al. 2010). HDL is a mediator of cholesterol transport from peripheral tissues to the liver and is considered as an important regulator of CHD risk (Parker et al. 1983; Dietschy and Turley 2004; Cohen et al. 2010) with an inverse relationship of HDL levels to CHD risk (Martin et al. 1986). HDL-C is an independent risk factor for CVD and superior than LDL-C as a predictor of CV events (Sever et al. 2003; Heart Protection Study Collaborative Group 2002; Colhoun et al. 2004). An increase of HDL-C by 1 mg/dL (0,026 mmol/L) was associated with a reduction of CVD risk by 2–3% (Curb et al. 2004). Paradoxically, despite the unequivocal role of HDL as an independent risk factor for CVD events, the failure of recent clinical trials with drugs aiming to increase HDL-C levels may suggest that the functionality of the HDL particles rather than the height of HDL-C levels is more relevant for the cardio- and atheroprotective efficacy of HDL particles. Due to the complex metabolism of HDL particles involving many different proteins and interaction with other lipoprotein particles, the impact of mutations in key proteins of the HDL-particle pathways and CV risk is not fully understood today. The role of HDL as a predictor of CV diseases is still valid in the sense that HDL-C is a predictor of incident CV events in the setting of secondary prevention in individuals who have already been diagnosed with CVD (Rader and Hovingh 2014). As a conclusion, low HDL-C plasma level may serve as a strong biomarker for CVD risk but does not predict HDL functionality or composition. Consequently, measuring of HDL-C as a metric for monitoring pharmacological efficacy for reduction of CVD events by modulation of HDL particles is not sufficient to evaluate and predict of the efficacy of a

drug approach elevating HDL levels (Kingwell et al. 2014). Recently, the results of a population study involving 2,924 adults free of CVD with a mean follow-up of 9.4 years investigating the association between HDL levels and functional parameters to primary endpoint of CVD (nonfatal MI, nonfatal stroke, or coronary revascularization or death from CVD) were published (Rohatgi et al. 2014). Whereas baseline levels of HDL were not found to be associated with CV events, the cholesterol efflux capacity (from macrophages) as a key component of the RCT process was identified as a reliable biomarker being strongly and inversely associated with the incidence of CV events in a population cohort. Little correlation was found between cholesterol efflux capacity and traditional CV risk factors, coronary artery calcium, or markers of inflammation. Cholesterol efflux from macrophages is predominantly catalyzed by ABCA1, underlining the importance of this ABC transporter for RCT. From these findings, it can be concluded that measurements of macrophage-specific cholesterol efflux using fluorescently labeled cholesterol could be used in the future as a reliable method to assess the severity of atherosclerosis and its clinical consequences and to predict the efficacy of a drug approach affecting HDL metabolism for reduction of CVD risk.

### 2.3 Plasma Triglycerides and CVD

Regarding a causative role of TG for CVD events, the picture is as well not really clear. Observational epidemiological studies and clinical trials using drugs targeting elevated TG levels and low HDL-C levels indicate a causative and predictive role of elevated TG plasma levels for the development of primary CHD. A recent systematic review and meta-regression analysis of 40 randomized controlled trials of lipid-modifying drugs with CHD events as outcome revealed that changes in plasma TG levels are predictive of CV events in randomized controlled clinical trials being significantly in primary prevention populations but not in secondary prevention populations (Stauffer et al. 2013). Because plasma TG levels and HDL-C are metabolically and mechanistically interconnected via lipid metabolizing and exchanging proteins like CETP, LPL, or EL, it is methodically difficult to determine the relative contribution of either TG or HDL-C on CVD risk. Loss-of-function mutations in the ABCA1 transporter decrease plasma HDL-C without an effect on TG levels or risk for CHD arguing against a direct relationship between HDL-C and CHD risk. As a potential explanation for the difference in the prediction power of changes in TG levels for CV events in primary and secondary prevention trials elevated plasma TG levels could still be a risk factor in secondary populations but of lower importance for the total risk of coronary events.



### 3 Current Treatment of Dyslipidemia

Today the armamentarium introduced in medical practice for the treatment of lipid disorders comprises five different modes of action and respective molecular targets with overall 24 different drug entities on the market, some of them available only in selected countries. The majority of approaches focus on the reduction of LDL-C levels by inhibition of cholesterol biosynthesis (HMG-CoA reductase inhibitors – statins), interruption of the enterohepatic circulation of bile acids (bile acid sequestrants), or inhibition of intestinal cholesterol absorption (ezetimibe). Nicotinic acid and derivatives thereof as well as agonists of the peroxisome proliferator activator receptor PPAR- $\alpha$  (fibrates) are efficacious drugs to increase HDL-C levels and to decrease elevated TG levels. Table 1 summarizes the main characteristics of these drug classes with regard to their activity on plasma lipid parameters.

#### 3.1 HMG-Co-Reductase Inhibitors (Statins)

The molecular target of statins is the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (EC 1.1.1.34), the rate-limiting enzyme in the biosynthesis pathway from mevalonate to cholesterol, sterols, isoprenoids, and other lipids like dolichol and ubiquinone. For a detailed overview about statins, some of the recent review articles are recommended (Sirtori 2014; Shitara and Sugiyama 2006). Statins are competitive enzyme inhibitors competing with 3-hydroxy-3-methylglutaryl-CoA for the catalytic binding site, thereby reducing biosynthesis rate of mevalonate and its downstream

**Table 1** Lipid-modifying drugs on the market

Class	Mode of action	Efficacy parameters			
		LDL-C (%)	HDL-C (%)	Triglycerides (%)	LIT
Statins	Inhibition of HMG-CoA reductase	–(18–55)	+(5–15)	–(7–30)	Toth (2010)
Ezetimibe	Inhibition of cholesterol absorption	–(18–20)	+(1–4)	–8	Toth (2010)
Sequestrants	Inhibition of bile acid reabsorption	–(9–28)	+(0–9)	+ (2–16)	Hou and Goldberg (2009); Martinez-Hervas et al. (2011)
Fibrates	PPAR-alpha	–(5–20)	+(10–20)	–(20–50)	Toth (2010)
Nicotinic acid	Multiple targets?	–(5–25)	+(15–35)	–(20–50)	Toth (2010)

**Table 2** Efficacy of Hmg-Coa reductase inhibitors (Ewang-Emukowhate and Wierzbicki 2013)

Drug	Total C (%)	LDL-C (%)	HDL-C (%)	TG (%)
Atorvastatin	–(27–39)	–(37–51)	+ (2–6)	–(20–28)
Rosuvastatin	–(33–40)	–(46–55)	+ (8–10)	–(20–26)
Simvastatin	–(20–33)	–(28–46)	+ (5–7)	–(12–18)
Fluvastatin	–(13–19)	–(17–23)	+ (0.9)	–(5–13)
Lovastatin	–(21–36)	–(29–48)	+ (7–8)	–(2–13)
Pravastatin	–(15–22)	–(20–30)	+ (3–6)	–(8–13)
Pitavastatin	–(28–32)	–(39–44)	+ (4–6)	–(14–19)

*Adverse side effects*

Mild elevation of transaminases in 1–3% of treated patients

Myalgia in 15–20% of treated patients (Toth et al. 2008)

Frequency of rhabdomyopathy 1/7,428 of statin-treated patients (Cholesterol Treatment Trialists' (CTT) Collaborators et al. 2008b)

Around 50% discontinuation after 1 year of treatment (Jackevicius et al. 2002)

products like cholesterol. The decrease in the intracellular cholesterol regulatory pool leads to an upregulation of LDL receptors in the hepatocyte plasma membrane, leading to an increased clearance rate of LDL particles from the blood and therefore a fall of plasma LDL-C levels. Overall, statins reduce LDL-C levels by 17–55%, concomitantly with an increase of HDL-C by 1–10% and a decrease of TG by 2–28% (Vigna and Fellin 2010; Toth 2010; Ewang-Emukowhate and Wierzbicki 2013) (Table 2). Based on dosage, atorvastatin and rosuvastatin are the most potent statins; the more polar compounds pravastatin and rosuvastatin are not or only metabolized by cytochrome P450 enzymes, particularly Cyp3A4, and are therefore less prone to drug–drug interactions with other medications due to cytochrome P450 metabolism. With high-dose therapy of statins, reduction of LDL-C up to 60% can be achieved. The findings however that whenever the statin dose is doubled LDL-C levels are reduced by only additional 6% (“the role of 6%”) limits the clinical efficacy of statin use because of a steep increase in the incidence of adverse side effects by high statin doses; elevations of plasma transaminase levels and muscle toxicity by myalgia or severe rhabdomyolysis have been associated with high statin doses, thereby limiting the tolerable dose of a statin and the achievable reduction of plasma LDL-C levels. In addition to the beneficial effects of statins on plasma lipid profiles, further activities of statins are discussed to contribute to their efficacy in reduction of CVD events, but a final proof of the causative role of these pleiotropic statin effects still has to be proven: statins improve endothelial function leading to vasodilation (Förstermann and Li 2011), inhibit inflammatory processes with reduction of circulating CRP levels (Plenge et al. 2002), as well as stabilize plaques maybe by inhibition of myocyte infiltration and inhibition of metalloproteinase secretion (Massaro et al. 2010). Since inhibition of the biosynthesis cascade to cholesterol by statins occurs at a very early step, a potential risk was seen in this approach due to the decrease of other lipids like ubiquinone and dolichol and manifestation of these decreases of non-sterol lipids in adverse side effects in different organs including the liver, muscle, eye, or brain. Today, after more than 25 years of broad clinical use with millions of patients treated,

statins can be considered as very effective and safe drugs; currently more than 25 million American people with dyslipidemia are taking statins, and according to a recent recommendation for use, another 13 million are expected to benefit from statin treatment; for all patients with known CVD, the new guidelines by the American College of Cardiology and the American Heart Association for the management of cholesterol recommend statin treatment regardless of their LDL-C levels (Pencina et al. 2014). If the public health guidelines being currently discussed in the USA and UK would be fully implemented, one third of the middle-aged and adult population in these countries will be recommended for statin therapy (Pencina et al. 2014). Safety and tolerance of statin treatment is good with the exception of myalgia as the major adverse side effect. Indeed, statin-induced myalgia is a very frequent phenomenon in clinical practice observed in 15–20% of patients treated with statins (Toth et al. 2008), whereas fortunately, the frequency of the severe and life-threatening rhabdomyolysis is low (1 out of 7,428 patients treated) (Cholesterol Treatment Trialists' (CTT) Collaborators et al. 2008). Statins are taken up in the liver into hepatocytes by the organic anion transporting polypeptide OATP1B, and it was found that genetic variants of the *SLCO1B1* gene are associated with the risk of myopathy upon statin treatment (Carr et al. 2013).

Some of these variants exert a diminished affinity and transport activity for statins, leading to a reduced hepatic uptake resulting in higher blood levels and increased muscle exposure with an increased off-target activity at the neuromuscular endplate: statins are powerful inhibitors of chloride channels at muscle cell membranes (Pierno et al. 2006) potentially leading to paralysis; additional mechanism possibly being involved in statin-induced myopathy are diminished ubiquinone levels in plasma and muscle due to inhibition of their synthesis in the liver, induction of atrogen-1 being involved in skeletal muscle atrophy (Hanai et al. 2007), or direct antagonistic effects of statins on muscle differentiation (Martini et al. 2009). Mild elevation of serum transaminase occurs in only 1–4% of statin-treated patients dose-related, indicating a high liver safety of statins (Vigna and Fellin 2010). Evidences for a slightly increased incidence for type 2 diabetes and acute renal failure were reported from observational data: 1 additional case of type 2 diabetes may occur for every 225 individuals treated with statins for 2 years, indicating a 20–30% increase risk to develop diabetes (Sattar et al. 2010). Acute renal failure maybe a dose-related adverse side effect of statins of low incidence (Dormuth et al. 2013). During development of statins, cataracts were an issue due to findings from dog studies; a recent retrospective analysis gave evidence for a slightly increased incidence for cataract formation in patients treated with statins (Leuschen et al. 2013). Today there are no evidences regarding early concerns that statin use may impair cognitive functions (Shepherd et al. 2002), but in contrast, patients treated with statins were shown to have a reduced risk to develop Alzheimer's disease (Corrao et al. 2013). As well, no evidence for an increased risk for cancer or cancer mortality was found for treatment with statins (Ridker 2014). The introduction of inhibitors of HMG-CoA reductase, the statins, into clinical practice has dramatically changed the landscape and treatment regimens for the treatment of lipid disorders. Coronary events and stroke can be

reduced by 25–30% and 10–15%, respectively (Law et al. 2003), and several meta-analyses of statin trial revealed reduction of CVD risk for each 1 mmol/L LDL-lowering by 21% (Cholesterol Treatment Trialists' (CTT) Collaborators et al. 2012) and of overall mortality by 14%, respectively (Taylor et al. 2013). Nevertheless, the overall mortality by CHD will increase in the future due to the increasing incidence of metabolic disorders like obesity, diabetes, and the metabolic syndrome.

Despite optimized drug therapy with statins, a significant proportion of patients fail to achieve the recommended levels of LDL cholesterol (Schechtman and Hiatt 1996; Hsu et al. 1995) as defined by the European and US guidelines (Catapano 2009). The reasons for these treatment failures are manifold including the following factors:

- A poor compliance due to discomfort and side effects; still around 50% of statin-treated patients discontinue their therapy after only 1 year (Jackevicius et al. 2002).
- The recommended target for LDL-C in high-risk patients and FH patients (1.8–2 mmol/L) cannot be achieved with tolerable statin doses due to the “rule of 6%” phenomenon, leading to insufficient dosing to avoid a steep increase of adverse side effects upon dose escalation.
- Inefficiency of a drug class or statin intolerance (Hsu et al. 1995; LaRosa et al. 2005).

## 3.2 Bile Acid Sequestrants

Bile acid sequestrants are polymeric anion-binding resins that strongly bind the negatively charged bile acids in the lumen of the small intestine, thereby interrupting the enterohepatic circulation of bile acids (Out et al. 2012). By blocking intestinal bile acid reabsorption, the amount and/or composition of bile acids recirculating to the liver is changed, leading to an increase in hepatic bile acid synthesis from cholesterol via involvement of the nuclear receptors FXR, SHP, and Lrh-1; the depletion of the regulatory hepatic cholesterol pool induces a stimulation of cholesterol biosynthesis via upregulation of HMG-CoA reductase and LDL-receptor expression at the hepatocyte surface, the latter leading to an increase in hepatocellular uptake of LDL particles with a consequent decrease of plasma LDL-cholesterol levels.

Currently there are three bile acid sequestrants on the market – cholestyramine, colestipol, and colesevelam. Cholestyramine and colestipol are used in daily dosages up to 30 g, whereas recommended daily dose of colesevelam is 3.75 g; the high necessary doses of cholestyramine and colestipol are caused by occupation of the majority of the anion-binding sites by chloride anions in competition to anionic bile acids. Whereas cholestyramine and colestipol show a preference for dihydroxy bile acids, colesevelam binds bile acids by both ionic and hydrophobic interactions, thereby increasing binding affinity and specificity resulting in

significantly lower necessary dose. The treatment with sequestrants is safe, and due to their nonsystemic mode of action within the lumen of the intestine, sequestrants do not show systemic toxic effects; because of their non-absorbability, they can also be used in pregnancy. The necessary high doses of the 1st-generation sequestrants cholestyramine and colestipol are responsible for the major adverse side effects in the gastrointestinal tract with constipation, abdominal pain, flatulence, and nausea being the cause for the compliance issues and high rate of treatment discontinuation up to 40–60% with first-generation sequestrants (Andrade et al. 1995); colessevelam due to its improved binding characteristics has only few adverse side effects and a high compliance of 88–93% (Davidson et al. 1999). Because of the binding characteristics for anionic and hydrophobic compounds, chronic use of sequestrants may decrease the absorption of fat-soluble vitamins and nutrients and interfere with the intestinal absorption of drugs and influencing their pharmacodynamics, particularly important for drugs with a narrow therapeutic window such as anticoagulants, digitalis,  $\beta$ -blockers, thiazides, or thyroxine. In these cases, a time lack between intake of the respective drug and the sequestrant is recommended. By monotherapy, sequestrants can decrease LDL-C levels by 9–28% and increase HDL-C levels up to 9% (Hou and Goldberg 2009). First-generation sequestrants may lead to an undesired increase of plasma triglyceride levels which is not or significantly less the case with colessevelam. By the change in the composition of the bile acid pool with an enrichment of trihydroxy bile acids due to a higher binding affinity of dihydroxy bile acids to cholestyramine and colestipol, the less potent trihydroxy bile acids as FXR agonists lead to a decreased FXR activity such as a decrease in the expression of ApoC-II as an activator of lipoprotein lipase or reduction of the FXR-mediated inhibition of hepatic TG synthesis via Shp and Srebp1c (Kast et al. 2001). As a result, the increased TG synthesis and the decreased TG clearance in the vasculature induces an overall increase of circulating TG. Bile acid sequestrants have proven their efficacy in reducing CHD (Hou and Goldberg 2009). CVD events were reduced by cholestyramine by 18% with an additional 8% effect upon coadministration to a statin (The Lipid Research Clinics Investigators 1992). The additive activity of bile acid sequestrants makes them ideal partners for co-medication to statins in patients where statin therapy is not sufficient enough: coadministration of colessevelam to statins significantly lowered LDL-C levels by additional 16% so that more than 39% of the patients reached their therapeutic target of <100 mg/dL LDL-C compared to only 10% treated with a statin alone (Bays et al. 2006). Colessevelam is able to reduce as well serum levels of CRP (Bays et al. 2006) and to increase LDL particle size, indicating an additional inhibitory effect on inflammatory processes involved in atherogenesis (Deveraj et al. 2006).

Recent studies have strengthened early observations that bile acid sequestrants in addition to their cholesterol-lowering activities can improve glycemic control in patients with type 2 diabetes (Handelsman et al. 2010), and colessevelam was approved as the first bile acid sequestrant for the treatment of type 2 diabetes: in a 16-week clinical trial with prediabetic individuals, colessevelam in addition to its improvement of lipid profiles reduced fasting blood glucose levels by 4% and HbA1c values by 2%, respectively. Fasting blood glucose levels and HbA1c values

in patients treated with antidiabetic drugs could be further reduced by 10% upon coadministration of colesevelam (Goldberg et al. 2008). The mechanisms whereby bile acid sequestrants improve glycemic control are not yet fully understood but may involve the following contributions:

- Increased secretion of incretins like GLP-1 due to spillover of bile acids into the colon (Suzuki et al. 2007)
- Changes in the composition of the bile acid pool with activation of FXR downstream activities like repression of hepatic gluconeogenesis and increasing of insulin sensitivity (Zhang et al. 2006)
- Activation of TGR5 receptor in the colon by bile acid spillover with stimulation of energy expenditure (Thomas et al. 2009)

The proven efficacy of bile acid sequestrants on improvement of atherogenic lipid profiles and reduction of CHD events, their safety profile and nonsystemic mode of action, and their beneficial effects on glucose homeostasis strengthens the role and value of bile acid sequestrants particularly for the treatment of patients with statin intolerance, type 2 diabetes, or the metabolic syndrome.

### 3.3 Ezetimibe

Ezetimibe exerts its hypolipidemic action by inhibiting intestinal cholesterol absorption. Ezetimibe targets the NPC1L1 pathway which is a key regulator of cholesterol uptake from enterocyte brush border membranes and reuptake of cholesterol from bile into hepatocytes.

Cholesterol solubilized in mixed micelles is transferred to the enterocyte brush border membrane via a protein-mediated process (Cai et al. 2002; Kramer et al. 2005) and moved to detergent-resistant microdomains from which cholesterol absorption occurs then by clathrin-dependent receptor-mediated endocytosis involving a complex of clathrin, adaptor protein 2 complex, and NPC1L1 (Wang and Song 2012) with storage in the so-called endocytic recycling compartment. NPC1L1 is a cholesterol-sensing protein cycling between this compartment and the brush border membrane dependent on the cholesterol levels sensed in the brush border membrane; when intracellular cholesterol levels are low, NPC1L1 is with the aid of microfilaments recycled to the brush border membrane, thereby initiating and stimulating the microdomain endocytotic process. Ezetimibe specifically inhibits this NPC1L1 pathway. There is a controversy with regard to the primary molecular target of ezetimibe. It is postulated that the molecular target of ezetimibe is the NPC1L1 protein itself (Betters and Yu 2010). However, a direct binding of ezetimibe to NPC1L1 by labeling techniques was never really demonstrated. By use of a fluorescent ezetimibe glucuronide, a specific binding to the surface of HEK 293 cells expressing NPC1L1 was shown (Garcia-Calvo et al. 2005), but this can as well be explained by a role of NPC1L1 in forming of the protein complex necessary for clathrin-mediated endocytosis (Wang and Song 2012), thereby allowing access

of ezetimibe to its primary molecular target putatively being different from NPC1L1. In contrast, a direct and specific binding of ezetimibe to aminopeptidase N (CD13) was demonstrated by extensive photoaffinity labeling studies with various photoreactive ezetimibe analogues as well as by isolation of the ezetimibe-binding protein by affinity chromatography with an ezetimibe affinity matrix. Sequence analysis of the purified radiolabeled ezetimibe-binding protein or the affinity-purified ezetimibe-binding protein unequivocally demonstrated its identity with aminopeptidase N (CD13), whereas no amino acid sequences of NPC1L1 were found in the isolated ezetimibe-binding proteins (Kramer et al. 2004, 2005; Frick et al. 2003), indicating that aminopeptidase N is the primary target for ezetimibe in the process of NPC1L1-mediated endocytosis of cholesterol from cholesterol-rich microdomains of the enterocyte brush border membrane (Skov et al. 2010):

- a. Membrane impermeable ezetimibe analogues are able to inhibit cholesterol uptake into CaCo<sub>2</sub> cells as well in vivo, indicating that the binding to the outside of the enterocyte brush border membrane is sufficient for inhibition of cholesterol internalization (Kramer et al. 2004, 2005).
- b. The cellular localization of APN between the brush border membrane and an intracellular storage compartment – the so-called deep apical tubules – is cholesterol-dependent as is the case for NPC1L1.
- c. Besides its enzymatic activity, APN can act as a receptor involved in endocytotic processes (Hansen et al. 2003) and is involved in the endocytosis of various virus classes like corona, SARS, or cytomegaly (Yeager et al. 1992).
- d. The uptake of cholesterol from mixed micelles by CaCo<sub>2</sub> cells can specifically be inhibited by masking APN with APN-specific antibodies (Kramer et al. 2004, 2005) independently whether the cells were cholesterol-depleted with cyclodextrin or not, whereby the concentration of NPC1L1 in the brush border membrane is largely changed by cyclodextrin treatment.

These findings indicate that specific binding of ezetimibe to APN blocks the internalization of the cholesterol-rich microdomains, thereby preventing cholesterol absorption by interruption of the NPC1L1-cholesterol sensing pathway (Skov et al. 2010).

Ezetimibe is the only drug available inhibiting intestinal cholesterol absorption. In monotherapy, ezetimibe decreases serum LDL-C up to 20%, decreases TG up to 8%, and has a small increasing effect on HDL-C by 1–4% (Bruckert et al. 2003). Ezetimibe does not influence intestinal absorption of bile acids, fat-soluble vitamins, or carotenes. Ezetimibe does not show any significant adverse side effects and has no influence on the activities of cytochrome P-450 enzymes, therefore being devoid of significant pharmacokinetic interactions with other drugs. The effect of ezetimibe on LDL-C is additive to statins; therefore, combination of ezetimibe with a statin can overcome the “rule of 6%” by doubling of a statin dose. Ezetimibe/simvastatin combinations with 10 mg ezetimibe and 10, 20, 40, or 80 mg simvastatin reduced LDL-C by 51%, 57%, and 59%, respectively

(Ballantyne et al. 2005). The additive effect to statins and the lack of necessary titration of the statin dose makes combinations of ezetimibe with statins an important options of patients resistant or intolerant to statins. Large clinical trials, however, like ENHANCE in patients with heterozygous FH did not reveal a statistically significant effect on carotid intima media thickness compared to simvastatin alone despite a higher LDL-C lowering efficacy of the combination (Kastelein et al. 2008). In two further trials SEAS (Simvastatin and Ezetimibe in Aortic Stenosis) and SHARP (Study of Heart and Renal Protection), however, a significant risk reduction for CVD events was observed proportional to the reduction of LDL-C (Rossebo et al. 2008; Baigent et al. 2011). A conclusive answer regarding a clinically relevant beneficial effect of ezetimibe on cardiovascular morbidity and mortality is awaited by the ongoing IMPROVE-IT study covering 18,000 patients with acute coronary syndrome, comparing treatment regimens of simvastatin vs a combination with ezetimibe.

### 3.4 Fibrates

Peroxisome proliferator activator receptor  $\alpha$  (PPAR $\alpha$ , NR1C1 (nuclear receptor subfamily 1, group C)) is a nuclear transcription factor being a major regulator of lipid metabolism in the liver, exerting its activity by modifying the expression of a large number of target genes predominantly involved in fatty acid transport and metabolism and gluconeogenesis. Endogenous ligands comprise fatty acids and eicosanoids including leukotriene B<sub>4</sub>. Binding of an agonist to PPAR $\alpha$  to the ligand-binding domain induces a conformational change, leading to the sequestration of a number of co-activator and corepressor proteins activating the receptor and heterodimerization with retinoid X receptor (RXR) followed by binding to PPAR responsive elements (PPREs) in the promoters of the target genes initiating modification of their expression. Fibrates are synthetic ligands selectively mimicking natural fatty acid ligands activating PPAR $\alpha$  and thereby orchestrating via the PPAR $\alpha$  target genes the adaption of the body to energy deprivation with stimulation of mitochondrial and peroxisomal  $\beta$ -oxidation, inhibition of hepatic VLDL production by inhibition of DGAT-2 activity, and activation of LPL to deliver free fatty acids for metabolism and energy generation, the latter mediated by reduction in the expression of the LPL inhibitor ApoC-III and induction of the LPL-activator ApoC-II. As a result, TG hydrolysis in VLDL and chylomicron particles is stimulated leading to a strong decrease in circulating TG levels; the resulting TG-poor LDL particles and remnants show a higher affinity to the LDL receptor resulting in an increased clearance with an accompanying decrease in LDL-C levels; fibrates modify LDL-particle size from small dense LDL-particles – characteristic for diabetic dyslipidemia – to large less atherogenic ones (Gazi et al. 2007). Additionally, HDL-C levels are increased by fibrates due to a stimulation in hepatic expression and production of apolipoproteins ApoA-I and ApoA-II ----as well as by a diminished loading of HDL particle with TG whereby the resulting HDL particles become less sensitive to catabolism by hepatic lipase.



Overall, reverse cholesterol transport is stimulated by these processes. Currently three fibrates are in clinical use – fenofibrate, gemfibrozil, and bezafibrate: fenofibrate is the preferred drug owing to its lack of PK interaction with statins. In clinical use, fibrates efficiently reduce serum TG concentration by 20–50%, increase HDL-C levels by 10–20%, and have a modest LDL-C lowering effect of 5–20%. Due to their lipid-modifying profile, fibrates can be particularly useful in monotherapy or in combination with statins for the treatment of patients with diabetic dyslipidemia or the metabolic syndrome (Fievet and Staels 2009). In large outcome studies in diabetic patients – FIELD (fenofibrate intervention and event lowering in diabetes) involving 9,795 type 2 diabetic patients (The FIELD study Investigators 2005) and ACCORD (action to control cardiovascular risk in diabetes) with 5,518 diabetic patients receiving either simvastatin (40 mg/day) or a combination of 40 mg/day simvastatin with 160 mg/day fenofibrate (The ACCORD Study Group 2010) – no reduction in macrovascular events like CHD, coronary death, or nonfatal MI could be demonstrated. However, microvascular events including retinopathy, nephropathy, and neuropathy were significantly reduced. Meta-analysis revealed that patients with low HDL-C (<0.9 mmol/L) and high TG plasma levels (>2.3 mmol/L) benefit from treatment with fenofibrate (Sacks et al. 2010). The tolerability and safety of fibrates are acceptable: Adverse side effects include myopathy, upper GI-symptoms, mild elevation of serum transaminase and homocysteine levels, and an increased incidence for cholesterol gallstones (Prueksaritanont et al. 2002a). The most severe adverse side effect is muscle toxicity with rhabdomyolysis (Davidson et al. 2007). Particularly gemfibrozil treatment in combination with statins is associated with an increased risk for myopathy and hepatotoxicity caused by its competition with statins for the glucuronidation elimination pathway, thereby decreasing the elimination of statins (Prueksaritanont et al. 2002b).

### 3.5 Nicotinic Acid

The lipid profile-modifying effects of vitamin B3 – nicotinic acid or niacin – are since nearly 60 years introduced into clinical practice (Carlson 2005). At pharmacological doses up to 2 g/day, niacin exerts significant changes in most of the different lipoproteins: increases in HDL-C of 25–30% and Apo-AI production (Lamon-Fava et al. 2008) combined with decreases of LDL-C by 15–20%, TG by 20–40%, Lp(a) by about 30%, as well as reduction of fibrinogen and plasminogen activator inhibitor 1 (PAI 1). Niacin is currently the most potent drug on the market to increase HDL-C and the only approved drug with significant reduction of the independent CV risk factor Lp(a). From that perspective, the profile of niacin with reduction of atherogenic lipoproteins, increase of antiatherogenic lipoproteins, and reduction of prothrombotic factors principally is close to an ideal drug target profile to treat dyslipidemias, particularly in patients with diabetic dyslipidemia.

However, these clinical benefits on lipid profiles of niacin are overshadowed by a number of shortcomings hampering its therapeutic value in clinical practice. These include:

- (a) A poor compliance due to the unpleasant side effect of flushing mediated by activation of the prostaglandin receptor PGD2R.
- (b) Glucose control is negatively affected with increases of fasting blood glucose (4–17%), HbA1c levels, insulin levels, and a decrease in insulin sensitivity for several weeks at high niacin doses necessary to achieve the above beneficial effects on lipid patterns; normalization of glucose controls was observed after 48 weeks (Goyal et al. 2014) indicating that short- and long-term treatment with niacin worsens glycemic control limiting its use particularly in patients with diabetic dyslipidemia; low doses of nicotinic acid have only minimal effects on glucose tolerance but as well only a moderate effect on lipid profiles.
- (c) Increases in plasma levels of uric acid promoting the risk for gout attacks.
- (d) Liver toxicity and gastrointestinal side effects. To overcome the clinically most prominent and uncomfortable side effect, flush slow release forms of niacin (niaspan) were developed, improving handling of nicotinic acid and the benefit/adverse side effect ratio.

Another approach to increase the compliance of niacin use was the inhibition of flushing by co-medication with the PGD2 inhibitor laropiprant; the incidence of flush was significantly reduced but did not disappear completely due to the fact that flush can also be induced by prostaglandin E2 (Vosper 2011).

Despite its clinical use since decades, the molecular mechanism(s) by which niacin mediates its pleiotropic effects on lipid mechanism is not fully understood. A whole plethora of molecular targets and mechanisms has been reported for niacin; whereas its effects on TG metabolism can quite well be explained in molecular terms, the mechanisms whereby niacin increases HDL-C are more speculative. Scientific arguments for the following mechanisms for niacin have been reported:

#### A. Inhibition of TG Levels

Niacin potently inhibits lipolysis in adipose tissue by activation of the nicotinic acid receptor GPR109A, triggering an inhibition of adenylate cyclase, and consequently the decrease of cAMP levels prevents activation of PKA with inhibition of hormone-sensitive lipase resulting in a decrease of lipolysis (Julius and Fischer 2013) and plasma free fatty acids. In the liver, the diminished flux of free fatty acids from adipose tissue leads to a suppression in the expression of PPAR- $\gamma$  coactivator-1b (PGC-1b) as well as of apolipoprotein C3, resulting in a decreased secretion of VLDL particles. A direct inhibition of the enzyme DGAT-2 by nicotinic acids limits the delivery of TG for secretion as VLDL particles and was proposed as the most relevant molecular target for niacin action of TG synthesis (Kamanna et al. 2013). The decrease in the content of TG in the VLDL particles increases resulting LDL particle size, and additionally, by

stimulation of ApoB catabolism, plasma LDL and VLDL levels were decreased further.

#### B. Increase of HDL-C Levels

The HDL effects of niacin probably are not related to activation of the nicotinic acid receptor GPR109A; investigations with GPR109A k.o. mice or specific high-affinity ligands for GPR109A demonstrated that this receptor – being clearly involved in niacin’s pharmacological activity in TG metabolism – does not play a significant role for the HDL effects of niacin (Lauring et al. 2012). This explains the clinical failure with highly specific GR109A agonists; MK-1903 and SCH900271 strongly inhibited lipolysis and decreased plasma FFA levels but had no significant effects on serum lipid profiles, particularly HDL levels (Lauring et al. 2012). Recent studies explain the niacin effects on HDL by an inhibition of CETP-mediated lipid exchange resulting in cholesterol-enriched HDL particles, reduction of hepatic clearance of HDL particles, as well as a stimulation of reverse cholesterol transport through an increased flux of cholesterol to HDL. Mechanisms suggested to be involved is an increase in ABCA1 expression and production of nascent HDL particles and a reduction in the expression of the putative hepatic HDL-receptor  $\beta$ -chain ATP synthase resulting in an inhibition of HDL-particle clearance (Kamanna et al. 2013).

#### C. Effect on Flush

The discovery of GPR109A as a specific nicotinic acid receptor strongly triggered the search for non-niacin-related high-affinity ligands for this receptor led by the assumption that the pleiotropic beneficial effects of niacin are mediated via this receptor. Unfortunately, these new drugs failed in increasing HDL levels. GPR109A is not only expressed in adipocytes but as well in immune cells, spleen, and the Langerhans cells of the epidermis (Kamanna et al. 2009). Activation of the latter by nicotinic acid increases the production of PGD2 as the causative agent of the undesired side effect flush; therefore, flush is a non-dissociable adverse side effect from inhibition of lipolysis with GPR109A agonists like niacin, making the search for a “better” niacin with less side effects unlikely.

In large clinical trials, however, niacin failed to exert significant beneficial effects on CVD events. The AIM HIGH study performed in high-risk patients with CVD and low HDL-C, their LDL-C being optimally treated with statins, did not reveal a significant reduction in CVD events despite strong increases in HDL-C and reductions in TG (Investigators AIM-HIGH et al. 2011). On the other hand, a combination of niacin with bile acid sequestrants and lovastatin resulted in a regression of CAD (Kane et al. 1990). In the largest hitherto performed drug trial with niacin – the HPS2-THRIVE trial – in more than 25,000 adults with vascular disease, their LDL-C levels were adjusted with 40 mg simvastatin or 40 mg simvastatin plus 10 mg ezetimibe; afterwards, the patients were randomly assigned either to a treatment group receiving 2 g niacin and 40 mg laropiprant or a placebo arm. Over an observation period of 3.9 years at average, LDL-C was lowered by additional 0.25 mmol/L and HDL-C increased by 0.16 mmol/L. However, no

significant effect on the incidence of major CVD events could be observed (13.2% vs 13.7%). In contrast, discontinuation rate was significantly higher in the niacin/laropiprant group (25.4% vs 16.6%), and adverse side effect reactions were as well increased in the treatment group (55.6% vs 52.7%); patients with diabetes had a 55% proportional increase in disturbances of glucocontrol, and a 32% higher incidence of newly diagnosed diabetes was found in the niacin/laropiprant group (The HPS2-THRIVE Collaborative Group 2014). As a conclusion, the addition of a slow-release niacin-laropiprant combination to patients treated with statins had no additional beneficial effect on vascular events. This unfavorable outcome finally led to the withdrawal of niacin/statin/laropiprant combinations from the market, leaving behind significant doubts regarding benefits of niacin treatment for dyslipidemic patients.

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## 4 Novel Approaches for the Treatment of Dyslipidemias

Various meta-analyses of clinical trials performed with statins indicate that even with an optimized LDL-C reduction by use of statins, the risk for CVD can be reduced by about 30%, indicating persistence of a 70% residual risk for the development and progression of CHD. Despite the fundamental change in the treatment of lipid disorders by the introduction of statins being the standard treatment today, a number of open questions remain, and for prevention of atherosclerosis, new treatments are needed:

- Will an reduction of LDL-C by other mode of actions yield similar outcome benefits on CVD morbidity and mortality?
- Will lowering of TG-rich lipoproteins and remnant cholesterol result in a reduction of major CVD events?
- Will pharmacological stimulation of reverse cholesterol transport lead to an improvement of CVD risk?

Particularly, an improvement regarding the following shortcomings of statin therapy should be addressed by new lipid-modifying drugs:

- Achievement of target LDL-C in high-risk patients, i.e., improved efficacy and additional beneficial effects such as lowering of Lp(a)
- More efficient treatment of patients with FH
- Overcoming the “rule-of 6%” hurdle of statins
- Effective treatment and reaching LDL-C targets in patients with statin intolerance/resistance

Therefore, an ideal drug for the treatment of dyslipidemias would concomitantly more efficaciously lower LDL-C and TG as well as stimulate reverse cholesterol transport (RCT) via the HDL pathway with less side effects than the existing therapies defining the medical need for future lipid-modifying drugs:

- A higher efficiency in lowering of LDL cholesterol
- Addressing additional risk factors for cardiovascular diseases
- Less adverse side effects
- Inhibition of atherosclerosis progression or even reversion
- Higher responder rate
- Higher compliance
- Low costs

For approval of each new agent to treat lipid disorders, a positive benefit-to-risk ratio for CHD development and progression probably will have to be clinically demonstrated. With statins as the current standard therapy, most novel hypolipidemic investigational drugs will have to be tested probably as an add-on therapy to statins or in patient cohorts not being efficiently treatable with statins.

As of April 2015 (analyzed by publically available sources and the actual official development pipelines published on the homepages of more than 70 pharmaceutical companies), 53 novel drug candidates ((NME) new medical entities) acting on around 27 different molecular targets or pathways are reported to be in clinical development (Tables 3, 4, 5, and 6). The tissue localization of the mode of action of the drug candidates in clinical development is illustrated in Fig. 1.

**Table 3** Lipid-modifying drugs in clinical development: LDL-lowering (as primary target)

Target	Approach	Drug candidate	Company	Development stage
PCSK 9	MoAb	Alirocumab	Sanofi/Regeneron	Phase III, under approval
		Evolocumab	Amgen	Phase III, under approval
		Bococizumab	Pfizer/Rinat	Phase III
		RG7652	Roche/Genentech	Phase II
		LY-3015014	Eli Lilly	Phase II
	Adnectin	BMS-962476	BMS	Phase I
	siRNA	ALN-PCS	Alnylam	Phase I
Small molecule	CAT-2003	Catabasis Pharmaceuticals	Phase II	
	K-312 (PCSK9/CETP-Inh)	Kowa	Phase I	
MTP	Small molecule	Lomitapide	Aegerion	Approved
		SLx-4090/KD-206	Surface Logix/Kadmon	Phase II
AMPK/ATP-citrate lyase	Small molecule	ETC-1002	Esperion Therapeutics	Phase II
TR- $\beta$ 1 receptor	Small molecule	MGL-3196/VIA-3196	Madrigal Pharmaceuticals	Phase I
ApoB	Antisense	Mipomersen	Sanofi	Approved
Apo(a)	Antisense	ISIS-APOARx	ISIS Pharmaceuticals	Phase II

**Table 4** Lipid-modifying drugs in clinical development: HDL-increasing (as primary target)

Target	Approach	Drug candidate	Company	Development stage
CETP	Small molecule	Anacetrapib	Merck	Phase III
		Evacetrapib	Eli Lilly	Phase III
		DRL-17822	Dr Reddy	Phase II
		DEZ-001/TA-8995	Dezima/Mitsubishi Tanabe	Phase II
	Vaccine	ATH-03	AFFIRIS	Phase I
CETP/PCSK9	Small molecule	K-312	Kowa	Phase I
BET	Small molecule	RVX-208	Resverlogix	Phase II
Niacin analogue	Small molecule	ARI-3037MO	Arisaph Pharmaceuticals	Phase II
HDL mimetics	Peptide-lipid complexes	MDCO-216	The Medicines Company	Phase II
		CSL 112	CSL Ltd.	Phase II
		CER 001	Cerenis Therapeutics	Phase II
ApoE mimetic	Peptide	AEM-28	Capstone Therapeutics	Phase I
rec-LCAT	Protein replacement	MEDI6012 (ACP-501)	MedImmune/AlphaCore	Phase I
FGF-21 analogues	Protein	LY2405319	Eli Lilly	Phase I
	Protein-PEG conjugate	?	BMS	Phase II

Whereas the today used drugs for treatment of lipid disorders are all orally taken and are with the exception of the bile acid sequestrants throughout small organic molecules, the current clinical development pipeline shows a much greater heterogeneity with regard to the characteristics of the drug candidates: Out of the 52 clinical candidates, 20 of them are biologics (Table 7):

- Six (monoclonal) antibodies: five addressing the PCSK9 pathway, one being a vaccine against CETP
- One monobody (adnectin): PCSK9
- Five oligonucleotide drugs: PCSK9 (1), ApoB (1), Apo(a) (1), ApoC-III (1), ANGPTL3 (1)
- Three HDL mimetics: complexes of ApoA-I with phospholipids
- One peptidomimetic: ApoE
- Three therapeutic recombinant proteins: LCAT, FGF21, FGF21-PEG conjugate
- One gene therapy: lipoprotein lipase

All of these drug candidates have to be applied by an invasive delivery, i.e., by s.c. injection or infusions. The balance between efficacy and safety of these new

**Table 5** Lipid-modifying drugs in clinical development: triglyceride lowering (as primary target)

Target	Approach	Drug candidate	Company	Development stage
Lipoprotein lipase	Gene therapy	Glybera	UniQure	Approved
DGAT-1	Small molecule	Pradigastat	Novartis	Phase II
		P7435	Piramal Enterprises	Phase I
LpPLA2	Small molecule	Darapladib	GSK	Phase III
Apo CIII	Antisense	ISIS-APOC-III <sub>Rx</sub>	ISIS Pharmaceuticals	Phase III
ANGPTL3	Antisense	ISIS-ANGPTL3 <sub>Rx</sub>	ISIS Pharmaceuticals	Phase I
PPAR-alpha/delta	Small molecule	GFT-505	Genfit	Phase II
PPAR-alpha	Small molecule	ZYH7	Cydus Cadia	Phase I
		K-877	Kowa	Phase II/III
		NP-136	Mitsubishi Tanabe	Phase I
PPAR-delta	Small molecule	MBX-8025	CymaBay Therapeutics	Phase II
		CER-002	Cerenis Therapeutics	Phase I

biological approaches to reduce CVD events and the discomfort for the patients to receive repeated injections or infusions (at least once a month) either by medical doctors or self-medication with appropriate injection devices will finally determine the acceptability of these new treatment regimens for lipid disorders.

According to the mode of action, the drug candidates can be classified with regard to their primary focus in their interference with lipoprotein metabolism:

LDL-increase:

- Inhibition of cholesterol and triglyceride synthesis
- Inhibition of lipoprotein assembly
- Enhancement of lipoprotein clearance
- Antisense approaches

HDL-increase:

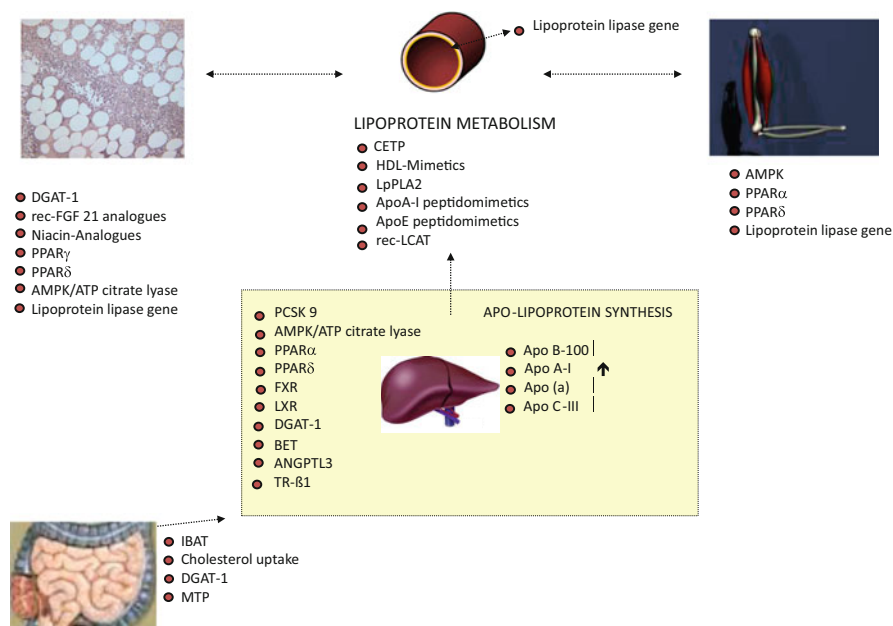
- Inhibition of lipoprotein assembly
- Enhancers of ApoA-I action

Concerning the mechanisms and molecular targets, there are two top activities in the lipid field: (a.) the development of approaches inhibiting the action of PCSK9 for LDL-C lowering and (b.) the development of inhibitors of CETP to increase HDL-C levels.

**Table 6** Lipid-modifying drugs in clinical development: miscellaneous targets and indications

Drug candidate	Company	Target	Approach	Indication	Development stage
SHP626/LUM002	Shire	IBAT-inhibitor	Small molecule	NASH	Phase I
SHP625/LUM001	Shire	IBAT-inhibitor	Small molecule	PBC	Phase II
Elobixbat	Albireo/Ferring/Ajinomoto	IBAT-inhibitor	Small molecule	Chronic constipation	Phase III
A4250	Albireo	IBAT-inhibitor	Small molecule	Cholestatic liver disease	Phase II
2330672	GSK	IBAT-inhibitor	Small molecule	T2 diabetes, cholestatic pruritus	Phase II
Px-104	Phenex Pharmaceuticals	FXR-agonist	Small molecule	NASH	Phase II
Obeticholic acid	Interecept	FXR-agonist	Small molecule	NASH, PBC, PSC	Phase II/III
S-556971	Shionogi-Kotobuki	NPC1L1-pathway	Small molecule	Hypercholesterolemia	Phase II
BMS-852927	BMS/Exelixis	LXR-Agonist	Small molecule	Metabolic Syndrome	Phase I
BMS-823778	BMS	? (MGAT2-Inh.?)	Small molecule	Dyslipidemia	Phase I
PF-06427878	Pfizer	?	Small molecule	Hyperlipidemia	Phase I





**Fig. 1** Organ localization of the targets and mode of actions of lipid-modifying drugs currently in clinical development

### 4.1 LDL- and Apolipoprotein B-Lowering Approaches

Familial hypercholesterolemia (FH) is an inherited lipid disorder with a prominent elevation of plasma LDL-C levels and CV risk caused by loss-of-function mutations in the genes for the LDL receptor, apolipoprotein B-100, or gain-of-function mutations for proprotein convertase subtilisin/kexin type 9 (PCSK9). With a prevalence of 1:500 in western populations, heterozygous FH is one of the most abundant inherited diseases, whereas homozygous FH is very rare (1 in a million) (Yuan et al. 2006). Current therapy options for the FH patients rely on statins and their combinations with ezetimibe and/or bile acid sequestrants, but still 5–10% of these patients do not reach LDL-C target levels. LDL apheresis is today the only available therapy for hoFH patients which is effective, but very costly with a limited access for patients to clinical centers performing apheresis. Therefore, inherited forms of hypercholesterolemia caused by mutations in the genes for apolipoprotein-B and PCSK9 are principally ideally suited for pharmacological approaches blocking these proteins, either by inhibition of the respective protein or by a gene-silencing therapy, whereas homozygous forms with loss of the LDL-receptor function can only be approached by gene therapy.

**Table 7** Classes of lipid-modifying drugs in clinical development

	Number of targets	Small molecules	Biologics							Sum of NCE's
			MoAb	Peptides	Proteins	Oligonuc	Vaccine	Gene therapy		
LDL-lowering	6	6	6			3				15
HDL-increasing	7	6		4	3			1		14
TG-lowering	8	9				2			1	12
Miscellaneous	6	11								11
	27	32	6	4	3	5	1	1		52

*Overall*

52 NCE's with 27 targets/modes of action

32 Small molecules

20 Biologics

### 4.1.1 Inhibition of Cholesterol and Triglyceride Synthesis

#### Inhibitors of Diacylglycerol Acyltransferases (DGAT)

A rapidly growing field in drug discovery for the treatment of dyslipidemia, obesity, diabetes, and the metabolic syndrome is the search for DGAT inhibitors. DGATs catalyze the final step in the biosynthesis of TG. In mammals, two nonhomologous enzymes encoded by different genes – DGAT-1 and DGAT-2 – are involved in TG synthesis; DGAT-1 is localized predominantly in the endoplasmic reticulum, whereas DGAT-2 also associates with lipid droplets (Liu et al. 2012). The interest in DGAT inhibitors was stimulated by the phenotype observed in DGAT k.o. mice. DGAT-1 k.o. mice have reduced TG levels, are resistant to diet-induced obesity, and show an increased insulin sensitivity (Smith et al. 2000; Chen and Farese 2000; Chen et al. 2002). DGAT-2 k.o. mice show a similar phenotype but exert severe skin abnormalities questioning the usefulness as a potential therapeutic target. However, antisense approaches to DGAT-2 have demonstrated decreases in body weight, adipose tissue, hepatic TG, and insulin resistance without skin abnormalities which may indicate that DGAT-2 inhibitors may be beneficial for the treatment of metabolic diseases, particularly of hepatic steatosis and nonalcoholic steatohepatitis (NASH). Currently at least four small molecule DGAT-1 inhibitors are in clinical and preclinical development, LCQ-908 in phase III being mostly advanced for the treatment of the rare familial chylomicronemia syndrome (hyperlipoproteinemia type I) ([www.novartis.com/downloads/newsroom/corporate-fact-sheet/2a\\_Pharmaceuticals\\_EN.pdf-2013-01-22](http://www.novartis.com/downloads/newsroom/corporate-fact-sheet/2a_Pharmaceuticals_EN.pdf-2013-01-22)). In randomized, placebo controlled clinical studies, pradigastat given to healthy human volunteers for 14 days suppressed postprandial TG dose-dependently with a maximal suppression of 90%; concomitantly, the number of postprandial chylomicron particles measured by ApoB48 levels was also reduced associated with modest reductions in fasting TG and cholesterol levels (Myers et al. 2012a). In a small clinical study with six patients suffering from familial chylomicronemia syndrome FCS, a 3-week treatment with 20 mg pradigastat was able to reduce plasma TG levels from 1,212 mg/dL by 38.4% nearly exclusively by a reduction in chylomicron TG; the compound was well tolerated at all doses tested (10–40 mg) with mild and transient gastrointestinal side effects in all patients. No serious adverse side effects or discontinuation from the study occurred (Myers et al. 2012b). Dosing for 14–28 days caused weight loss, improved insulin sensitivity, decreased lipid disposal after a meal, and reduced hepatic steatosis (Aicher et al. 2010). For inhibition of DGAT-2, an antisense drug is in preclinical development as an attempt for the treatment of NASH awaiting filing of an IND application early 2013 ([www.isispharm.com/Pipeline/index.htm](http://www.isispharm.com/Pipeline/index.htm); Drugs in development).

#### Activation of AMPK and Inhibition of ATP-Citrate Lyase

ETC-1002 (8-hydroxy-2,2,14,14-tetramethylpentadodecan-1,15-dioic acid) is a novel regulator of lipid and carbohydrate metabolism exerting a unique dual mode of action, namely, activation of AMPK and inhibition of ATP-citrate lyase, the latter mechanism exerted by formation of a coenzyme A thioester of ETC-1002

within the liver which is a direct inhibitor of ATP-citrate lyase (Pinkovsky et al. 2013). ETC-1002 was investigated in various clinical trials including four phase IIa double-blind placebo-controlled studies in cohorts of hypercholesterolemic patients of different characteristics (Newton et al. 2014a).

- a. One hundred and seventy-seven hypercholesterolemic patients with normal or elevated TG levels receiving 40, 80, or 120 mg ETC-1002 for 12 weeks showed reductions of 17.9–26.6% in LDL-C and 20% in hsCRP vs baseline.
- b. In 60 type 2 diabetes patients receiving up to 120 mg for 4 weeks, LDL-C and hsCRP levels were reduced by –42.9% and 40.5%, respectively.
- c. Fifty-six patients with statin intolerance receiving up to 240 mg ETC-1002 for 2 weeks showed reduction of 32 and 42% in LDL-C and hsCRP vs baseline.
- d. In 58 hypercholesterolemic patients treated with 10 mg/day atorvastatin, additional treatment with up to 240 mg/day ETC-1002 could reduce plasma levels of LDL-C and hsCRP by further 21.9% and 23.5% vs baseline.

The drug was well tolerated, and hypercholesterolemic patients with a history of statin intolerance treated with ETC-1002 had compared to placebo lower rates of muscle-related adverse events (27% vs 32%) and fewer number of discontinuation because of muscle-related adverse effects (0% vs 16%) and did not show signs of liver toxicity (increases in transaminases  $<3 \times \text{ULN}$ , CPK  $>5 \times \text{ULN}$ ) (Newton et al. 2014b). In a phase 2b study including 349 hypercholesterolemic patients for 12 weeks, the efficacy of ETC-1002 was investigated as monotherapy vs ezetimibe and in combination with ezetimibe. In monotherapy, ETC-1002 at 120 or 180 mg/day lowered LDL-C by 27% and 30% and hsCRP by 30% and 40% vs baseline compared to 21% and 11% for 10 mg ezetimibe. Patients treated with 10 mg ezetimibe receiving additional 120 or 180 mg/day ETC-1002 showed reduction in LDL-C and hsCRP of 43–48% and 28–38%, respectively (Press Release Esperion 2014). No clinically relevant changes in HDL-C or TG levels were observed, and the compound was well tolerated and not associated with any dose-limiting side effects. These data indicate that ETC-1002 is a potential safe alternative treatment of hypercholesterolemic patients.

### 4.1.2 Inhibition of Lipoprotein Assembly

#### Inhibition of the Microsomal Triglyceride Transfer Protein (MTP)

The assembly of VLDL particles from apolipoprotein B and lipids in the endoplasmic reticulum of hepatocytes and enterocytes is catalyzed by MTP, and consequently, inhibition of this protein would reduce the secretion of VLDL particles by the liver and of chylomicrons by the small intestine. Due to the catabolism of these lipoproteins to LDL, an inhibition of MTP results in a strong decrease of plasma LDL particles. MTP inhibitors are very efficacious in lowering both atherogenic ApoB-containing lipoproteins and plasma triglycerides, but their development is hampered and limited by its mechanism-based adverse side effects of lipid accumulation in the liver and the intestine with the drawbacks of liver toxicity by

hepatic steatosis and its sequelae. However, patients with homozygous FH are unresponsive to current lipid therapy and have to rely on LDL apheresis only. For these patients, the reduction in the synthesis rate of LDL particles by inhibition of VLDL/chylomicron assembly would be a new therapeutic option. In December 2012, the FDA approved lomitapide for the treatment of patients with homozygous FH as an adjunct to diet and other lipid-lowering therapies including LDL apheresis. In a phase III clinical trial, an average reduction of 40% in LDL-C was achieved after 26 weeks with LDL reduction up to 80% in patients receiving 40–60 mg of lomitapide daily. In addition, triglycerides were reduced by 54% compared to placebo. Most patients in the study showed mild to moderate gastrointestinal adverse effects with abdominal discomfort, diarrhea, and nausea (Cuchel et al. 2013). In order to limit the mechanism-based appearance of hepatic steatosis, SLx-4090 inhibits MTP in the enterocytes only because the compound is inactivated upon entry into systemic circulation by metabolization to an inactive metabolite. Results of a phase II clinical trial of SLx-4090 in combination with metformin for 12 weeks in type 2 diabetes patients indicate a significant reduction of plasma triglycerides by 35%, reduction of postprandial free fatty acids and HbA1c, and a weight loss of 1.3% (Tong et al. 2010).

### 4.1.3 Enhancement of Lipoprotein Clearance

#### Phospholipase A2 Inhibitors

Phospholipases are involved in the reshaping of lipoproteins and the pathogenesis of atherosclerosis. Phospholipase A2 (PLA2) occurs in two forms – secretory PLA2 (sPLA2) and associated to lipoproteins (LpPLA2). Overall, PLA2 occurs in 12 isoforms and 6 of them have been found associated with atherosclerotic lesions (Rosenson and Hurt-Camejo 2012). Elevated LpPLA2 levels are linked to an increased CVD risk (Packard 2009). PLA2 hydrolyzes glycopospholipids at the surface of VLDL and LDL particles, yielding lysophosphatides, oxidized unesterified free fatty acids, and eicosanoids, the latter being further processed to proinflammatory mediators like prostanoids, leukotrienes, and lipoxins. Additionally, the resulting VLDL and LDL particles are less efficiently cleared by the ApoB and ApoE receptors in the liver leading to an increased LDL-C level. These PLA2-modified LDL particles are avidly bound to macrophages with subsequent receptor-mediated internalization of cholesterol fostering foam cell formation. Due to these, functions of PLA2 in atherogenesis inhibitors to both forms of PLA2 have been in clinical development for a long time. Darapladib was tested overall in more than 23,000 patients whether it could significantly reduce the rate of strokes and heart attacks. In the IBIS-2 study, no effect on inflammatory markers, arterial stiffness, or atheroma volume could be discovered (Serruys et al. 2008). In patients with CHD, darapladib showed a dose-dependent inhibition of LpPLA2 activity up to 66% accompanied with a mean decrease of the inflammatory markers IL-6 and CRP by 12.3% and 13%, respectively. After 12 months of treatment with 160 mg/day darapladib, however, no differences between treatment and placebo groups were found with regard to plaque stability and plasma high-sensitive CRP. Whereas the

necrotic core volume of the plaques increased in the placebo group, darapladib produced a decrease in the core volume by  $5.2 \text{ mm}^3$  without significant differences in total atheroma volume (Serruys et al. 2008).

Recently published data from clinical trials with PLA2 inhibitors were disappointing. Neither with varespladib nor with darapladib (SOLID-TIMI 52 trial with 13,026 patients with acute coronary syndrome; STABILITY trial with 15,000 patients with chronic CHD) significant reductions on major coronary events could be found (The STABILITY Investigators 2014). A meta-analysis of 32 studies with PLA2 inhibitors involving overall 79,000 patients showed a correlation of both, PLA2 mass and PLA2 activity, with CVD events; however, after adjustment of ApoB levels, there was no longer a correlation.

Since statins reduce the number of ApoB-containing lipoproteins, a potential additional effect of PLA2 inhibition probably is marginal. Furthermore, inflammatory pathways are highly redundant, and pro- and anti-inflammatory effects may overlap, imposing significant doubts on the rationale and probability of success of this approach.

### Thyromimetics

Thyroid hormones, particularly triiodothyronine T3, have profound effects on lipid metabolism via various mechanisms such as increase in hepatic LDL-receptor expression, stimulation of cholesterol conversion to bile acids by activation of the cholesterol-7 alpha hydroxylase pathway, increase in hepatic cholesterol secretion by upregulation of ABC transporters ABCG5/ABCG8, and stimulation of RCT. Consequently, a liver-selective thyromimetic compound shows principally all desired actions for the treatment of dyslipidemias: decreases in LDL-C and TG and stimulation of RCT. Thyroid hormones exert these pleiotropic activities by means of thyroid receptors. The cholesterol-lowering activity is mainly mediated by the TR $\beta$ -1 receptor isoform predominantly expressed in hepatocytes, whereas the TR $\alpha$ -1 receptor is responsible for the heart rate increasing effects; therefore, a high selectivity for the TR $\beta$ -1 receptor is mandatory to avoid any CV adverse side effects (Gullberg et al. 2002). After initial trials with naturally occurring thyroid hormones, the observed spectrum of beneficial effects for dyslipidemias stimulated the search for safe and liver-selective thyromimetics. Eprotirome (KB-2115) was investigated in phase III trials for the treatment of hypercholesterolemia and dyslipidemia. A 12-week study in 189 patients on statin therapy with 25–100  $\mu\text{g}$  eprotirome/day showed promising results with reductions of LDL-C, TG, and Lp (a) of 22–32%, 27–43%, and 16–33%, respectively, with a small decrease in HDL-C by 6%. No significant adverse side effects on heart and cardiovascular function were observed (Ladenson et al. 2010). The development of the compound was terminated due to safety concerns by reason of cartilage damage after a 12-month toxicology study in dogs (Press Release Karo 2012). Another thyroid hormone receptor agonist, soberitome, was investigated in a phase I clinical trial, showing LDL-C reductions up to 41% in healthy volunteers (Scanlan 2010), but the compound is also no longer in development. The continuing interest in thyroid hormone analogues to overcome the mechanism-based issues is documented by the

recent entry of two new thyromimetic compounds – MGL-3196/VIA-3196 and ZYT1 – into phase I clinical trials. MGL-3196 is a liver-directed  $\beta$ -selective TR agonist lacking activity on the  $\alpha$ -TR receptor without any observed cardiovascular activity. In a phase I clinical trial in 48 healthy volunteers receiving daily doses of 50–200 mg MGL-3196 for 2 weeks, the compound was found to be safe without significant adverse side effects: Plasma lipid profiles were beneficially influenced with decreases of 30% in LDL-C, 28% in non-HDL-C, 24% in ApoB, and 60% in TG, indicating a promising profile for the treatment of mixed dyslipidemias (Taub et al. 2013). The major challenge for thyromimetics for the treatment of dyslipidemias remains the unequivocal demonstration of their long-term safety.

### **Inhibitors of Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9)**

The clinical efficacy of statins is limited by the so-called rule of 6%: each time the dose of a statin is doubled, the resulting decrease in LDL-C lowering is only additional 6%. Elevations of plasma transaminase levels and muscle toxicity have been associated with high statin doses, thereby limiting the tolerated dose of a statin and the achievable reduction in plasma LDL-C levels. As a consequence, a significant percentage of statin-treated patients does not reach the recommended LDL-C target levels. The findings that around 3% of Afro-Americans are heterozygous for loss-of-function mutations of PCSK9 associated with very low plasma LDL cholesterol levels and a reduction of CV events by 80–90% (Konrad et al. 2011) has made PCSK9 one of the most intensively investigated novel targets to treat hypercholesterolemia. PCSK9 is a serine protease synthesized by the liver and the intestine as a 692 amino acid precursor protein. After autocatalytic cleavage in the endoplasmic reticulum, the cleaved prodomain acts as a chaperone tightly bound to the mature 62 kDa PCSK9 protein permitting the entry of this complex into the secretory pathway (Lambert et al. 2012). The action of PCSK9 is twofold: (a) in the post-endoplasmic reticulum compartment, PCSK9 guides the LDL receptor for degradation into lysosomes and (b) mature 62 kDa PCSK9-protein is secreted and binds to the LDL receptor at the surface of the hepatocyte. The complex composed of PCSK9, LDL receptor, and the adaptor protein ARH is then internalized into endosomes. Binding of PCSK9 prevents the LDL receptor from being recycled back to the cell surface, shifting the PCSK9/LDL-receptor complex to the acidic compartment of lysosomes for degradation. By this dual action, PCSK9 reduces the surface expression of LDL receptors and thus decreases the clearance rate of LDL particles resulting in an increase of LDL-C plasma levels. The height of circulating PCSK9 levels is predictive for cardiovascular events in patients with CAD based on its positive correlation to plasma TG levels (Werner et al. 2014), and thus plasma PCSK9 levels may be useful as a biomarker for CVD risk assessment. It is obvious from these mechanisms that by inhibition of the action of PCSK9, the endogenous “brake” in LDL-receptor-mediated LDL clearance can be released. The activity of PCSK9 is not restricted to the LDL receptor but also to a variety of other receptors like VLDLR, ApoER2 (LRP8), LRP1, BACE1, and hepatic receptor CD81 (Norata et al. 2014); the degradation of these receptors probably involves specific cellular pathways not directly linked with cholesterol

metabolism, such as modulation of genes involved in proliferative, apoptotic, and inflammatory pathways (Norata et al. 2014).

Various observations contribute in a convergent manner to the high attractivity of PCSK9 as a (the ?) major regulator to treat hypercholesterolemia:

- Natural loss-of-function mutations in human lead to maximal reduction in plasma LDL-cholesterol levels without apparent adverse side effects. However, until now homozygous mutations have been described only in a few cases not allowing a generalization of safety perspectives to large populations being treated with a PCSK9 inhibitor. Additionally to its function in regulating cholesterol homeostasis PCSK9 may be involved in further cellular processes: Controversial results have been published with regard to a potential increase in BACE 1 and  $\beta$ -amyloid levels in the brain of PCSK9 k.o. mice raising the possibility of an increased risk to develop Alzheimer's disease upon PCSK9 inhibition treatment (Jonas et al. 2008; Liu et al. 2010).
- PCSK9 may be necessary for a normal  $\beta$ -cell function and therefore, loss of PCSK9 may predispose to diabetes treatment (Mbikay et al. 2010).
- PCSK9 reduces the expression of CD 81, the receptor mediating the cellular entry of the hepatitis C virus (Labonte et al. 2009) indicating a potential risk for HCV infection upon treatment with PCSK9 inhibitors.
- In one study it was shown that PCSK9 is necessary for liver regeneration (Zaid et al. 2008). This is particularly important in the light of co-medication with drugs having a potential for liver toxicity.
- PCSK9 inhibits also the VLDL receptor and PCSK9 k.o. mice show adipocyte hypertrophy and fat accumulation (Roubtsova et al. 2011).

So far no reports regarding these potential adverse effects have been reported in humans. Nevertheless, these potential physiological roles of PCSK9 have to be further investigated to ensure a safe long-term treatment with PCSK9 inhibitors. The mode of action of PCSK9 by extracellular binding to the LDL receptor avoids the necessity of a hepatocyte or organelle-specific drug targeting. Cholesterol-lowering drugs can increase plasma levels of PCSK9, thereby limiting their pharmacological efficacy and explaining the underlying biochemistry of the “rule of 6%” found with doubling of a statin dose (Mayne et al. 2008; Dubuc et al. 2010). PCSK9 expression is mainly modulated by sensing intracellular cholesterol levels and subsequent activation of SREBP-2 leading to a concomitant increase in the expression of LDL receptor, HMG-CoA reductase, and PCSK9 to ensure cellular cholesterol homeostasis (Dong et al. 2010). Statins, particularly at higher dosages, can strongly induce PCSK9 up to 47% with 80 mg/day atorvastatin (Welder et al. 2010). For fibrates and ezetimibe, controversial results regarding their effect to increase PCSK9 levels were reported (Konrad et al. 2011). An optimal therapy to lower LDL-C levels would therefore be possible by combination of a statin or other LDL-C-lowering drugs with a PCSK9 inhibitor to counteract attenuation of statin potency.



Due to the mechanisms of PCSK9 action, the following approaches are principal options for drugs interfering with the PCSK9 pathway:

- A. Silencing of PCSK9 expression
- B. Inhibition of autocatalytic processing of the PCSK9 precursor protein
- C. Prevention of PCSK9 secretion
- D. Prevention of the interaction of secreted PCSK9 with the LDL receptor
- E. Prevention of endosome targeting to lysosomes

#### Monoclonal Antibodies Against PCSK9

Most advanced is approach D aiming to inhibit the interaction of secreted PCSK9 with the LDL receptor, either by neutralizing antibodies against PCSK9 or peptidomimetics mimicking the contact points between PCSK9 and the LDL receptor. Currently there are at least 5 different monoclonal anti-PCSK9 antibodies in clinical development. For the three most advanced antibodies – evolocumab (AMG-145), alirocumab (SAR236553/REGN727), and bococizumab (RIN 316) – extensive data from clinical studies in phase I and II (all 3) as well as III for evolocumab and alirocumab have been published (an excellent overview about all performed and running clinical data can be found in the paper of Dadu et al. (2014)). In phase I clinical trials in monotherapy, these antibodies reduced LDL-C by 61–81% concomitantly with a reduction of ApoB by 48–59%. A further cardiovascular risk factor, Lp(a), was reduced by all three drugs by 27–50%. Alirocumab additionally decreased TG levels by 16% and increased HDL-C by 18%, whereas no effect on these parameters were found for evolocumab. No significant differences regarding adverse side effects were found for all three antibodies: the most common adverse side effects were injection site reaction with pain and localized rash (2–9%), upper respiratory tract infection (6–10%), nasopharyngitis (4–15%), and gastrointestinal disturbance like diarrhea (4%) and nausea (4–6%). When used in combination with other lipid-lowering drugs like statins or ezetimibe, the PCSK9 antibodies could further decrease LDL-C by 50–60%. In phase II clinical trials in combination with statins or ezetimibe, the outstanding efficacy of blocking PCSK9 with antibodies could be further underpinned: In addition to statins, alirocumab reduced LDL-C by 35–68% and Lp(a) by 13–32% compared to 42–66% and 11–29% for evolocumab, respectively. The effect of both drugs on HDL-C with increases of 13–32% for alirocumab and 11–29% for evolocumab and reductions of TG levels by 6–18% and 4–33% were as well comparable. In a placebo-controlled trial in statin-treated patients after 24 weeks, bococizumab at doses of 150 or 300 mg twice monthly produced mean changes of LDL-C of 67% and 55% from baseline, respectively (Ballantyne et al. 2014). Whereas in the phase II trials primarily patients with a primary hypercholesterolemia on stable statin doses were included, the ongoing and planned phase III trials include a much wider spectrum of patients with dyslipidemias such as statin-intolerant and naive on lipid therapy with and without combinations with statins, ezetimibe, or fibrates.

The recently published data from the first two Phase III clinical trials allow a first anticipation regarding the potential impact and quantum leap in the treatment of lipid disorders and potential prevention of atherosclerosis. In the ODYSSEY long-term trial, 2,431 patients of a high CV risk (18% of them with heterogeneous FH) being treated with a statin or another lipid-modifying therapy were treated for 78 weeks in a placebo-controlled study with 150 mg of alirocumab s.c. every fortnight. After 24 weeks, mean reduction in LDL-C was 61% vs. 0.8 increase in the placebo group. Seventy-nine percent of treated patients achieved the target of at least 50% reduction in LDL-C from baseline, and discontinuation rate was similar in the verum and placebo arms with 6.2% vs 5.5%, respectively. The alirocumab group shows a 54% risk reduction in the absolute rate of CV events (1.4% vs 3%) (Robinson et al. 2015). Treatment of hypercholesterolemic patients with 150 or 300 mg alirocumab every 4 weeks resulted in similar reductions of LDL-C as fortnightly injections (Press Release Sanofi 2015).

Within the DESCARTES study 901, hypercholesterolemic patients were stratified into four groups treated with diet alone, 10 mg atorvastatin, 80 mg atorvastatin, or 80 mg atorvastatin plus 10 mg ezetimibe, and received additional 420 mg of evolocumab s.c. every 4 weeks. Mean add-on reductions of 55.7%, 61.2%, 56.8%, and 48.5% could be achieved with supplementation of evolocumab with additional reduction in ApoB, non-HDL-C, Lp(a), and TG plasma levels (Blom et al. 2014). In patients with hoFH, PCSK9 antibodies are also efficacious as shown recently by the TESLA study: In 50 patients with a mean LDL-C of 348 mg/dL after 12 weeks of treatment with 420 mg of evolocumab, LDL-C were reduced by 30.9% compared with an increase by 8% in the nontreated group, in patients with mutations in the LDL receptor, a reduction by 41% could be demonstrated, whereas the drug had no effect in patients devoid of the LDL receptor (Raal et al. 2015). Huge phase III clinical outcome trials are running for alirocumab and evolocumab involving 23,500 (ODYSSEY) and 22,000 (FOURIER) patients, respectively, and for bococizumab as well a phase III trial involving 22,000 patients is planned (for a detailed overview regarding the clinical trials with PCSK9 antibodies, see the excellent article by Dadu et al. (2014). Conclusive results from these trials are expected in 2018, allowing a definite judgment regarding the clinical benefit of PCSK9 inhibition for cardiovascular diseases. Primary outcome parameters of these megatrials will be the time of occurrence of one of the following clinical events: death from CHD, nonfatal myocardial infarction, stroke, or hospitalization due to unstable angina. In summary, the clinical findings obtained so far with PCSK9 neutralizing antibodies are encouraging. It will have to be demonstrated that these impressive reductions in LDL-C and Lp(a) levels are translated into measurable clinical benefits for the patients in terms of reduction of mortality and prevention from CHD and that this antibody therapy possesses a high long-term safety.

### Alternative Approaches to Inhibit PCSK9 Activity

An alternative approach to monoclonal antibodies for blocking the protein–protein interaction between PCSK9 and the LDL receptor is the design of peptides or peptidomimetics interfering with the different contact surfaces between the two proteins. Drug candidates mimicking the C-terminus of PCSK9 (SX-PCK9), the EGF-A domain of the LDL receptor, or an adnectin based on a scaffold for human fibronectin with an engineered domain to prevent the interaction between PCSK9 and the LDL receptor (BMS-962476) are evaluated in early preclinical and clinical trials, respectively (Mullard 2012). Both monoclonal antibodies and PCSK9 mimetics tackle the function of the secreted PCSK9 protein only. Since PCSK9 is also intracellularly involved in the regulation of LDL-receptor surface expression approaches, silencing the PCSK9 gene should theoretically be even more effective. Both an antisense (BMS-PCSK9Rx-2) and gene-silencing approaches are evaluated. Most advanced in this regard is the investigational drug CAT-2003 in phase 2 clinical trials: CAT-2003 is an orally active small molecule – a covalent conjugate between niacin and eicosapentaenoic acid. The molecule is systemically inert, after uptake into cells the prodrug is intracellularly hydrolyzed into its pharmacologically active drugs. The molecular mechanism whereby CAT-2003 inhibits PCSK9 is the inhibition of the processing of the SREBP 120 kDa precursor protein into active SREBPs, thereby inhibiting the activation of SREBP-regulated target genes such as PCSK9 (Zimmer et al. 2014). In HepG2 cells, expression of SREBP2 is reduced by 50% as well as PCSK9-mRNA. Due to the pleiotropic action of SREBPs, the actions of other genes like HMG-CoA-reductase, SCD-1, ACC-2, S1P, or S2P are as well inhibited. In ApoA3 Leiden mice being treated for 16 weeks with the compound plasma levels of PCSK9, total cholesterol and TG were reduced by 61%, 41%, and 33%, respectively; histological evaluation of the livers showed an increase in the expression of LDL receptor by 104%. In a phase I clinical trial (PATHWAYS I), normal healthy volunteers ( $N = 99$ ) receiving 300–2,000 mg CAT-2003 for up to 14 days showed a decrease of TG levels up to 90% at each dose tested with a concomitant reduction of the plasma levels of PCSK9, non-HDL-C and LDL-C (Donovan et al. 2014). Plasma levels of NEFAs were not increased, indicating that the compound did not activate the nicotinic acid receptor GPR109a. Furthermore, no flushing was observed, demonstrating no systemic activity of niacin via the PDGR2 receptor. Further clinical trials are ongoing, among them a 12-week study in patients with chylomicronemia (hyperlipoproteinemia type I) to assess further efficacy and safety.

A small molecule originally designed as a CETP inhibitor K-312 – currently in phase 1 clinical trials – was found to suppress also the production of PCSK9; in the hepatocyte cell line, HepG2 K-312 decreased PCSK9 mRNA by 90% by inhibiting SREBP binding to the sterol response element SRE in the promoter region of the PCSK9 gene and suppressing SREBP expression. As a potential mechanism of K-312 was suggested that it interferes with the binding of the transcription factors SREBP  $\frac{1}{2}$ , HNF $\alpha/\beta$ , and HINFP to their closely neighboring binding sites in the PCSK9 promoter region (Shibata et al. 2012; Miyosawa et al. 2012).

### **Apolipoprotein E Mimetics**

AEM-28 is a synthetic 28mer peptide combining the a 18mer amphipathic lipid-binding domain of ApoE and the binding domain for the human ApoE receptor. AEM-28 has the ability to insert into the phospholipid surface of ApoB containing lipoproteins, targeting them to the liver where it is docked via the heparansulfate proteoglycan receptor (syndecan 1). In normocholesterolemic and hypercholesterolemic cynomolgus monkeys, AEM-28 was able to reduce total cholesterol levels up to 70% after a 2-h infusion (Goldberg et al. 2014). In a first-in-man study, the compound showed an acceptable safety profile with reductions of VLDL and TG up to 76% vs baseline within the first 12 h after infusion (Press Release Capstone 2014). Treatment with AEM-28 maybe an effective treatment option for patients that lack a functional or dysfunctional LDL-receptor pathway such as homozygous and heterozygous FH or severe refractory hypercholesterolemia.

#### **4.1.4 Inhibition of Lipoprotein Lp(a)**

Epidemiological and genetic studies as well as meta-analyses have manifested a causal and independent role of apolipoprotein Lp(a) for CVD, particularly myocardial infarction and stroke (Kolski and Tsimikas 2012) with Lp(a) levels linearly linked to the risk of CVD. Lp(a) levels show a strong heterogeneity primarily due to genetic variations between individuals and cannot be modulated by diet or lifestyle changes. Lp(a) mediates its atherogenicity through both its LDL and its apolipoprotein (a) moiety, the latter being atherogenic because of the proinflammatory properties of the Kringle domains. The most effective therapy to reduce elevated Lp(a) levels is plasma apheresis resulting in Lp(a) reductions up to 75%. Currently no drugs are approved for specific lowering of Lp(a), but some drugs like nicotinic acid show a certain efficacy in lowering of Lp(a) levels. Several lipid-modifying drugs in clinical development are as well investigated for their effects on Lp(a). Mipomersen recently approved for the treatment of FH showed a Lp(a) reduction by 42% and 50% in patients with heterozygous and homozygous familial FH, respectively (Visser et al. 2012). A specifically designed antisense nucleotide against apolipoprotein A was able to reduce its expression in animals more than 80% (Merki et al. 2011). The MTP inhibitor lomitapide decreased Lp(a) modestly by 17% (Samaha et al. 2008). Antibodies against PCSK9 like AMG-145 and SAR236553 reduced Lp(a) levels in hypercholesterolemic patients by 15–44% (Dias et al. 2012; Stein et al. 2012a). The CETP inhibitor anacetrapib or the thymimetic eprotirome also reduced Lp(a) levels by up to 40%. A novel and efficacious approach to reduce Lp(a) seems possible with agonists of the farnesoid X receptor (FXR) (Chennamsety et al. 2011); obeticholic acid (Neuschwander-Tetri et al. 2014) and Px-104 (Press Release Phenex 2012) are FXR agonists currently in clinical development for the treatment of NASH.

### 4.1.5 Gene Modulation Approaches

#### Antisense and Gene Silencing

Patients with statin intolerance and heterozygous FH do not reach the ATP III goals for LDL-C (Rana and Boeckholdt 2010), indicating a significant medical need for efficacious lipid management for these patients. Principally, antisense oligonucleotide (ASO) or RNA silencing technologies are ideally suited to achieve a high specificity in inhibiting the specific function of a particular protein by downregulating its synthesis on the translation level, thereby minimizing the risk of drug–drug interactions due to lack of involvement of cytochrome P450 enzymes in drug metabolism. This is of particular importance for multimorbid patients often receiving a multidrug therapy. Major disadvantages of antisense-directed therapies are their limited stability against serum and cellular nucleases, their limited permeability across biological membranes, as well as their off-target effects by interacting with Toll-like receptors (Jones 2011). Therefore, RNA-based and antisense drugs have to be applied parenterally, and thus the delivery to their target tissues and cells is still a major challenge and obstacle for RNA and ASO drugs. After an i.v. injection, the majority of ASOs are accumulated in the liver and the kidney, thus making therapeutically interesting drug targets localized in these organs a priority for ASO therapy. On the other hand, this organotropism remains a major challenge for RNA and DNA-based drugs acting in other tissues than the liver and the kidney. Fortunately, the liver is the major organ regulating lipid homeostasis and metabolism, and therefore ASO therapies are a reliable approach to treat dyslipidemias by blocking the expression of target genes localized in the hepatocyte such as apolipoprotein B-100, apolipoprotein(a), PCSK9, or MTP. In particular, locked nucleic acid-derived ASOs are promising candidates for a wider scope of application due to their improved compound characteristics such as cellular uptake via gymnosis (Stein et al. 2010) and the avoidance of liposome technologies with their associated toxic lipid reagents.

#### Apolipoprotein B

##### ApoB Antisense Approaches

The most advanced nucleic acid-based drug to treat dyslipidemias is mipomersen (Toth 2011), which obtained FDA approval for treatment of patients with homozygous FH in early 2013 (Press Release Sanofi 2013). Mipomersen is an Apo B100 ASO made of 20 2'-O-methyl-ethyl-nucleotides. Several clinical trials have demonstrated the efficacy of mipomersen to reduce Apo B-100, LDL-C, total cholesterol levels, as well as Lp(a) levels. In healthy volunteers, Apo B-100 and LDL-C levels were reduced by 21% and 34% (Akdin et al. 2010). Addition of 200 mg mipomersen s.c. once weekly to heterozygous FH patients being treated with the maximal possible statin doses reduced their LDL-C levels further by  $-28 \pm 5.2\%$ , and 49% of treated patients achieved their LDL-C target of 100 mg/dL (2.6 mM) compared to only 4.9% in the group treated with statins only. Efficacy of mipomersen was better in females than in men (mean LDL-C reduction  $-40.6\%$

vs  $-20\%$ ). The dropout rate of  $10.8\%$  was mainly caused by injection-site reactions, and  $4.9\%$  of treated patients showed an increase in liver fat (Stein et al. 2012b). In patients with homozygous FH treated with the maximally tolerated dose of statins,  $200\text{ mg}$  of mipomersen reduced LDL-C by further  $24.7\%$  (Raal et al. 2010).

Adverse side effects were elevations of transaminases, flu-like symptoms, and injection site reactions as well as signs of hepatic steatosis frequently found in FH patients with genetic deficiencies of Apo B-100, limiting the maximal tolerable dose. The FDA voted for approval of mipomersen as an add-on therapy for patients with homozygous FH being already treated with another lipid-lowering therapy on a low-fat diet (Press Release Sanofi 2013), whereas the European Agency EMA did not approve mipomersen due to safety concerns. Mipomersen is a new and attractive alternative to LDL apheresis for these patients.

### ApoB RNAi-Approaches

For apolipoprotein B-100, several RNA-silencing drugs are investigated in clinical trials. Using the SNALP technology (Rozema et al. 2007), the liposomal siRNA drug PRO-040201 (Press Release Tekmira 2009) tested at doses of  $0.03\text{--}1\text{ mg/kg}$  in 23 probands was well tolerated without severe adverse side effects or injection site reactions; one proband expressed “flu-like symptoms” probably caused by an innate immune response to the ApoB SNALP drug product with dose-limiting cytokine release leading to termination of its development (Press Release 2009). The development of a follow-up compound with adjustments to the ApoB siRNA (TKM-ApoB) to minimize any immune stimulatory properties is reported to continue.<sup>1</sup>

## PCSK9

### PCSK9 RNAi Approaches

For PCSK9, several RNA-based drug approaches are in preclinical and clinical development. ALN-PCS is an RNAi drug delivered as a stable nucleic acid lipid particle (SNALP delivery technology) (Rozema et al. 2007) aiming to silence the PCSK9 gene. A phase I trial was performed with healthy volunteers having elevated LDL-C levels  $>110\text{ mg/dL}$  with a single injection of doses of  $0.015\text{--}0.4\text{ mg/kg}$ . After 4 weeks, plasma levels for PCSK9 and LDL-C were reduced up to  $84\%$  and  $50\%$ , respectively, with no effect on HDL-C levels. The compound was reported to be safe without severe adverse side effects (Fitzgerald et al. 2014). Importantly, no elevations in liver enzymes were observed tempting to speculate that PCSK9 may be a better target for antisense approaches to lower LDL cholesterol levels with regard to liver toxicity compared to apolipoprotein B. In a multidose study with a dose of  $2\text{ mg/kg}$ , circulating PCSK9 levels were reduced by  $95\%$  leading to a  $67\%$

<sup>1</sup>Homepage Tekmira Pharmaceuticals Corporation. <http://www.tekmira.com/programs/Products.asp#apob>

decrease in LDL-C (Fitzgerald et al. 2014). With a new drug delivery platform based on GalNAC-siRNA conjugates, subcutaneous application with a long duration of action become possible; in nonhuman primates, a single dose of 3–10 mg/kg of ALN-PCS s.c. knocked down PCSK9 levels up to 96% and LDL-C up to a 77% with a reduction of LDL-C of >50% even 90 days after injection. These data support attempts for an effective treatment of hypercholesterolemia with injection of a PCSK9 RNAi drug only once a month or even once per quarter (Press Release Alnylam 2015). On a preclinical level, a 19mer gene-silencing oligonucleotide for PCSK9 mRNA shows after s.c. administration a significant specific reduction in the concentration of PCSK9 mRNA without affecting expression of ABCA1, ABCG1, and LXR (Press Release Idera 2011).

### PCSK9 Antisense Approaches

Two antisense drug candidates for PCSK9 – Santaris SPC 5001 BMS/ISIS-394814, both being phosphothioates to work via an RNase H mechanism – have been investigated in phase I clinical trials, but their development has been terminated for undisclosed reasons. Recently, a case of acute kidney injury (with recovery) was reported in one patient having received weekly injections of SPC-5001 for 3 weeks with multifocal tubular necrosis and signs of oligonucleotide accumulation in the kidney (van Poelgeest et al. 2013). It is known that phosphothiate ASOs bind particularly to proteins in plasma and various tissues interacting with polyanions such as PDGF or VEGF resulting in high concentrations in these tissues and therefore a high efficacy. On the other hand, the resulting high concentrations in the liver, kidney, and spleen can lead to cellular toxicity with transient inhibition of the clotting cascade (Kurreck 2003), thereby limiting the therapeutic window of phosphothioate-based ASOs.

## Other RNA-Based Drug Candidates

### Antisense Approaches

Further molecular targets for antisense approaches for the treatment of dyslipidemias in development are apolipoprotein CIII (ISIS-ApoC-III<sub>Rx</sub>), lipoprotein Lp(a) (ISIS-Apo(a)<sub>Rx</sub>), and angiopoietin-like III (ISIS-ANGPTL3<sub>Rx</sub>). Chylomicronemia is a rare inherited disease with 3–5,000 patients worldwide; due to increased levels of apolipoprotein CIII as a noncompetitive inhibitor of lipoprotein lipase, TG-rich lipoproteins cannot be catabolized leading to extremely high TG plasma levels up to 2,000 mg/dL strongly increasing the risk for acute pancreatitis and diabetes. Polymorphisms in ApoC-III have been associated with hypertriglyceridemia; ApoC-III loss-of-function results in higher TG hydrolysis rates and consequent increased TG clearance leading to lower TG and VLDL levels in heterozygous individuals of ApoC-III loss-of-function, and recently it was shown that rare loss-of-function variants in ApoC-III seem to be cardioprotective (Tachmazidou et al. 2013). In a phase II clinical program, ISIS-APOC-III<sub>Rx</sub> was investigated in patients with extremely high TG levels, type 2 diabetics, and FCS (familial chylomicronemia syndrome) receiving 300 mg weekly. In single therapy after 13 weeks, plasma levels of ApoC-III and TG

were reduced by 88% and 69%, respectively, whereas HDL-C increased by 42%. In addition, glucose control was significantly improved with reduction of 1.2% in HbA1c (placebo-adjusted) as well as reductions in serum fructosamine and glycated albumin (Alexander et al. 2014), suggesting a potential of this drug to improve peripheral insulin sensitivity in patients with diabetic dyslipidemia. ISIS-APO(a)Rx investigated in a phase I trial in healthy volunteers with Lp(a) levels ranging from 10 to 98 mg/dL decreased Lp(a) levels dose-dependently by 95% and reduced oxidized pro-inflammatory phospholipids by up to 59%.<sup>2</sup>

### RNAi Approaches

Using the GalNAC siRNA conjugate platform RNAi drugs silencing PCSK9 (see above), ApoC-III (ALN-AC3), and ANGPTL3 are investigated in preclinical investigations. In mouse models matching human genetics, a single s.c.-administration of ALN-AC3 knocked down ApoC-III levels by >95% and reduced TG levels up to 68% with a persistence of the pharmacodynamic effect for more than 20 days. ANGPTL3 is an independent risk factor for CV diseases, regulating lipid, glucose, and energy metabolism; individuals with increased levels of ANGPTL3 exert hyperlipidemia with an increased risk of heart attacks, increased arterial wall thickness, and metabolic disease including insulin resistance. ANGPTL3 is an inhibitor of cellular lipases produced by the liver, and genetic investigations have demonstrated that loss-of-function in ANGPTL3 is associated with decreased levels of both TG and LDL-C (Musunuru et al. 2010). S.c. applications of ALN-ANG to ob/ob mice resulted in 95% knockdown of ANGPTL3 and decreases in TG, LDL-C, and total C levels of 95%, 85%, and 60%, respectively. In addition to the RNAi drug ALN-ANG, also an antisense drug ISIS-ANGPTL3Rx is in phase 1 clinical trials. If these findings could be confirmed in humans, silencing/k.o. of ANGPTL3 could become an important therapeutic option for the treatment of severe hypertriglyceridemia and mixed dyslipidemias.

### Gene Therapy

For the treatment of patients with the rare disease FCS (“familial chylomicronemia syndrome,” hyperlipoproteinemia type 1a) being deficient in the enzyme lipoprotein lipase which is essential to breakdown circulating chylomicron particles, the drug adipogene tiparvovec (Glybera) was approved in 2012 in Europe as the first gene therapy drug (Melchiorri et al. 2013) to restore LPL activity. Glybera contains the gene for the human LPL-variant S447X with a tissue-specific promoter in a non-replicating AAV1 vector with a high affinity for muscle tissue. For gene therapy, Glybera was applied by multiple injections at doses of  $10^{11}$  to  $10^{12}$  gene copies/kg body weight into muscles of the lower extremities under spinal anesthesia or strong sedation of the patients. Three days before and 12 weeks after Glybera injection, an immunosuppressive therapy with cyclosporin (3 mg/kg  $\times$  day) and mycophenolat mofetil ( $2 \times 1$  g/day) is recommended to avoid antibody formation.

<sup>2</sup> Homepage ISIS Pharmaceuticals. [www.isispharma.com/Pipeline/index.htm](http://www.isispharma.com/Pipeline/index.htm)



Overall, 27 patients received the gene therapy; 2–12 weeks after injection in some patients, reduction of fasting TG levels >40% could be achieved, and after 4 months, the starting TG levels reappeared. Muscle biopsies after 6 months demonstrated the expression of the LPL gene and the presence of secreted active LPL enzyme. A follow-up observation of 12 patients with earlier episodes of pancreatitis up to 3 years indicated a tendency to less frequent and less severe acute attacks of pancreatitis ([www.ema.europa.eu/docs/de\\_DE/document\\_library/EPAR-Product\\_Information/human/002145/WC500135472.pdf](http://www.ema.europa.eu/docs/de_DE/document_library/EPAR-Product_Information/human/002145/WC500135472.pdf)).

## 4.2 Novel Approaches to Increase HDL

The inverse relationship of HDL-C levels and the incidence of CHD (Parhofer 2005; Gordon et al. 2000) indicate that an elevation of HDL levels should exert antiatherogenic properties. A major mechanism for the antiatherosclerotic properties of HDL is their role in RCT as the key pathway to transport cholesterol from peripheral tissues such as cholesterol-laden macrophages to the liver for excretion into bile. RCT is a very complex pathway not fully understood involving a sequence of events:

1. Synthesis of ApoA-1 and formation of nascent pre- $\beta$ -HDL particles.
2. Uptake of cholesterol from peripheral cells (macrophages) by mediation through the ABC transporter ABCA1 and ABCG1.
3. Esterification of cholesterol taken up to cholesterol esters (CE) by lecithin-cholesterol acyl transferase (LCAT) to avoid cholesterol efflux from the HDL particles and leading to formation of CE-rich  $\alpha$ 2 HDL and  $\alpha$ 3 HDL.
4. CE transfer from HDL particles to ApoB-rich lipoproteins in exchange for triglycerides catalyzed by CETP resulting in the formation of pre- $\alpha$  and pre- $\alpha$ 1 HDL particles. The resulting TG-rich HDL is susceptible to lipolytic modification by hepatic lipase and endothelial lipase leading to the formation of smaller HDL particles more sensitive to faster catabolism.
5. Hydrolytic release of phospholipids from these HDL particles by the enzymes hepatic lipase (HL), endothelial lipase (EL), and soluble phospholipase A2 (sPLA2).
6. Hepatic uptake of remaining CE from HDL particles by the scavenger receptor SR-BI releasing ApoA-1 for cholesterol reloading in peripheral tissues or being filtered in the kidney into urine.

There are three monogenic causes known of elevated HDL levels with loss of function of CETP, EL, and ApoC-III (Larach et al. 2013):

- Loss of function of CETP blocks the transfer of CE to ApoB-containing lipoproteins and prevents therefore the formation of more atherogenic VLDL and LDL particles.

- Loss of function of ApoC-III as a noncompetitive inhibitor of lipoprotein lipase stimulates hydrolysis of TG-laden lipoprotein particles, and due to the reciprocal interrelationship of high TG and low HDL, plasma levels of HDL are increased.
- Endothelial lipase catalyses the hydrolysis of phospholipids in HDL particles, thereby destabilizing HDL particles which show an increased catabolism; loss of function of EL prevents this EL-mediated catabolism of HDL particles.

However, the role of HDL particles is more complex than just being a carrier for water-insoluble lipids in plasma in the course of RCT. In addition to this key function, HDL particles exert antioxidative, anti-inflammatory, and antithrombotic activities (Grunfeld and Feingold 2008) as well as improvement of endothelial function (Terasaka et al. 2008). The functionality of HDL particles is as important as the magnitude of plasma HDL levels for protection from atherosclerosis. HDL is also able to inhibit the formation of oxidized LDL, and lipid peroxides from oxidized LDL particles (oxLDL) can be transferred via CETP to HDL particles leading to a rapid clearance of the resulting lipid peroxide-laden HDL particles from circulation, indicating a role of HDL in the elimination of oxidized LDL as well (Garner et al. 1998). Additionally, HDL and ApoA-1 can protect endothelial cells from injury by inhibiting signaling pathways involved in apoptosis of endothelial cells triggered by oxLDL or proinflammatory cytokines (Suc et al. 1997). The findings that people with certain ApoA-1 mutations are not at a higher risk for CVD despite very low overall HDL levels underline the essential role of HDL particle functionality. The complex physiology of the HDL metabolism offers in a variety of drug approaches such as:

- Prevention of HDL particle reshaping by inhibition of lipid-transfer proteins like CETP or inhibition of phospholipid-hydrolyzing enzymes like HL, EL, or sPLA2.
- Increasing production of ApoA-I.
- Substitution by exogenous reconstituted HDL particles or ApoA-I peptidomimetics.
- Stimulation of cholesterol efflux from macrophages by increasing the expression of ABCA1 and ABCG1.
- Stimulation of hepatic CE uptake mediated by the scavenger receptor SR-BI.
- In addition, modulation of the antioxidative, anti-inflammatory, or antithrombotic properties of HDL particles by interference with targets like the apolipoproteins E, J, A2, or A3 and enzymes like paraoxonase (PON1) or LCAT can be considered.

The negative outcome with the CETP inhibitor torcetrapib may indicate that not every way of raising HDL cholesterol levels automatically translates into a clinical benefit. The nearly complete and irreversible inhibition of CETP may have eliminated the portion of RCT of CE by VLDL and LDL to such an extent that any beneficial effect was eliminated (Tall 2007). It may be that the dynamics of lipoprotein modulation have to be maintained.

This complexity of HDL pathways shows that simply measuring HDL levels is not a viable parameter for drug efficacy and more sophisticated methods to monitor the different aspects of HDL function are needed as was recently demonstrated by a detailed analysis of HDL parameters and their correlation to endpoints of CVD in a population cohort (Rohatgi et al. 2014).

#### **4.2.1 Inhibitors of Cholesterol Ester Transfer Protein (CETP)**

CETP catalyzes the transfer of CE from HDL particles to VLDL or LDL, yielding cholesterol-rich apoB-containing lipoproteins which contribute to an increased deposition of cholesterol into the peripheral arterial wall. The reverse translocation of TG from VLDL and LDL to HDL yields TG-rich HDL particles which are more prone to hydrolysis of their lipids by hepatic lipase. By this mechanism, the HDL particle is destabilized and more rapidly cleared by the kidney, resulting finally in decreased HDL plasma levels. Consequently, a high CETP activity leads to decreased plasma HDL levels. Therefore, a shift in partitioning of cholesterol between LDL/VLDL and HDL particles by blocking of CETP is one option to increase plasma HDL levels. The finding that certain individuals with loss-of-function mutations in the CETP gene showed elevated HDL levels and a decreased incidence of CHD (Koizumi et al. 1985; Inazu et al. 1990) triggered the search for CETP inhibitors. Other findings, however, showed that certain mutations in the CETP gene are associated with an increased risk of CHD despite high HDL-C levels (Zhong et al. 1996). Additionally, animal experiments also show conflicting results with regard to beneficial and detrimental effects of CETP inhibition. Torcetrapib was the first CETP inhibitor being evaluated in human clinical trials. Monotherapy with a dose of 60 mg/day was able to increase HDL, HDL<sub>2</sub>, and HDL<sub>3</sub> levels by 33%, 74%, and 26%, respectively, with a reduction of LDL-C by 8% (McKenney et al. 2006). A large clinical trial with 60 mg/day torcetrapib on top of 10–80 mg/day atorvastatin showed an increase of HDL-C levels by 51.9% with an additional lowering of LDL-C of 20.6% compared to atorvastatin alone (Kastelein et al. 2007). In these and further studies, however, no effect of torcetrapib on progression or regression of atherosclerosis could be demonstrated despite a 60% increase in HDL-C levels. Torcetrapib increased systolic and diastolic blood pressure by 1.3–2.2 and 0.9–1.1 mmHg and was associated with an increase in the number of deaths from both cardiovascular and noncardiovascular causes in the torcetrapib–atorvastatin group compared to the atorvastatin group alone (McKenney et al. 2006; Davidson et al. 2006), finally leading to the termination of its clinical development. Another CETP inhibitor, dalcetrapib, weaker in its pharmacological efficacy compared to torcetrapib, showed HDL-C increases by 25–30% without any effects on blood pressure. Later on it could be demonstrated that the hypertensive activity of torcetrapib is independent on its mechanism on CETP and is an off-target effect possibly by induction of elevated aldosterone and cortisol levels (Forrest et al. 2008). Dalcetrapib was investigated in a phase III clinical trial with 15,000 patients for its ability to reduce cardiovascular morbidity and mortality in patients with a recent acute coronary syndrome event (Schwartz et al. 2009). After an interim analysis, the development of the drug was

discontinued not because of adverse side effects but due to disappointing results in the efficacy outcome not disclosed further yet. Despite these failures, the search for efficacious and safe CETP inhibitors is still one of the most active drug discovery fields for novel anti-atherosclerotic drugs. At least four small molecule CETP inhibitors are currently in clinical investigation. Anacetrapib now in phase III showed in a double-blind, placebo-controlled 18-month trial at a dose of 100 mg/day on top of a statin an increase of HDL-C by 138% and a decrease in LDL-C of 40% without any effect on blood pressure, serum electrolytes, or aldosterone levels without any cardiac adverse effects. Additionally, Lp(a) levels were decreased by 36.4% (Cannon et al. 2010). In a direct comparative trial, 300 mg/day anacetrapib could increase HDL-C by 100% compared to 30% with 2 g/day nicotinic acid (Yvan-Charret et al. 2010), the most potent HDL-C increasing drug available today. A huge trial involving 30,000 patients (REVEAL, NCT01252953) is under way to elucidate whether anacetrapib can reduce the rate of cardiovascular events in patients with optimized statin therapy. Evacetrapib in monotherapy increased HDL-C by 53.6–128.8% concomitantly with a decrease of LDL-C by 13.6–35.9% at doses of 30–500 mg/day. In combination with a statin a dose of 100 mg/day, evacetrapib increased HDL-C by 78.5–88.5% with an additional decrease of LDL-C of 11.2–13.9% (Nicholls et al. 2011a). These clinical trials with anacetrapib and evacetrapib demonstrate a clear beneficial change of the lipid profiles. However, both drugs also reduce LDL-C and Lp(a) which makes it difficult to differentiate and elaborate the contribution of the beneficial effect of HDL increase and potential reduction of CVD risk due to CETP inhibition.

Masking of CETP action with an antibody raised against a fusion protein of a certain CETP epitope linked to a T cell epitope of tetanus toxin (CETi-1) is an alternative approach. In a phase II study, 90% of treated patients showed 1 year after vaccination with CETi-1 an immune response with an increase of HDL-C by 8% (Omori 2004). CETi-1 is no longer in development, but a new CETP vaccination approach with ATH-03 using a small peptide fragment of the CETP protein acting as a B cell epitope has entered phase I trials to assess its safety and immunogenicity (NCT01284582) (Homepage AFFiRiS 2009). An interesting approach is the combination of the CETP-inhibitory action with PCSK9 downregulatory activity in one molecule. K-312, currently in phase I clinical trials, has a unique mode of action to suppress PCSK9 transcription in part by inhibiting SREBP binding to SRE on the PCSK9 promoter and suppression of SREBP expression (Shibata et al. 2012; Miyosawa et al. 2012). In summary, it still remains to be demonstrated whether a pharmacological intervention raising plasma HDL levels by inhibition of the enzymatic activity of CETP exerts a robust reduction of atherosclerosis and cardiovascular morbidity and mortality.

#### **4.2.2 Enhancer of Apolipoprotein A-1 Activity**

Owing to the key role of ApoA-1, several approaches aim to increase its contribution to RCT, either by stimulation of its biosynthesis or by mimicking HDL function with synthetic ApoA-1 mimetics or reconstituted HDL formulations.

### HDL Replacement Therapy

A mutant form of ApoA-1 – ApoA-1 Milano – was found to be associated with very low HDL-C levels without an increased risk for CVD (Sirtori et al. 2001; Franceschini et al. 1980). A small clinical trial with an i.v. application of ApoA-1 Milano reconstituted in phospholipid vesicles was reported to induce a significant reduction of coronary atherosclerosis in patients with ACS after only 6 weeks of treatment with a decrease of atheroma volume by 14.1% (Nissen et al. 2003). This surprising result triggered the research for efficient HDL replacement therapies. The ApoA-1 Milano phospholipid formulation MDCO-216 was investigated in a phase 1 clinical trial for the treatment of atherothrombotic diseases and ACS as well as for prevention of restenosis after CABG and balloon angioplasty.<sup>3</sup> Twenty-four healthy individuals and 24 patients with confirmed CAD received a 2-h infusion of MDCO-216 in doses of 5–40 mg/kg and followed for 30 days. Half-lifetime ranged from 45 to 59 h. In both groups ApoA-1, phospholipids and prebeta HDL levels were increased with an increase in HDL particle size accompanied by decreases in ApoE; in dosages above 20 mg/kg, however, increases in TG and decreases in HDL-C were observed (Bellibas et al. 2014). Overall the drug was well tolerated with no serious adverse events or safety issues. CSL-111 is a synthetic HDL particle reconstituted from ApoA-1 isolated from human plasma with soybean phosphatidylcholine. The compound failed in phase II trials to reduce plaque volume in coronary arteries in patients suffering from a recent episode of ACS (Tardif et al. 2007). In patients with ACS receiving a single infusion of 80 mg/kg CS-111 over 4 h, no significant difference could be detected in the surrogate markers of forearm venous occlusion plethysmography despite a 64% increase in HDL-C and a 23% decrease in LDL-C (Chenevard et al. 2012). A reformulation of CSL-111 – named CSL-112 – is currently in phase II clinical trials in stable CAD patients (Gille et al. 2012; Diditchenko et al. 2012). In 57 healthy volunteers, having received a single dose of 5–135 mg/kg CSL-112 increased prebeta 1-HDL by 3,600% and stimulated macrophage cholesterol efflux mediated by ABCA1 by 630% and increased efflux capacity by 192% (Gille et al. 2014). Mechanistic investigations suggest that the infused CSL-112 particles fuse with HDL in plasma with subsequent release of lipid-poor ApoA-1 (Diditchenko et al. 2014). The effects lasted at least for 72 h indicating that a once or twice weekly infusion could be an appropriate treatment regimen (Gille et al. 2012). Additionally, CSL-112 exerted anti-inflammatory effects with a strong inhibition in the expression of the adhesion molecule ICAM-1 (CD-54) on monocytes and neutrophils and a decrease in proinflammatory cytokines (TNF- $\alpha$ , Il-1 $\beta$ , Il-6) and chemokines (Il-8, RANTES, Mip-1 $\beta$ ) (Diditchenko et al. 2012). CER-001 is an HDL-mimetic, comprise of recombinant ApoA-I complexed with phospholipids and designed to mimic the beneficial properties of natural nascent pre- $\beta$  HDL particles. In healthy volunteers, CER-001 increased plasma ApoA-1 levels after a single infusion in a dose-dependent manner with similar increases in HDL-C (Keyserling et al. 2011). In

<sup>3</sup> Homepage The Medicines Company. <http://www.themedicinescompany.com/page/pipeline>

rabbits CER-001 was at least 10–20 times more potent than ETC-216 to mobilize cholesterol from macrophages (Kastelein 2015). In a placebo-controlled phase 2b study (CHI-SQUARE) with 507 patients with ACS given CER-001 for 6 weeks with weekly infusions, the drug failed to show that this HDL mimetic can reduce total atheroma volume; intravenous ultrasound analysis (IVUS) scans conducted 3 weeks after the last infusion showed that CER-01 reduced total atheroma volume from baseline, but the change was not significantly different from the change from baseline seen in the placebo group (Press Release Cerenis 2014a; Tardif et al. 2014). In a further clinical trial in patients with familial primary hypoalphalipoproteinemia, however, CER-001 was able to reduce carotid artery mean vessel wall area as measured by magnetic resonance imaging after treatment of 23 patients with 9 doses of CER-001 over 4 weeks. Furthermore, in 23 patients with hofFH having received biweekly infusions of CER-001 over 6 months on top of optimized LDL-C, lowering therapy including apheresis carotid artery mean vessel wall area was as well statistically reduced (Press Release Cerenis 2014b; Kootte et al. 2015) leading to orphan drug approval by the European Medicines Agency EMA in September 2014.

As of today, the clinical trials with HDL replacement therapy have delivered mixed results. The efficacy, safety, and cost-effectiveness of this approach has to be shown in future studies. As well, the invasive route of administration will limit its widespread use both due to reasons regarding patient compliance and the need for a physician for intervention.

### **Apolipoprotein A-I Peptidomimetics**

Several ApoA-1 mimetics mimicking at least one of the amphipathic helices of ApoA-1 and therefore being able to associate lipids showed promising results in animal studies after oral application such as stimulation of RCT, LCAT activity, and off-loading of cholesterol in the liver via the SR-BI receptor (Navab et al. 2004; Carballo-Jane et al. 2010). The 18mer peptide D-4 F containing four D-amino acids orally applied to mice was able to inhibit lipid peroxidation, to increase PON1 activity, and to stimulate cholesterol efflux (Navab et al. 2006; Xie et al. 2010; Vakili et al. 2010). A phase I clinical trial in 50 patients with CAD receiving 30–500 mg of APP-018 (D-4 F) orally showed only a very low bioavailability (Bloedon et al. 2008; Watson et al. 2011). Efficacy data in this study and a related one using the peptide L-4 F are inconsistent with regard to changes in total cholesterol, LDL-C, HDL-C, or TG despite some decrease in the HDL inflammatory index for D-4 F. FX-5A is a bihelical peptide derived from ApoA-I where five nonpolar amino acids on the hydrophobic side of the helix were substituted with alanine, thereby reducing the lipid affinity of the second helix. This peptide modification was found to reduce the peptide's cytotoxicity and increase its specificity to remove cholesterol from cells. I.v. application of FX-5A peptide complexed with phospholipids to ApoE k.o. mice was able to raise HDL, promote RCT, and reduce atherosclerosis (Reuters 2012).

### ApoA-1 Upregulators

A completely new and innovative approach to increase HDL-C is pursued by Resverlogix's drug RVX-208 which exerts its activity via an epigenetic mechanism (Bailey et al. 2010). RVX-208 is an inhibitor of BET proteins (bromodomain and extracellular domain) including BRD4, a member of the BET protein family (Belkina and Denis 2012). One of the key epigenetic mechanisms involves the modification of chromosomal proteins by acetylation, methylation, or phosphorylation catalyzed by specific enzymes. The modified amino acids of histones serve as baits for other proteins including BET proteins for binding and reading this epigenetic code. BET proteins contain two specific sites – the bromodomain and extracellular domain – which can recognize acylated lysine residues of histones bound to DNA. By binding of BET proteins to these modified histones, additional cofactors regulating gene activity are recruited leading to an increase in gene transcription as the ApoA-1 apolipoprotein. RVX-208 mimics the binding of acylated lysine residues to BET, thereby triggering the cascade of increased ApoA-1 gene transcription and ApoA-1 formation. A phase IIb clinical trial (SUSTAIN) with 176 patients with established atherosclerosis RVX-208 significantly raised HDL-C with increases in ApoA-1 levels and large HDL particles, thereby stimulating RCT (Nicholls et al. 2011b). Statin-treated patients with CHD being treated with 50–150 mg/day RVX-208 for 12 weeks showed an increase of 0.1–5.6% for ApoA-I, 3.2–8.3% in HDL-C, and 11.1–21.1% in the number of large HDL particles (Nicholls et al. 2011b). In 10% of patients, transient and significant increases in liver transaminases were observed. In a phase 2b placebo-controlled clinical trial, 323 patients with low HDL levels and established CHD were randomized to a placebo group ( $n = 80$ ) or a treatment group ( $n = 243$ ). All patients received either 10–40 mg/day atorvastatin or 5–20 mg/day rosuvastatin, whereas the treatment group received 100 mg bid RVX-208 for 6 months. RVX-208 failed in achieving its primary and secondary endpoints to increase HDL-C and ApoA-I or to reduce arterial plaques (European Society of Cardiology (ESC) 2013). HDL-C and ApoA-I were increased by 10.9 and 12.8% vs 7.7 and 10.6% in the placebo group. No differences were found for LDL-C (–16 vs –17.6), large HDL particles (38.1 vs 38), or hsCRP (–32.7% vs –33.8%), whereas liver transaminase increases were more often reported in the RVX-208 group (7% vs 0%). In the primary endpoint, the medium change percent of atheroma volume, the RVX-208 group showed a change of –0.4% vs –0.3% in the placebo group. The reasons for failure are not yet clear: lack of efficacy, a too short duration of treatment, or the inability to improve to beneficial effects of statin treatment. Due to their crucial role in cell gene control, BET inhibitors may depress transcription of endogenous human retroviruses because the BET protein-containing transcription regulator complexes can also exert corepressor function (Belkina and Denis 2012). Since the efficacy data of RVX-208 on HDL-C are so far disappointing, further studies will be necessary to determine the validity of this epigenetic approach to increase HDL levels. Overall, the mystery regarding HDL continues (Virani and Ballantyne 2011), and the efficacy of HDL increases by pharmacological intervention as an

approach to reduce cardiovascular morbidity and mortality has still to be demonstrated in further clinical endpoint trials.

### 4.3 Therapeutic Proteins

#### 4.3.1 LCAT Replacement

Lecithin-cholesterol acyltransferase (LCAT) increases plasma levels of HDL by converting free cholesterol into cholesterol esters, the latter becoming substrates for CETP-catalyzed transfer to TG-rich ApoB-containing lipoproteins in exchange for TG, thereby generating pre- $\alpha$  and pre- $\alpha$ 1 particles. Therefore, increasing of LCAT activity by stimulation, increase of expression, or exogenous delivery could be a way to increase functional plasma HDL levels and stimulate RCT. A proof of concept was recently demonstrated in one patient with the rare genetic disease familial LCAT deficiency (125 patients so far reported). Patients with LCAT deficiency have dramatically low levels of HDL-C with a pathophysiological phenotype including corneal opacities (“fish-eye disease”), anemia, splenomegaly, and severe kidney disease, the latter being the major cause of morbidity and mortality. A 53-year-old patient with baseline HDL levels lower than 5 mg/dL ( $<0.13$  mmol/L) received increasing doses of recombinant LCAT (ACP-501) (0.9, 3, and 9 mg/kg) as single infusions for 2 weeks followed by an infusion of 9 mg/kg every 1–2 weeks. After each dose, LCAT levels increased, remaining stable for 4 days. Plasma HDL-C levels rose up to 24 mg/ml (0.62 mmol/L); markers for renal function improved significantly (30% decrease in blood urea, 17% decrease of cystatin C, 25% improvement of anemia) without any significant adverse side effects (Shamburek et al. 2013). From a phase 1 single-dose escalation study with 16 volunteers with stable atherosclerosis receiving a single dose of ACP-501, an elevation of HDL-C with no adverse side effects was reported after 28 days of follow-up observation (Business Wire Press 2012). These findings may stimulate the search for compounds upregulating or stimulating LCAT activity as a novel approach to increase HDL levels and RCT.

#### 4.3.2 FGF 21 Analogues

Fibroblast growth factor 21 (FGF 21) is a 181 amino acid protein secreted by the liver, white and brown adipose tissue, and exocrine pancreas, regulating insulin sensitivity and lipid and energy metabolism (Kharitononkov et al. 2005). Administration of exogenous FGF 21 to obese diabetic rhesus monkeys exerted profound beneficial effects on glucose and lipid levels as well as on body weight (Kharitononkov et al. 2007). LY2405319 is a stable analogue of FGF 21 and was investigated in a placebo-controlled clinical trial in 47 diabetic patients who received daily s.c. injection of 3–20 mg for 28 days. LDL-C levels at doses of 10 and 20 mg were reduced by 29.5% and 20.2% and of total cholesterol by 19.2% and 15.4%, respectively. Apolipoproteins B and CIII were reduced by 25.1% and 34% (ApoB) and 21.6% and 35.4% (ApoC-III). Furthermore, HDL-C levels were increased by 15–20%, whereas TG levels were strongly decreased by 46.2% and



44.6%. Additionally, the 4-week treatment was accompanied by a weight loss of 1.3–1.5 kg and a strong increase in circulating adiponectin levels, whereas no significant effects were observed for fasting plasma glucose levels (Gaich et al. 2013). These beneficial efficacy of LY2405319 on multiple metabolic parameters deviated in metabolic disorders may indicate that FGF 21 analogues could evolve if proven to be a safe as novel effective drugs for the treatment of dyslipidemia associated with metabolic disorders like diabetes, obesity, or the metabolic syndrome.

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## 5 Conclusions

The introduction of statins into clinical practice with lovastatin as the first drug of this class in 1987 (Henwood and Heel 1988) has revolutionized the treatment of lipid disorders and made reduction of LDL-C as the primary goal in the treatment of dyslipidemia and prevention of CVD (National Cholesterol Education Program 2002; Graham et al. 2007b). The tremendous clinical efficacy of antibodies against PCSK9 to reduce LDL-C far beyond what was possible with statins marks a potential quantum leap in the treatment of elevated LDL cholesterol levels by modification of the PCSK9 pathway. Despite the clinically established inverse relationship between HDL and CVD risk, the enormous efforts have not yet translated these findings into effective HDL-modifying drug approaches with unequivocal clinical benefit on CVD morbidity and mortality. Recent findings highlighting the functionality of HDL as probably the most relevant factors underlying the protective effects of HDL will help to develop HDL-modifying drugs with beneficial clinical efficacy on cardiovascular endpoints to prevent and combat atherosclerosis and its clinical consequences. A major need for the clinical development of novel lipid-modifying drugs is the development of new noninvasive measurements and biomarkers for monitoring their efficacy in halting the progression or inducing regression of atherosclerosis to allow smaller, focused clinical trials with stratified patient populations for clinical proof-of-concept of novel drug approaches.

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