

Expert Opinion

1. Background
2. Medical need and existing treatment
3. Therapeutic class review
4. Current research goals
5. Scientific rationale
6. Competitive environment
7. Potential development issues
8. Expert opinion

Emerging antidyslipidemic drugs

Rebecca L Pollex, Tisha R Joy & Robert A Hegele[†]

University of Western Ontario, Blackburn Cardiovascular Genetics Laboratory,
Robarts Research Institute and Schulich School of Medicine and Dentistry, Ontario, Canada

Background: Many patients at high risk for coronary heart disease (CHD) fail to reach target lipid levels with currently available medications, and a small but clinically relevant proportion of patients experience adverse effects. Thus, additional pharmaceutical strategies are required to fill these gaps in efficacy and tolerability. **Objective:** To provide an overview of both current and emerging antidyslipidemic drugs. **Methods:** For the current antidyslipidemic drugs, we focus primarily on statins, bile acid sequestrants, fibrates, ezetimibe, and niacin. Emerging antidyslipidemic drugs herein discussed were identified by searching the Pharmaprojects database for 'hypercholesterolemia drugs' (Phase II or Phase III), 'HDL-based therapies', and 'PCSK9 inhibition'. **Results/conclusions:** Combinations of currently existing medications are most easily applicable. Meanwhile, strategies to raise HDL-C rely on a deep understanding of the complexity of HDL metabolism. Furthermore, novel approaches to further reduce LDL-C warrant careful evaluation of benefit-risk ratio. Finally, the medical community will have to rely on late-phase CHD outcome studies as the final arbiter of clinical application for any new antidyslipidemia treatment.

Keywords: biotherapeutics, cholesterol, combination therapy, coronary heart disease, dyslipidemia, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, statins, triglyceride

Expert Opin. Emerging Drugs (2008) 13(2):363-381

1. Background

Dyslipidemia refers to a spectrum of metabolic disorders characterized by either an excess or a deficiency of lipoprotein particles, resulting in elevated plasma concentrations of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) or triglyceride (TG), and/or depressed high-density lipoprotein cholesterol (HDL-C). The central lipid actors are TG and cholesterol, which are either absorbed from food or synthesized endogenously. These hydrophobic lipids navigate their way through the plasma in hydrophilic, spherical structures called lipoproteins. Lipoprotein particles harbor distinguishing surface proteins called apolipoproteins, which function as structural components, cofactors for enzymes and ligands for cell-surface receptors. An accumulation of pro-atherogenic particles or a deficit in antiatherogenic particles can increase coronary heart disease (CHD) risk.

Dyslipidemias are categorized as being primary or secondary (Table 1), and are further characterized by particular lipid or lipoprotein disturbances. Primary dyslipidemias often result from single or multiple genetic mutations affecting critical metabolic pathways. For example, mutations in the LDL receptor gene (*LDLR*) cause heterozygous familial hypercholesterolemia (HeFH), an inherited condition that affects around 1/500 individuals, which is characterized by elevated plasma LDL-C and early CHD. Primary dyslipidemias are suspected clinically in the context of such manifestations as lipid deposits in skin or tendons, early

informa
healthcare

Table 1. Primary and secondary causes of dyslipidemia.

Primary	Secondary
Genetic	Obesity
Elevated LDL-C	Diabetes mellitus
<i>LDLR</i> mutation (autosomal dominant familial hypercholesterolemia)	Alcohol overuse
<i>PCSK9</i> mutation (variant FH)	Nephrotic syndrome
<i>APOB</i> mutation (familial defective apoB-100)	Chronic renal failure
<i>ARH</i> mutation (autosomal recessive hypercholesterolemia)	Hypothyroidism
Polygenic influences, e.g., <i>APOE</i> , <i>PCSK9</i> SNPs	Dysgammaglobulinemia (SLE, multiple myeloma)
Elevated TG	Primary biliary cirrhosis and other cholestatic liver diseases
<i>LPL</i> , <i>APOC2</i> , <i>APOA5</i> mutations	Antihypertensive medications (thiazide diuretics and β -blockers)
Polygenic influences, e.g., <i>APOA5</i> , <i>APOC3</i> SNPs	Corticosteroid therapy (or severe stress that increases endogenous corticosteroids)
Depressed HDL-C	Progestin or anabolic steroid treatment
<i>ABCA1</i> , <i>APOA1</i> , <i>LCAT</i> mutations	Highly active antiretroviral agents
Polygenic influences, e.g., <i>CETP</i> , <i>ABCA1</i> SNPs	

ABCA1: ATP binding cassette transporter A1 gene; *APOA1*: Apolipoprotein A-I gene; *APOA5*: Apolipoprotein A-V gene; *APOB*: Apolipoprotein B gene; *APOC2*: Apolipoprotein C-II gene; *APOC3*: Apolipoprotein C-III gene; *APOE*: Apolipoprotein E gene; *ARH*: Autosomal recessive hypercholesterolemia gene; *CETP*: Cholesteryl ester transfer protein gene; HDL-C: High-density lipoprotein cholesterol; *LCAT*: Lecithin cholesterol acyltransferase; LDL-C: Low-density lipoprotein cholesterol; *LDLR*: Low-density lipoprotein receptor gene; *LPL*: Lipoprotein lipase gene; *PCSK9*: Proprotein convertase subtilisin/kexin type 9 gene; SLE: Systemic lupus erythematosus; SNPs: Single nucleotide polymorphisms; TG: Triglyceride.

CHD onset, severe biochemical perturbances, and/or a family history of either dyslipidemia or early CHD.

However, most cases of dyslipidemia result from secondary causes, such as a sedentary lifestyle with excessive dietary fat intake, diabetes mellitus, alcohol overuse and hypothyroidism. But a component of genetic susceptibility is still likely since not all individuals with these secondary factors develop dyslipidemia to the same degree. The initial clinical manifestation is often symptomatic CHD. From a recent American survey of 6814 men and women, aged 45 – 84 years, from four ethnicities (non-Hispanic White, Chinese, Black and Hispanic), the overall prevalence of dyslipidemia was 29.3% [1]. The prevalence of dyslipidemia was lowest among Chinese women (21.0%) and highest among non-Hispanic white men (36.9%). Overall, men were about 30% more likely than women to have dyslipidemia.

CHD accounts for > 30% of all deaths in North America [2,3]. Direct and indirect financial costs attributable to CHD are estimated to reach \$448.5 billion in the United States this year [3]. Early control of dyslipidemia can potentially decrease CHD events by up to 35% [4], saving millions of lives each year globally.

1.1 LDL-C and CHD

The predominant atherogenic lipoprotein is cholesterol-rich (~ 50% by weight) LDL. LDL-C accounts for approximately 70% of total circulating plasma cholesterol. The precursor of LDL is very-low density lipoprotein (VLDL), a TG-rich particle secreted by the liver. VLDL is metabolized

to intermediate-density lipoprotein (IDL) by the action of lipoprotein lipase (LPL), and further metabolized to LDL by hepatic triglyceride lipase (HTGL). VLDL and LDL particles each carry a single apolipoprotein (apo) B-100 molecule. LDL is normally cleared by the liver in a highly regulated process mediated by apoB and LDL receptors. Excess circulating LDL can become oxidized and ectopically taken up in an unregulated manner by macrophage scavenger receptors within the arterial wall. Cholesterol-laden macrophages – ‘foam cells’ – cluster and accumulate, forming fatty streaks and eventually plaques. As plaques impinge into the arterial lumen, blood flow becomes compromised, especially when a lipid-rich plaque suddenly ruptures, leading to such CHD end points as myocardial infarction (MI) or stroke.

Numerous population studies have demonstrated a strong direct relationship between plasma LDL-C concentration and CHD events [5]. Based on epidemiologic and clinical trial evidence, treatment guidelines have been developed, such as those from the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III [6]. According to these guidelines, the LDL-C goal for individuals at high CHD risk is < 100 mg/dl (< 2.6 mmol/l) [6]. However, even more aggressive targets have recently been recommended for individuals at very high CHD risk, with an LDL-C goal of < 70 mg/dl (< 1.8 mmol/l). Since lifestyle measures rarely reduce plasma LDL-C by > 15%, pharmaceutical strategies are needed to help patients reach these goals.

1.2 HDL-C and CHD

HDL consists of a family of particles that are heterogeneous in shape, density, size and antiatherogenic properties. The major structural protein on HDL is ApoA-I, which is secreted by the liver and intestine. ApoA-I is present on virtually all HDL particles, accounting for around 70% of the protein content on the HDL particle [7].

Epidemiological studies showed a clear inverse relationship between plasma HDL-C and CHD [8-10]. Men in the bottom three quartiles of HDL-C (≤ 52 mg/dl or 1.33 mmol/l) had about 70% more MIs than men in the top quartile, while women in the lowest HDL-C quartile (≤ 46 mg/dl or 1.18 mmol/l) had a sixfold increased MI risk compared with women in the top quartile [9]. Furthermore, CHD mortality in both genders was inversely related to HDL-C [10]. Thus, raising HDL-C could have health benefits.

1.2.1 Reverse cholesterol transport

While HDL has favorable anti-inflammatory, antioxidant, antithrombotic, and endovascular properties, the major mechanism by which HDL protects from atherosclerosis is called 'reverse cholesterol transport' (RCT). RCT refers to the movement of cholesterol from the peripheral cells to the liver for ultimate biliary excretion. RCT is a complex process involving the concerted activities of four organs/tissues (liver, intestine, arteries and kidney), six enzymes (lecithin:cholesterol acyltransferase (LCAT), LPL, cholesteryl ester transfer protein (CETP), HTGL, endothelial lipase (EL), and secretory phospholipase A2 (sPLA2)), three receptors (ATP binding cassette transporter A1 (ABCA1), ATP binding cassette transporter G1 (ABCG1) and scavenger receptor – BI (SR-BI)) together with surface apolipoproteins, lipids and phospholipids [11]. Early steps in RCT involve the interaction of ApoA-I with phospholipids, forming a lipid-poor HDL particle that can effectively accept cholesterol from foam cells. Once accepted onto the HDL particle, cholesterol undergoes several modifications. Cholesterol esters (CE) from HDL are exchanged for TG from TG-rich lipoproteins via CETP, which together with remaining CE on HDL are taken up by the liver (See Figure 1). Thus, targeting key points in RCT, such as apoA-I-cellular interactions or CETP activity, may prove to be an effective means of raising HDL-C.

1.3 TG and CHD

TG-rich lipoprotein particles include chylomicrons (80 – 95% TG) and VLDL (55 – 80% TG) [12]. Chylomicrons are responsible for the transportation of dietary fat and fat-soluble vitamins. Following a meal, TG and cholesterol absorbed into the cells of the small intestine are incorporated into the cores of nascent chylomicron particles with the help of apoB-48, a truncated form of apoB-100, and microsomal triglyceride transfer protein

(MTP). The newly formed chylomicrons are then secreted into the lymph system and then enter the circulation, traveling to adipose tissues and muscles where the core TG is hydrolyzed and the fatty acids are taken up by the fat cells for storage, or used by the muscle cells for energy. The secretion of chylomicrons is modulated by the regulation of *APOB* transcription, apoA-IV levels, and enhanced lipid synthesis [13].

VLDL particles are assembled and secreted by the liver, again with the aid of MTP and also full-length apoB-100 [14]. VLDL TG are derived from glycerol and fatty acids taken up from the plasma or synthesized by the liver. Overproduction of VLDL or chylomicrons leads to increased TG levels which, via an exchange process mediated by CETP, results in low HDL-C levels, and the generation of small, dense LDL-C from VLDL, creating a highly atherogenic state (see [15] for a more comprehensive review of lipid metabolism).

While LDL-C and HDL-C have both been well established as independent risk factors for CHD, the role for TG remains more controversial, due to its inverse correlation with HDL-C. However, the small, apoB-48-carrying, chylomicron remnants have been implicated in the progression of coronary lesions [16], and more recent studies have also shown non-fasting TG levels to be associated with CHD, independent of traditional cardiac risk factors [17,18].

2. Medical need and existing treatment

CHD is a leading cause of death and lost productivity [19]. In 2005, approximately 267 million people in seven major markets had plasma TC concentration > 200 mg/dl (5.17 mmol/l), which is set to rise to around 286 million people by 2015 [20]. The primary focus of dyslipidemia treatment to reduce CHD risk has been lowering plasma LDL-C, since intervention trials showed relative risk reductions in CHD morbidity and mortality of around 25% [4,21-24]. However, despite recent reductions in CHD mortality due to earlier prevention and treatment of CHD risk factors, future CHD mortality may increase if current trends of increasing obesity and metabolic syndrome remain unchecked. More importantly, some small trials of combination therapy that reduced LDL-C and raised HDL-C demonstrated significant additive benefit: relative CHD risk reduction using a combination strategy was 71%, compared with 27% for statin monotherapy alone [25]. These rather limited data suggest that novel antidyslipidemic strategies might achieve even more substantial reductions in CHD risk.

Current antidyslipidemia drugs include statins, fibrates, niacin, ezetimibe, and bile acid binding resins (Table 2). These drugs target one component of the lipid profile, with smaller additional effects on other parameters. For instance, statins and fibrates produce sizable reductions

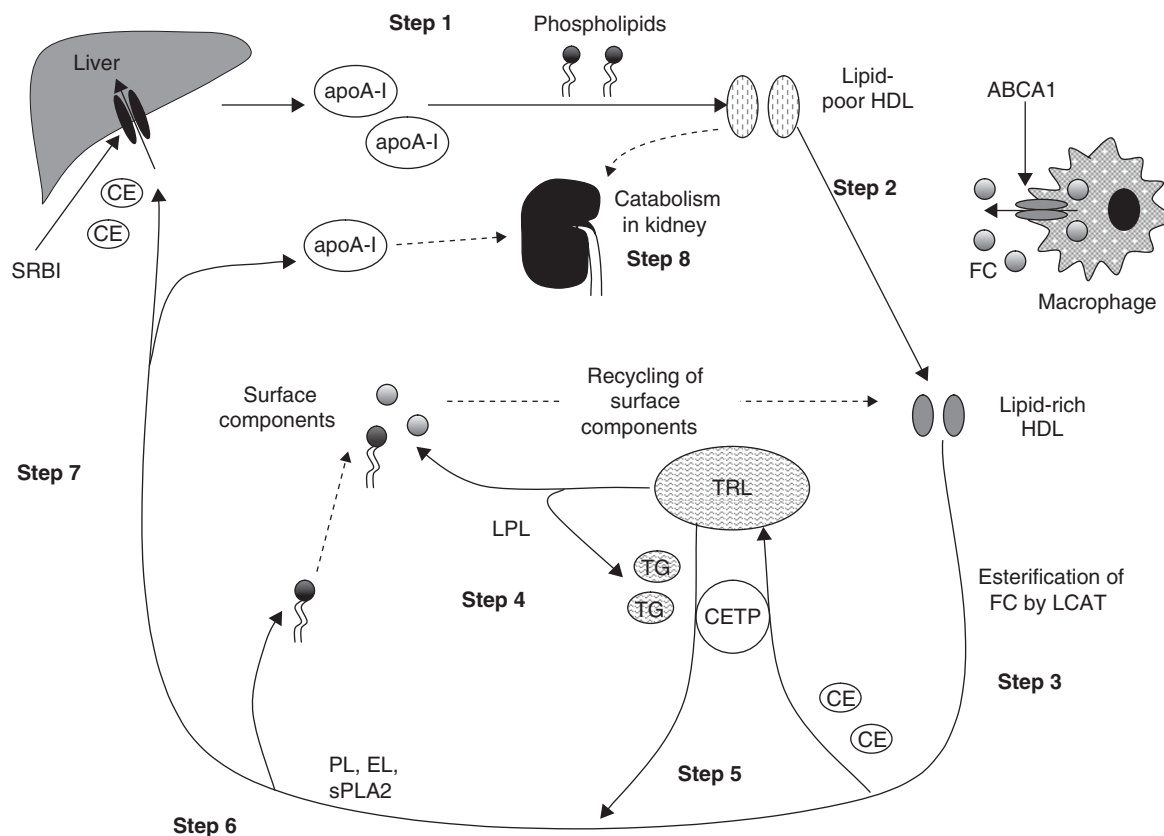


Figure 1. Reverse cholesterol transport. Apolipoprotein A-I (apoA-I) secreted by the liver and intestine combines with phospholipids to form small lipid-poor HDL particles, which are then ready to accept cholesterol (**step 1**). Free cholesterol (FC) is effluxed from places such as macrophages in the arterial wall via the ABCA1 and possibly ABCG1 transporters. The addition of FC transforms lipid-poor HDL to lipid-rich HDL (**step 2**), which then undergoes several modifications, including esterification of FC by lecithin: cholesterol acyl transferase (LCAT) (**step 3**). Lipoprotein lipase (LPL) hydrolyzes the triglyceride (TG) carried on TG-rich lipoproteins (TRL) to provide surface components (phospholipids (PL), FC, and apolipoproteins) to the HDL particle (**step 4**). This TG is exchanged for CE on HDL through cholesterol ester transfer protein (CETP) (**step 5**). As plasma enzymes – hepatic lipase (HL), endothelial lipase (EL), and secretory phospholipase A2 (sPLA2) – hydrolyze and remove PL from the newly formed HDL, surface apoA-I protein is recycled (**step 6**). CE from HDL is taken up by the liver via scavenger receptor-BI (SRBI) (**step 7**) with further recycling of HDL surface components. Finally, free apoA-I and lipid-poor HDL are catabolized by the kidney for excretion in the urine.

primarily in plasma LDL-C and TG, respectively. Meanwhile, niacin has the greatest HDL-C raising capacity. However, many high CHD risk patients fail to reach strict guideline target levels with currently available drugs. A small but clinically relevant proportion of patients experience adverse effects. Thus, additional pharmaceutical strategies are required to fill these gaps in efficacy and tolerability.

3. Therapeutic class review

3.1 Statins

Statin drugs are the most widely used treatment for dyslipidemia, accounting for around 87% of the US market [20]. Statins inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting step in cholesterol biosynthesis. Depletion of hepatic cholesterol

stores increases *LDLR* expression, resulting in enhanced clearance of plasma LDL particles. Statins have been touted to have several lipid-independent beneficial effects, including increased endothelial nitric oxide production, decreased platelet aggregation, and decreased smooth muscle proliferation [26]. Moreover, statins can reduce levels of the non-traditional cardiovascular risk factor, C-reactive protein [27]. Thus, statins appear to have anti-inflammatory, antithrombotic, and antiproliferative properties that may provide additional cardiovascular benefits. Some landmark statin trials include the Heart Protection Study (HPS) [23], Treating to New Targets (TNT) [28], Pravastatin or Atorvastatin Evaluation and Infection Therapy (PROVE-IT) [24], and Scandinavian Simvastatin Survival (4S) Study [29]. A meta-analysis of 14 randomized trials, involving around 90,000 participants [30], concluded that statin therapy safely reduces the incidence of major CHD

Table 2. Currently available pharmaceuticals for dyslipidemia.

Medication	Effects on lipid parameters	Adverse effects
Statins (HMG-CoA reductase inhibitors)	↓↓ LDL-C, ↓ TG Minimal effects on HDL-C (rosuvastatin can increase HDL-C levels)	Myalgias Myositis/rhabdomyolysis Transaminitis
Fibrates (PPAR- α agonists)	↓ LDL-C, ↓↓ TG, ↑ HDL-C (mild)	Myalgias Rhabdomyolysis Cholelithiasis Elevations in serum creatinine
Ezetimibe (intestinal cholesterol absorption inhibitor)	↓ LDL-C, ↓ TG	Myalgias (very rare) Rhabdomyolysis (very rare)
Niacin	↓↓ TG, ↑↑ HDL-C, ↓↓ LDL-C, ↓↓ LP (a)	Flushing/vasodilation Impair insulin sensitivity Gout, gastritis
Bile acid resins (inhibitors of enterohepatic circulation)	↓ LDL-C	↑ TG Bloating, constipation Interference with absorption of other medications such as levothyroxine, warfarin, digoxin, statins

HDL-C: High-density lipoprotein cholesterol; HMG-CoA reductase: 3-Hydroxy-3-methyl-glutaryl-CoA reductase; LDL-C: Low-density lipoprotein cholesterol; LP(a): Lipoprotein (a); PPAR: Peroxisome proliferator-activated receptor; TG: Triglyceride.

events, with 12% reduction in all-cause mortality per mmol/l reduction in LDL-C [30].

Although statins are generally well-tolerated, about 10% of patients experience side effects, mainly muscle aches, while a much smaller proportion will experience elevations in serum creatine kinase (CK) and transaminases [31]. An extremely rare adverse effect – occurring in about 1/100,000 prescriptions – is rhabdomyolysis, which can typically be detected early by monitoring of patient symptoms and serum CK levels, with discontinuation of the statin as appropriate. Also, a significant proportion of patients on statins do not reach stringent LDL-C targets [32].

3.2 Bile acid sequestrants

Bile acids are amphiphilic molecules synthesized from cholesterol that facilitate intestinal absorption of dietary fat. Following their synthesis in the liver and transit through the biliary tree, bile acids enter the intestinal lumen, where they emulsify diet fats, aiding in their absorption. Bile acids are then reabsorbed by active ileal uptake and recycled through the enterohepatic circulation.

Bile acid sequestrants (BAS), such as colestipol and colestyramine, have been used to treat hypercholesterolemia with a good safety record for > 40 years [33,34]. BAS in combination with low-dose statins result in synergistic reduction of plasma LDL-C, through complementary mechanisms of action [35,36]. BAS are positively charged indigestible resins that bind to negatively charged bile acids in the intestinal lumen and are then excreted with bound bile acids in the feces. This depletes the endogenous bile acid pool by around 40%, stimulating an increase in bile acid synthesis from hepatic cholesterol stores and

increasing *LDLR* expression, resulting in enhanced LDL particle clearance and lowering of plasma LDL-C by approximately 15% [37]. Unfortunately, BAS are associated with increased plasma TG, and can also alter the absorption of medications such as levothyroxine or warfarin. Furthermore, BAS use is complicated by constipation and flatulence, reducing patient compliance. Newer BAS, such as colesevelam, have greater affinity for binding bile acids, with fewer side effects. Moreover, colesevelam has been shown to decrease C-reactive protein [38].

3.3 Fibrates

Fibrates are weak peroxisome proliferator-activated receptor (PPAR)- α agonists that decrease TG levels by up to 50%. Their effects on plasma LDL-C and HDL-C are more variable, with a decrease and increase of around 10 and 10%, respectively. Fibrates downregulate apo C-III expression, resulting in increased VLDL clearance, and upregulate apoA-I, SR-BI and ABCA1 expression, perhaps increasing RCT [39]. Fenofibrate may also decrease cholesterol absorption by interfering with the intestinal sterol transporter Niemann-Pick C1 like 1 (NPC1L1) [40]. Fibrates variably decrease plasma fibrinogen and C-reactive protein, two non-traditional CHD risk factors.

The commonly used fibrates, namely gemfibrozil, fenofibrate, bezafibrate and, in Europe, ciprofibrate, have inter-individual differences in both molecular and biochemical effects [39]. The main side effects of fibrates include myalgias, increased serum creatinine, cholelithiasis and increased risk of venous thrombosis. Since gemfibrozil and not fenofibrate has been demonstrated to inhibit statin elimination via inhibition of glucuronidation, rhabdomyolysis risk

appears to be increased when gemfibrozil is combined with statins [41,42].

Early fibrate trials demonstrated benefits for CHD risk. For instance, plasma HDL-C was increased by 11% and MI was reduced by 34% with gemfibrozil in the Helsinki Heart Study [43]. Meanwhile, gemfibrozil in the Veterans Affairs HDL Intervention Trial reduced fatal and non-fatal MI by 22% [44]. However, two larger trials demonstrated no difference in primary CHD end points with the use of either bezafibrate among those with known CHD or fenofibrate among those with diabetes [45,46]. In the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial, although the primary CHD end point was not reduced among those randomized to fenofibrate, total cardiovascular events were indeed reduced. Furthermore, meta-analysis of fibrate trials – excluding clofibrate – revealed significantly decreased odds of nonfatal MI by around 22% without significant effects on other outcomes such as CHD mortality or stroke [47].

3.4 Ezetimibe

Ezetimibe is an intestinal cholesterol absorption inhibitor whose primary target is the NPC1L1 intestinal cholesterol transporter. Ezetimibe also reduces plasma phytosterols, which are a minor contributor to serum sterols, except in the rare disorder sitosterolemia. Unlike BAS, ezetimibe does not interfere with bile acid absorption [48].

Inhibition of intestinal cholesterol absorption by ezetimibe leads to both an increase in cholesterol synthesis and LDL receptor expression. Ezetimibe monotherapy can reduce LDL-C levels by about 18%, as well as TG and apoB levels by around 5 and 15%, respectively. Small but statistically significant increases in HDL-C have also been observed [49,50]. Moreover, since statins suppress cholesterol synthesis, the combination of ezetimibe plus statin therapy makes great theoretical sense. Trials have shown that the addition of ezetimibe to existing statin therapy can result in LDL-C reductions equivalent to higher doses of statin; for example, addition of 10 mg ezetimibe to 10 mg atorvastatin results in similar LDL-C reduction of approximately 50% compared with 80 mg atorvastatin alone [51]. Meanwhile, the addition of ezetimibe to fenofibrate produces additional incremental decreases of 9.6, 13.4 and 4.2% for plasma TC, LDL-C and TG levels, respectively [52]. Ezetimibe is safe and well tolerated, with very rare instances of elevated transaminases or CK [53].

While it would seem theoretically certain that LDL-C reduction by ezetimibe should translate into clinical benefit, such evidence from CHD end point studies is presently lacking.

3.5 Niacin

Niacin is an effective broad-spectrum antidyplipidemic drug, which is associated with HLD-C increases of up to 30% as well as reductions in TC, TG, LDL-C and lipoprotein (a)

[Lp(a)] by 20 – 40% [54]. The key human pharmacological receptor for niacin was recently characterized as HM74A (GPR109A), a G protein-coupled receptor whose activation leads to decreased lipolysis through inhibition of adenylyl cyclase in adipocytes. As a result, free fatty acid levels decrease, leading to decreased TG levels [55-57]. Niacin has also been shown to reduce C-reactive protein and fibrinogen [54].

Use of niacin was associated with improved CHD outcomes in the Coronary Drug Project (CDP) and with regression of carotid intima-media thickness (cIMT) in Arterial Biology for the Investigation of the Treatment Effects of Reducing cholesterol trials (ARBITER 2 and 3) [58-60]. Side effects from niacin use may include insulin resistance, gout, or gastritis. However, the most limiting side effect from niacin use has been skin flushing and related vasodilatory symptoms. These have been reduced somewhat with extended-release niacin (ERN, Niaspan®). Also, niacin-related flushing is probably mediated by increased prostaglandin (PG) production and binding to the dermal prostaglandin D2 receptor subtype 1 (DP1), leading to development of DP1 inhibitors.

4. Current research goals

The ideal antidyplipidemia drug would effectively lower LDL-C and TG and raise HDL-C. It would be proven to reduce CHD events in large, well-designed end point studies. However, the probability of developing such a single agent is remote. Current research goals are to develop new drugs that are more effective than existing ones, with fewer side effects and improved CHD outcomes. To meet the growing need for new antidyplipidemia treatments, recent focus has included: i) strategic combinations of existing drugs to affect multiple lipid parameters; ii) novel pharmaceuticals within these existing traditional pharmaceutical categories that improve tolerance, efficacy, and choice; iii) innovative strategies to raise HDL-C levels in an attempt to further decrease cardiovascular risk; and iv) development of unique biological agents to reduce LDL-C, based on recent advances in the understanding of lipid metabolism.

5. Scientific rationale

Dyslipidemia represents a heterogeneous clinical entity that includes both isolated lipoprotein abnormalities or a combination of two or three of these lipid abnormalities. As a result, treatments for dyslipidemia need to target several different yet parallel lipid and metabolic pathways. Thus, the pharmaceutical industry has turned its attention to alternate pharmacologic and biologic strategies to further lower LDL-C, as well as to develop better tolerated and more efficacious agents to lower TG and raise HDL-C (Table 3).

Table 3. Competitive environment table.

Compound	Company	Phase	Mechanism of action	Side effects	Comments
Pitavastatin (NK104)	Kowa Pharmaceuticals	Phase III	HMG-CoA reductase inhibition	Myalgia, creatine kinase elevation, transaminase elevation	Typical statin side effects
Lapaquistat (TAK-475)	Takeda	Phase III	HMG-CoA reductase inhibition	Transaminase elevations	
Ezetimibe + atorvastatin	Schering-Plough (Merck & Co.)*	Phase III	Ezetimibe: NPC1L1 inhibition Atorvastatin: HMG-CoA reductase inhibition	Ezetimibe: occasional mild gastrointestinal side effects Atorvastatin: myalgia, creatine kinase elevation, transaminase elevation	
Atorvastatin + fenofibrate	LifeCycle Pharma	Phase II	Atorvastatin: HMG-CoA reductase inhibition Fenofibrate: PPAR- α agonist	Atorvastatin: myalgia, creatine kinase elevation, transaminase elevation Fenofibrate: creatinine elevation, gastrointestinal side effects	
Rosuvastatin + fenofibrate (ABT-335)	Abbott & AstraZeneca	Phase III	Rosuvastatin: HMG-CoA reductase inhibition Fenofibrate: PPAR- α agonist	Rosuvastatin: myalgia, creatine kinase elevation, transaminase elevation Fenofibrate: creatinine elevation, gastrointestinal side effects	Proprietary formulation of fenofibrate
MD-0727	Microbia	Phase II	Unknown, cholesterol absorption inhibitor	Possible gastrointestinal effects (constipation, abdominal distension)	
AVE-5530	Sanofi-Aventis	Phase II	Unknown, cholesterol absorption inhibitor	Possible gastrointestinal effects (constipation, abdominal distension)	
AEGR-733 BMS-201038	Aegerion	Phase II	MTP inhibition	Steatorrhea, elevated liver aminotransferase levels, hepatic fat accumulation	
Colestilan (MCI-196)	Mitsubishi Tanabe Pharma	Launched	Bile acid sequestrant resin	Gastrointestinal effects (constipation, abdominal distension), possibility of intestinal perforation and/or closure	
ALN-PCS01	Tekmira Pharmaceuticals (Alnylam)*	Preclinical	RNAi, PCSK9	None noted	
PCSK9 inhibitors	Bristol-Myers Squibb (Isis)*	Preclinical	RNAi, PCSK9	None noted	
Mipomersen sodium (ISIS 301012)	Genzyme (Isis)*	Phase III	Antisense oligonucleotide, inhibitor of apoB	Mild injection-site erythema	
FM-VP4	Forbes Medi-Tech	Phase II	Unknown, phytosterol analog	None noted in Phase I trial	
Anacetrapib	Merck & Co.	Phase III	CETP inhibitor	None noted in Phase I trials	No blood pressure elevation in Phase I trial
Tredaptive™ [laropiprant + niacin] (Merck 0524A)	Merck & Co.	Pre-registration	Laropiprant: prostaglandin D2 receptor antagonist	Flushing	Decreased flushing episodes by 50% compared to extended-release niacin

*Originator.

CETP: Cholesteryl ester transfer protein; HMG-CoA reductase: 3-Hydroxy-3-methyl-glutaryl-CoA reductase; MAPK: Mitogen-activated protein kinase; NPC1L1: Niemann-Pick C1 like 1; PCSK9: Proprotein convertase subtilisin/kexin type 9; PPAR: Peroxisome proliferator-activated receptor; RNAi: Ribonucleic acid interference.

Table 3. Competitive environment table (continued).

Compound	Company	Phase	Mechanism of action	Side effects	Comments
RVX-208	Resverlogix	Preclinical	Unknown; increases apolipoprotein A-I levels and cholesterol efflux	None noted in Phase I trial	
CRD-5	Liponex	Phase II	Directly increases hepatic apolipoprotein A-I secretion, possibly through MAPK and PPAR- α pathways	High dose (suspended) associated with gastrointestinal effects	

*Originator.

CETP: Cholesteryl ester transfer protein; HMG-CoA reductase: 3-Hydroxy-3-methyl-glutaryl-CoA reductase; MAPK: Mitogen-activated protein kinase; NCP1L1: Niemann-Pick C1 like 1; PCSK9: Proprotein convertase subtilisin/kexin type 9; PPAR: Peroxisome proliferator-activated receptor; RNAi: Ribonucleic acid interference.

5.1 Therapies focused on LDL-C

5.1.1 New agents to interfere with cholesterol biosynthesis

Given the success of statin drugs, one strategy has been to develop more potent 'super-statins', such as pitavastatin (Kowa Co., NK104). Another idea has been to develop inhibitors of other enzymes involved in cholesterol biosynthesis. One such target is squalene synthase, the enzyme responsible for the biosynthesis of squalene, through a reductive dimerization of two farnesyl diphosphate molecules. This is an attractive target point, as farnesyl diphosphate is located at the final branch point in the isoprenoid biosynthesis pathway and the conversion to squalene marks the first committed step in the formation of cholesterol. The most promising agent to exploit this mechanism is the squalene synthase inhibitor lapaquistat (Takeda, Inc., TAK-475) [61].

5.1.2 Cholesterol absorption inhibitors

The commercial success of ezetimibe – related to its efficacy for additional LDL-C reduction when combined with statins, together with its minimal profile of adverse events – has prompted a more detailed evaluation of analogous agents [62]. The question of whether newer agents referred to as 'cholesterol absorption inhibitors' (CAIs) act like ezetimibe or instead interfere with other stages of intestinal cholesterol absorption is unclear. NPC1L1 itself was discovered as part of a bioinformatic search for the actual ezetimibe target. Related molecular entities include acyl coenzyme A:cholesterol acyltransferase (ACAT), which was thought to be the target of ezetimibe before its cholesterol absorption inhibition was appreciated [62]. Interestingly, avasimibe, the first oral ACAT inhibitor, was shown not to have favorably influenced plaque visualized in the coronary arteries [63], which has thus dampened enthusiasm for ultimate CHD benefit that might arise through ACAT inhibition. Since two different ACAT enzymes (ACAT 1 and ACAT 2) exist, focus may be

shifted towards future development of selective ACAT 2 inhibitors for hypercholesterolemia, since ACAT 2 rather than ACAT 1 is primarily present in hepatocytes – typically at sites of lipoprotein particle secretion – thereby implying a critical role for ACAT 2 in cholesterol ester formation and secretion in lipoproteins [64].

5.1.3 Microsomal triglyceride transfer protein inhibition

Another approach to reduce plasma LDL-C is reduction of LDL production. A key player in LDL particle formation is MTP, an enzyme that transfers TG onto apoB within the intestine for formation of chylomicrons and within the liver during assembly of VLDL, the precursor to LDL [65]. Mutations in MTP result in abetalipoproteinemia, an autosomal recessive disorder characterized by extremely low levels of chylomicrons, VLDL and LDL [66]. Preclinical studies in animal models have shown that the inhibition of MTP significantly reduces plasma cholesterol [67,68].

5.1.4 Interrupting enterohepatic circulation

BAS interrupt the enterohepatic circulation leading to the diversion of hepatic cholesterol into bile acid synthesis, resulting ultimately in increased clearance of LDL from blood. But despite evidence for reduction of CHD end points and a long track record of safety, BAS suffer from low compliance due to adverse effects. Emerging therapies in this subgroup are focused on reducing adverse effects to improve compliance or using new mechanisms to interrupt enterohepatic circulation of bile acids, such as ileal bile acid transport inhibitors.

5.2 Therapies focused on HDL-C

5.2.1 CETP inhibitors

CETP, a hydrophobic glycoprotein secreted primarily from the liver, facilitates the movement of CE from HDL to LDL, IDL, and VLDL in exchange for TG during the latter stages of RCT. CETP inhibition was first recognized as a potential HDL-raising strategy when rodents lacking plasma

CETP activity had elevated HDL-C and resistance to diet-induced atherosclerosis [69]. Subsequently, patients with CETP mutations were found with elevated HDL-C and decreased CHD [70,71]. Thus, Japan Tobacco (JTT-705, now Roche R1658) and Pfizer (torcetrapib) independently began to develop CETP inhibitors.

Both R1658/JTT-705 and torcetrapib raised HDL-C and reduced atherosclerotic plaque area by around 70% in animal models [72,73]. However, human trials of torcetrapib revealed no changes in surrogate atherosclerosis markers despite significant increases in HDL-C levels by approximately 60% [74-76]. More importantly, the first randomized control trial evaluating the clinical effects of torcetrapib and atorvastatin versus placebo and atorvastatin alone among 15,067 individuals at high risk of CHD was halted due to increased CHD and mortality risk (hazard ratio 1.25) in those randomized to receive torcetrapib [77]. All torcetrapib trials were halted, and CETP inhibition as a strategy to raise HDL-C has been questioned. Recent articles have attributed the failure of torcetrapib to features of the molecule rather than the mechanism of CETP inhibition: torcetrapib was associated with sometimes marked elevations in systolic blood pressure, an effect not seen with R1658/JTT-705 [76,78,79]. Hence, many consider that CETP inhibition remains a viable strategy for HDL-C raising and CHD event reduction.

5.2.2 Apolipoprotein A-I

ApoA-I is the major structural protein on HDL particles that is secreted by the small intestine and liver. It contains 243 amino acids arranged in 10 amphipathic helices [80]. ApoA-I plays a key role in RCT, since the interaction of lipid-free apoA-I on HDL with the macrophage ABCA1 transporter leads to cholesterol efflux from foam cells. Moreover, apoA-I has antioxidant properties, as apoA-I infusion in mice and humans renders LDL resistant to oxidation [81]. Transgenic expression of human apoA-I in atherosclerosis-prone apo E^{-/-} mice led to a twofold increase in HDL-C and an 82% reduction in aortic atherosclerotic area after 8 months [82].

In animal models, small peptide analogs mimicking the lipid-binding domain of apoA-I have been shown to inhibit lipid oxidation, enhance cholesterol efflux, improve endothelial function and reduce lesion size by up to 79%, without an increase in HDL-C levels [83-87]. This indicates that raising HDL-C may not be absolutely required for positive clinical outcomes. While inconsistent effects on atherosclerotic lesions in humans have been seen with the orally stable apoA-I mimetic D-4F, recent attention has continued to focus on apoA-I-based strategies [84,87].

ApoA-I_{Milano} denotes a variant of apoA-I where cysteine replaces arginine at position 173 in the *APOA1* gene. Individuals with apoA-I_{Milano} have low HDL-C levels but no increased CHD risk [88]. The exact atheroprotective mechanisms for apoA-I_{Milano} remain unknown. Yet a

randomized clinical trial of intravenous infusion of apoA-I_{Milano} complexed with phospholipids into individuals with acute coronary syndrome demonstrated significant regression of coronary atherosclerosis compared to baseline values, after just 6 weeks [89]. Unfortunately, the requirement of intravenous administration limits the long-term applicability of this treatment, and efficacy may depend on optimizing the specific phospholipids complexed for administration. Thus, apoA-I_{Milano}, despite not significantly altering lipid levels, requires further investigation as an emerging CHD therapy.

5.3 Combination therapies

Combining other agents with statins is an attractive means to induce additional favorable biochemical changes. The best example is the niacin-statin combination, which has provided impressive end point reductions, albeit in very small studies [90]. The fixed dose lovastatin-ERN combination is called Advicor[®] (Kos Pharmaceuticals) and the fixed dose simvastatin-ERN combination (Simcor[®]), which was associated with the excellent outcomes in small angiographic studies [90], is under development.

Another fixed-dose combination that is very widely used is Vytarin[®] (simvastatin plus ezetimibe, from Merck-Schering). This combination tablet effectively reduces plasma LDL-C but so far has minimal clinical trial evidence of CHD reduction. The recent use of ezetimibe and simvastatin in the Hypercholesterolemia Enhances Atherosclerosis Regression (ENHANCE) study attempted to clarify this issue [91]. A total of 720 patients with HeFH were randomized to receive simvastatin alone or simvastatin plus ezetimibe. Despite > 20% further reduction in plasma LDL-C in patients who received the combination compared with simvastatin monotherapy, cIMT did not differ between the two treatments after 2 years. Thus, the results imply that addition of ezetimibe to statin therapy does not confer CHD benefit, using the surrogate marker of cIMT. However, the ENHANCE trial had several limitations: i) a rare and specialized demographic – HeFH patients – was studied; ii) the ultrasound device used in ENHANCE had less sensitivity and resolution compared to more modern instruments (Figure 2); and iii) most FH patients nowadays are not statin-naïve, so that cIMT may have already been maximally reduced with routine treatments before the study even began. The definitive answer on CHD benefit from the statin-ezetimibe combination awaits large end point trials.

Another unanswered question is whether the addition of a fibrate to baseline statin therapy confers additional protection from CHD. Importantly, FIELD safety data showed no rhabdomyolysis in > 1000 patients who took both fenofibrate and a statin [46]. Potential beneficial effects of the combination of a statin and fibrate in type 2 diabetes are currently being studied in the ongoing Action to Control Cardiovascular Risk in Diabetes (ACCORD) study [92].

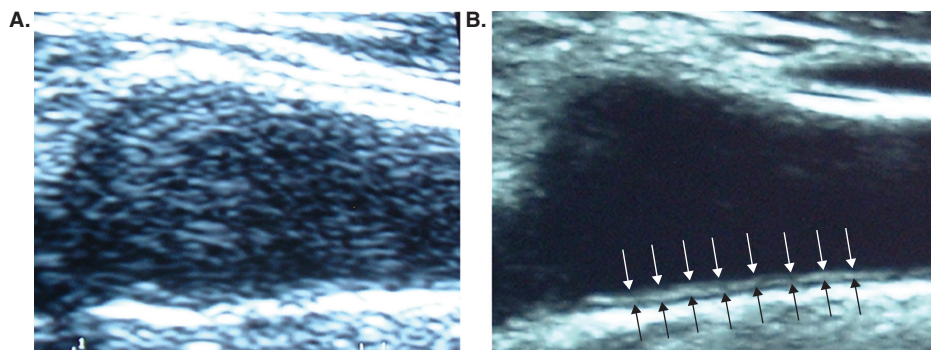


Figure 2. Comparison of ultrasound device sensitivity. The right carotid artery bulb in longitudinal view taken with two different ultrasound instruments over a half-hour period from the same individual (RAH). **A.** The image captured by the Acuson Aspen ultrasound device with the L7 transducer, comparable to the technology reported in the ENHANCE study [91]. **B.** The same region scanned by the Phillips model HD11 XE with the L12-5 transducer. The arrows indicate the anterior and posterior limits of 8 individual carotid intima-medial thickness (cIMT) measurements (mean ~ 0.55 mm) acquired over ~ 1 cm interval of the posterior wall of the carotid bulb. The same extent of cIMT is much more difficult to visualize with the technology used in the ENHANCE study (**A**).

5.4 Biotherapeutics

Biotherapeutic agents represent a new paradigm for treating dyslipidemia. Instead of pharmacological manipulation of a target protein, these newer biological methods, including antisense oligonucleotides (ASOs) and ribonucleic acid (RNA) interference (RNAi), interfere with translation by selectively degrading mRNAs.

An ASO is a single-stranded deoxyribonucleotide (~ 20 nucleotides long) that corresponds in a sequence-specific manner to target mRNA for the protein of interest [93]. After binding to the target mRNA, the ASO inhibits gene expression by: i) activating an enzyme, RNase H, which degrades the target mRNA while leaving the ASO intact; ii) interfering with ribosomal activity; and iii) interfering with maturation of mRNA by inhibition of splicing or destabilization of pre-mRNA [93].

RNAi is a natural process of gene silencing that occurs in organisms from plants to mammals. The process begins with *in vivo* introduction of a short double-stranded RNA (dsRNA) that perfectly complements the target mRNA. The dsRNA is then cleaved by an enzyme called Dicer at specific sites to form a small interfering RNA (siRNA), which binds to a protein complex called RNA-Induced Silencing Complex (RISC), thereby allowing for exposure of the antisense siRNA strand and recognition of target mRNA. The target mRNA is cleaved and degraded, resulting in the post-translational silencing of gene expression [94]. Not surprisingly, the potency, simplicity and specificity of this mechanism has spurred efforts to develop novel classes of biotherapeutics based on RNAi, despite two major hurdles: delivery and safety.

5.4.1 PCSK9 inhibition

Identified in 2003, proprotein convertase subtilisin/kexin type 9 (PCSK9) is a serine protease that mediates LDLR degradation [95]. Although the exact mechanism still

remains unresolved, the specific residues involved in LDLR recognition and binding have been elucidated [96]. The link between PCSK9 and plasma LDL-C was first established by the discovery of missense, gain-of-function mutations in *PCSK9* that were present in patients with HeFH [97]. Subsequently, loss-of-function mutations in *PCSK9* were identified that were associated with decreased cholesterol levels and reduced CHD risk [98]. Thus, recent strategies to reduce LDL-C focus on inhibiting PCSK9 through targeted mRNA degradation, since reduced PCSK9 will lead to increased persistence of the LDLR, promoting LDL particle clearance. Currently, industry is leapfrogging to develop biological methods for PCSK9 inhibition using gene silencing.

5.4.2 Apolipoprotein B knockdown

ApoB is an important structural protein that forms the backbone of all lipoproteins except HDL. From a single gene on chromosome 2, two apoB proteins are expressed. The smaller apoB isoform, apoB-48, is expressed in the intestine and is required for assembly of chylomicrons. The full-length apoB-100 is synthesized in the liver and provides structural integrity for VLDL, IDL and LDL particles. It also serves as a ligand for the LDLR. There is one copy of apoB-100 per lipoprotein particle; thus, plasma apoB-100 concentration roughly measures the number of atherogenic particles. Overproduction of apoB or reduced receptor-mediated clearance of lipoproteins leads to elevated plasma cholesterol levels and premature atherosclerosis. Over 50 truncation or nonsense mutations in *APOB* cause familial hypobetalipoproteinemia, which is associated with very low plasma LDL-C concentration. A few missense mutations in the *APOB* receptor-binding domain cause a disorder that clinically resembles HeFH.

The example of severe mutations in hypobetalipoproteinemia has suggested that major disruption of *APOB* synthesis

using gene silencing can reduce plasma cholesterol. For example, siRNAs to *APOB* significantly reduced the mRNA expression and plasma apoB-containing lipoprotein concentrations in nonhuman primates [99]. Development of siRNA-based targeting of apoB is less advanced than ASO-apoB (ISIS 301012), which has had numerous clinical trials. Dose-dependent reductions in apoB were observed, with the greatest reduction of 70% observed at the highest dose (400 mg) [100].

6. Competitive environment

6.1 Therapies focused on LDL-C

6.1.1 New agents to interfere with cholesterol biosynthesis

Among the 'super-statins', pitavastatin (Kowa Co., NK104) is currently available in Japan and India. However, no head-to-head trials have been performed between pitavastatin and rosuvastatin, which is currently the most efficacious statin drug. A potential concern is that potent pharmacological inhibition of HMG-CoA reductase has perhaps been maximized with current agents at their highest doses; the law of diminishing returns together with potential toxicities could limit this approach.

Developing inhibitors of other enzymes involved in cholesterol biosynthesis has been another area of exploration. The most promising agent to exploit this mechanism is the squalene synthase inhibitor, lapaquistat (Takeda, Inc., TAK-475) [61]. Lapaquistat appears to have fewer secondary effects that are mediated by non-cholesterol products of mevalonate metabolism distal to HMG-CoA reductase. However, elevated transaminases remain a concern and lower doses are being considered, potentially for administration in combination with statins. The place of squalene synthase inhibitors as a complement to or substitute for statins remains uncertain.

6.1.2 Cholesterol absorption inhibitors

Agents under development that are purported to act as inhibitors of cholesterol absorption include MD-0727 (Microbia), which has been described as a 'cholesterol transporter antagonist' whose target is possibly the sterol located on the villous surface of small intestinal epithelial cells, as with ezetimibe. Another agent is AVE-5530 (Sanofi-Aventis), a non-absorbable cholesterol absorption inhibitor [101], which is currently the subject of clinical trials [102]. While these new agents are likely to have beneficial effects on the biochemical surrogate of plasma LDL-C, the importance ultimately of CHD end point studies cannot be understated, just as for ezetimibe.

6.1.3 Microsomal triglyceride transfer protein inhibition

Last January, a dose-escalation study was carried out to examine the safety, tolerability, and efficacy of the MTP

inhibitor – Bristol-Myers Squibb (BMS)-201038 (since licensed to Aegerion) – in six patients with homozygous FH. The inhibitor was found to be effective; at the highest dose of 1.0 mg/kg/day, LDL-C levels decreased by 51% and apoB levels by 56% from baseline. However, there was also an elevation of liver aminotransferase levels and accumulation of hepatic fat [103]. This certainly is a major concern, and the effects of long-term inhibition of MTP on the liver will need to be carefully studied to determine the safety of this approach. Since MTP is also involved in the assembly and secretion of exogenous lipids via chylomicrons, another troublesome side effect that may affect compliance of this potential therapy is steatorrhea. Although intestinal MTP inhibitors are in development and an early study in guinea pigs reveals decreases in TG and LDL-C levels without increases in hepatic TG or enzyme levels, consequent intestinal chylomicron accumulation may still significantly limit the applicability of this potential drug in humans [104].

6.1.4 Bile acid sequestrants and ileal apical sodium-dependent bile acid transporters

A recently developed anion exchange resin, colestilan (also known as colestimide), synthesized from 2-methylimidazole and epichlorohydrin, has replaced cholestyramine in the treatment of hypercholesterolemia in Japan due to decreased adverse effects and improved compliance [105]. Colestilan was also tested in Japanese pediatric FH patients with positive results: all patients responded well, with significant decreases in both TC and LDL-C after 3 – 6 months [106]. Moreover, in a study comparing the lipid and glycemic effects of colestimide to pravastatin in patients with type 2 diabetes, both drugs showed favorable results for LDL-C and TC lowering but only colestimide demonstrated favorable reductions in plasma fasting glucose levels [107]. Since patients with diabetes often also have hyperlipidemia, colestimide therapy may have a clinically useful dual action in such patients.

Ileal apical sodium-dependent bile acid transporters (ASBT), which play a critical role in the reabsorption of bile acids in the ileum [108], have been considered an attractive target for cholesterol lowering [109]. Moreover, ASBT inhibitors would not be absorbed, since bile acid binding to ASBT occurs within the lumen of the most distal part of the ileum, thereby allowing for a potentially low risk for systemic toxicity or drug-drug interactions. Over the past decade, several potential compounds have been identified, including a recent novel inhibitor that reduced serum TC levels in hamsters by around 40% [110]. Clinical trials are underway.

6.1.5 Phytosterol analog

Disodium ascorbyl phytosterol phosphate (FM-VP4) is a water-soluble oral phytosterol mixture composed of sitosterol and campestanol ascorbate. Animal studies

of 8 – 12 weeks in duration have revealed significant reductions in TC of around 30 – 50%, LDL-C by > 95% in gerbils only, with no significant changes in HDL-C after FM-VP4 administration [111,112]. Compared with controls, decreases in TG levels were reported among apoE knockout mice, in addition to a 75% reduction in plasma TC level and 75% reduction in aortic atherosclerotic lesion area [113]. Although postulated mechanisms for FM-VP4 had included CETP inhibition, the exact mechanism of action for FM-VP4 remains unknown [111]. Instead, FM-VP4 has been recently shown to decrease cholesterol gastrointestinal absorption and accumulation, independently of pancreatic lipase activity or cholesterol incorporation into micelles [114,115]. FM-VP4 may also have applications for weight loss, since significant reductions in weight gain of 17 and 51% were seen in mice with dietary-induced obesity when fed a low-fat diet or high-fat diet, respectively [112].

From the company website, a Phase I trial for FM-VP4 was completed with no detection of adverse events [116]. The results of a US Phase II trial of 150 male and female individuals with mild-to-moderate hypercholesterolemia have been reported, but not yet published. In that randomized, double-blind, placebo-controlled trial, 50 patients were assigned to one of the following 3 groups: placebo, 450 mg FM-VP4, and 900 mg FM-VP4. At the end of the 12 weeks, only those receiving 900 mg FM-VP4 daily had a statistically significant 9% reduction in LDL-C. No serious adverse events were noted [116]. Thus, the main advantage of this emerging drug is its tolerability, and further studies on its potential effects of weight loss among humans may be warranted.

6.2 Therapies focused on HDL-C

6.2.1 CETP inhibitors

Torcetrapib was associated with systolic blood pressure (SBP) elevations of 2.8 – 5.4 mmHg in recent clinical trials [74-77]. Since prior studies have demonstrated an increase of around 25% in cardiovascular disease, stroke, and vascular death for every 10 mmHg increase in SBP, these SBP elevations with torcetrapib may have negated any potential benefit of HDL-C elevation [117,118]. Despite post-hoc analysis of aldosterone levels in one of the trials, the exact mechanism of SBP elevations remains unknown [77]. Yet, consequently, there has been intense evaluation of current CETP inhibitors for adverse events (particularly hypertension).

Recently, two Phase I trials for the CETP inhibitor, anacetrapib (MK-0859), have been published – one focused on dose-dependent lipid efficacy and the other on 24-h ambulatory blood pressure monitoring [119]. Both studies were double-blind, randomized, placebo-controlled studies. The dose-ranging study examined 50 individuals with dyslipidemia. After a 2-week diet run-in period, individuals were randomized to placebo or one of four doses of anacetrapib (10, 40, 150, or 300 mg) to be taken daily for 28 days. The 10-mg dose resulted in a 41% increase in

HDL-C, while the 300-mg dose increased HDL-C by 129% with an associated 38% reduction in LDL-C.

Anacetrapib 150 mg/d versus placebo among 22 healthy individuals in a 10-day randomized, double-blinded crossover trial demonstrated no statistically significant effect on blood pressure. In fact, anacetrapib was well tolerated, with no serious adverse events and no discontinuations in both studies [119].

These results suggest that anacetrapib may indeed be a useful emerging drug since it has relatively greater lipid-altering effects compared with either torcetrapib or R1658/JTT-705, and does not seem to affect blood pressure as torcetrapib had [74-77,79,119,120].

6.2.2 Improving tolerability of niacin

While niacin is the best HDL-C raising drug currently available, its use has been limited by side effects mediated through the DP1 receptor. The combination of extended-release (ER) niacin and the selective DP1 antagonist, laropiprant (MK-0524), has been shown to reduce niacin-related flushing by around 50% [121]. Tredaptive™ or MK-0524A (ER niacin combined with laropiprant) and MK-0524B (MK-0524A with simvastatin) are currently the focus of clinical outcome trials [122]. Thus, laropiprant may be an important addition allowing for better tolerability of and compliance with niacin.

6.2.3 Enhancing apoA-I

Anionically charged phosphatidylinositol (PI) inhibits LCAT activity, increases hepatic uptake of free cholesterol, increases cholesterol excretion in feces, reduces the synthesis and storage of CE, and decreases cholesterol synthesis and esterification [123,124]. Soy-based PI is an emerging therapy for raising HDL-C levels. In 16 normolipidemic subjects, 2 weeks of oral soy PI increased HDL-C and apoA-I by 18 and 6%, respectively, without apparent side effects. The high dose of soy PI (5.6 g/d) also resulted in a 36% reduction in TG levels [125]. PI-induced apoA-I secretion occurs through mitogen-activated protein kinase (MAPK) and PPAR- α pathways to induce apoA-I secretion [126]. Importantly, although PI does appear to activate PPAR- α , PI did not inhibit any major cytochrome P450 enzymes, indicating potential decreased risk for drug–drug interactions [126].

CRD-5 is the main player among PI-based therapies. In a 12-week Phase I/II trial of 50 dyslipidemic individuals with low HDL-C and high LDL-C levels, CRD-5 was associated with significantly elevated HDL-C (5%) [127], with > 20% of patients experiencing a > 10% increase in HDL-C, especially when baseline HDL-C was < 0.9 mmol/l [127]. Although there were adverse gastrointestinal effects with the now-suspended 5-g dose, the enteric-coated formulation tested in minipigs was well tolerated [128]. Since PI is derived from soy lecithin, potential adverse effects may include allergic responses. Further investigation in

humans is required, and will help determine the utility of this class of agents.

A novel small molecule, RVX-208 (developed by Resverlogix Corp.), is currently in human Phase I clinical trials [109-112]. RVX-208 has been shown to increase apoA-I production *in vitro* and *in vivo*. In Hep G2 cells exposed to RVX-208, levels of both endogenous apoA-I protein and mRNA were increased in a dose-dependent fashion. Furthermore, RVX-208 also induced activity of a transiently transfected reporter construct containing the human apoA-I promoter. These beneficial actions were not only observed *in vitro*, but also extended to a variety of rodent models including human apoA-I transgenic mice, C57/BL6 mice and Sprague-Dawley rats. These and other data laid the foundation for studies in the adult male African green monkeys (AGM), a model that may predict human response to antidiyslipidemia drugs. The AGMs were dosed orally once daily with RVX-208 at 60 mg/kg for 63 consecutive days; on the 64th day, administration of RVX-208 was stopped, followed by a 3-week washout. The animals were maintained on a primate chow diet throughout the study. Serum apoA-I increased by 52% (Figure 3, left bars) and HDL-C by 95% (Figure 4, left bars) from baseline within 28 days of exposure to RVX-208 and were sustained until day 63 (data not shown). As expected, both apoA-I and HDL-C returned to near baseline values (Figures 3 and 4, right bars) during the washout period. Such results formed the basis of a successful IND application enabling RVX-208 to be tested in human clinical trials, which are currently ongoing.

Serum opacity factor (SOF) is a substance produced by around 50% of clinical isolates of *Streptococcus pyogenes* and was named because of its ability to opacify mammalian serum. Recombinant SOF (rSOF) seems to interact with HDL apolipoproteins (A-I and A-II), resulting in the extrusion of HDL lipids and formation of a cholesteryl ester-rich microemulsion (CERM) as well as the release of a new HDL-like particle – neo HDL (enriched with phospholipid and protein) – and lipid free (LF) apoA-I. LF-apoA-I particles would then be free to participate as cholesterol acceptors in the early steps of RCT, while CERM could theoretically transport large amounts of cholesterol to the liver for excretion [129], meaning that rSOF could be useful for improving LDL-C and HDL-C.

6.3 Combination therapies

6.3.1 Ezetimibe + atorvastatin

Given the commercial success of Vytorin[®], and the fact that ezetimibe provides incremental LDL-C lowering when given in combination with a statin as separate medications, the fixed-dose combination tablet of ezetimibe plus atorvastatin (Schering-Plough) is actively being investigated. This combination tablet would be expected to give greater LDL-C reduction on a milligram basis compared with Vytorin[®], with a comparably acceptable adverse event

profile. The same caveat about the CHD end point benefit of combining a statin with ezetimibe, discussed above, would also apply to the atorvastatin-ezetimibe combination, although these combinations are extremely effective in helping patients to achieve the sometimes stringent target LDL-C levels.

6.3.2 Statin + fenofibrate

The ACCORD trial will provide CHD end point evidence for whether adding a fibrate to a statin will result in further benefit. Based on the possibility that this aspect of the trial will be positive, the atorvastatin-fenofibrate combination (LifeCycle Pharma) and the rosuvastatin-fenofibrate combination (ABT-335, Abbott and AstraZeneca) have entered into early-phase clinical trials. The combination seems appealing because the fenofibrate component addresses the abnormalities in TG and HDL-C in patients with complex, mixed dyslipidemia. The ACCORD trial data will probably be available sooner than the Vytorin[®] outcome trials. Depending on the aggregate of clinical outcome data, the statin-fenofibrate combinations may compete directly with Vytorin[®].

6.4 Biotherapeutics

6.4.1 PSCK9

The development of drugs to target PSCK9 has begun using the novel biotherapeutic approach. ALN-PCS01 (Tekmira) is a RNAi therapeutic comprising a siRNA encapsulated in a cationic liposomal nanoparticle formulation. ALN-PCS01 has demonstrated PSCK9 antagonism in primates, with a reduction of around 70% in PSCK9 plasma levels, 40 – 60% reduction in LDL-C, and 30 – 40% reduction in apoB levels. The single intravenous injection was well tolerated, and the effects lasted for up to 3 weeks [130]. In mice, ALN-PCS01 silenced the *PSCK9* gene, as demonstrated by > 70% reduction in mRNA levels, compared with controls, and also significantly decreased cholesterol levels [130]. The drug is currently entering Phase I clinical trials. Also in the pipeline is an ASO targeting *PSCK9*, which is in the preclinical stages. Studies in mice showed increased LDLR levels and reduced LDL-C concentrations [131].

6.4.2 Apolipoprotein B

The first ASO for the treatment of dyslipidemia is ISIS 301012 (mipomersen sodium), a 20-nucleotide ASO that targets *APOB*. It directly binds to the apoB-100 mRNA sequence and targets the bound mRNA for degradation via the activation of endogenous RNase H enzymes, thus reducing both apoB and LDL-C levels [132].

A double-blinded, randomized, placebo-controlled, dose-escalation Phase I trial in 36 volunteers, with an initial single dose of 50 – 400 mg of ISIS 301012 administered via subcutaneous injection, followed by a 4-week multiple-dosing regimen with the same assigned dose [133],

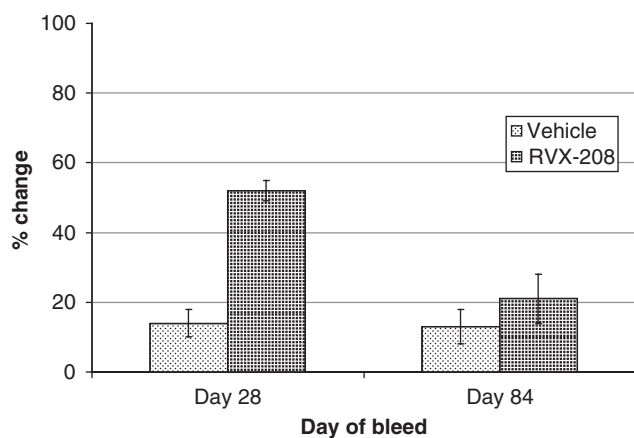


Figure 3. Percentage change in apo-A1 from African green monkeys dosed with RVX-208 at 60 mg/kg orally daily. African green monkeys were dosed orally once daily with RVX-208 at 60 mg/kg for 63 consecutive days, followed by a 3-week washout. Serum apoA-I increased by 52% (left bars) from baseline within 28 days of exposure to RVX-208. During the washout period (day 84, right bars), apoA-I returned to near baseline values.

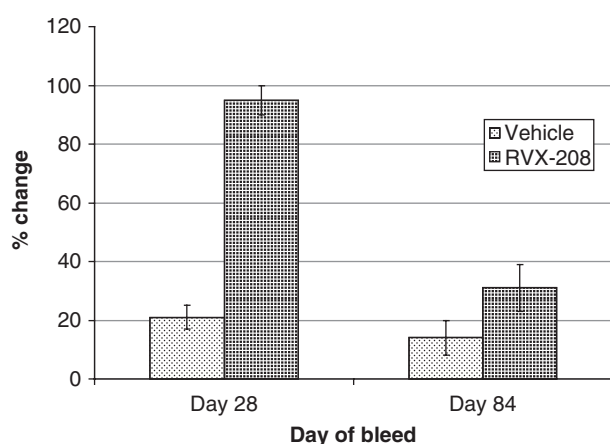


Figure 4. Percentage change in HDL-C from African green monkeys dosed with RVX-208 at 60 mg/kg orally daily. African green monkeys were dosed orally once daily with RVX-208 at 60 mg/kg for 63 consecutive days, followed by a 3-week washout. Serum HDL-C increased by 95% (left bars) from baseline within 28 days of exposure to RVX-208. During the washout period (day 84, right bars), HDL-C returned to near baseline values.

showed that apoB was reduced by 14 – 47% and LDL-C by 6 – 40% at 55 days; both LDL-C and apoB remained below baseline at 3 months [133]. The most frequent adverse event was mild injection-site erythema (21/29 subjects). Elevated serum alanine aminotransferase was also observed in four patients.

Two recent Phase II trials examined the safety, efficacy, and pharmacokinetics of ISIS 301012 as add-on therapy in patients with HeFH [134] or homozygous FH [135].

Again, effective lowering of LDL-C levels was found, and no serious adverse effects were noted beyond painless mild-to-moderate injection-site reactions. Similarly, trials examining ISIS 301012 added to statin therapy [136] showed a 48% further reduction in LDL-C with 3 months on ISIS 301012 therapy (200 mg/week). Currently, a Phase III clinical trial is underway for the treatment of HeFH; if found to be safe and effective, this may be an important therapeutic option in this high-risk group for CHD.

7. Potential development issues

Long-term and more recent experiences with various lipid-altering medications indicate that despite their mechanistic attractiveness, with beneficial effects on biochemical or even imaging surrogate markers, only unassailable clinical trial evidence showing improved CHD outcomes can transform clinical practice. This was certainly the case with statin drugs, which were viewed with suspicion by many cardiologists and general practitioners between 1987 and 1994. It was only after the 4S study showed unequivocal simvastatin-related reductions in both major CHD events and overall mortality that statins began to experience widespread clinical application. Since 1994, clinical dyslipidemia research has focused on intensified LDL-C lowering using higher doses of more potent statins or combination drugs. But given the experience with the ENHANCE study, it would appear even LDL-C reduction by a non-statin mechanism still requires end point studies to ease doubts, providing the final 'stamp of approval'.

The power of CHD end point studies is further exemplified by the negative FIELD trial, which has resulted in a dramatic reduction in the use of fibrates, despite their biochemical efficacy for TG reduction. Similarly, theoretical advantages of torcetrapib were rendered almost meaningless in the face of increased CHD mortality. CHD end point studies for every new agent under development will likely be required to settle the issue.

Similarly, for all other mechanisms of correcting the lipid profile discussed above, no matter how elegant based on biochemical or imaging surrogate markers, each new agent discussed above will be required to demonstrate a positive benefit-to-risk ratio for CHD. It will be difficult for any new treatment to evade providing this standard of evidence. Also, because CHD end point studies are resource-intensive, and because contemporary control groups benefit from lower CHD risk due to modern treatments, performing end point studies for any new class of lipid-altering drug will become even more challenging.

8. Expert opinion

Management of dyslipidemia, particularly with statin drugs, has revolutionized clinical cardiology. Many of the new mechanisms for lipid-lowering drugs discussed here followed

from clever insights derived from a range of experiments. Given the spectrum of different mechanisms involved, together with development of non-pharmaceutical molecular approaches, it is very likely that at least some of these treatments will provide an incremental benefit over statins alone.

Most promising for relatively early application would seem to be combinations of existing classes of medications – particularly statin-fibrate, statin-CAI and statin-ERN-DP1 inhibition – to enhance lipid parameters and perhaps translate into improved outcomes. Strategies to raise HDL-C are more questionable, given the complexity of the HDL metabolic pathway: CETP inhibition might not be the best approach, while apoA-I-based treatments appear more promising.

New approaches to further reduce LDL-C, such as PCSK9 or APOB knockdown, while attractive mechanistically, will need to be carefully evaluated for potential ‘off-target’ effects or undesirable ‘on-target’ effects when targets and indeed aspects of biology are incompletely characterized. Such treatments, in addition to inhibition of MTP or squalene synthase, will need to have careful evaluation

of benefit–risk ratio in both early- and late-phase trials. Finally, for any new treatment, the medical community will have to rely on late-phase CHD outcome studies as the final arbiter of clinical application.

Declaration of interest

One of the authors is supported by the Jacob J Wolfe Distinguished Medical Research Chair, the Edith Schulich Vinet Canada Research Chair (Tier I) in Human Genetics, a Career Investigator award from the Heart and Stroke Foundation of Ontario (CI-5710), and operating grants from the Canadian Institutes for Health Research (FRN-13430 and MOP-79533), the Heart and Stroke Foundation of Ontario (PRG-5967, NA-6059 and T-6018), the Ontario Research Fund and by Genome Canada through the Ontario Genomics Institute. We would also like to acknowledge the contribution of Norman Wong, Professor of Medicine, University of Calgary, who provided us with novel data regarding the effect of RVX-208 on apoA-I and HDL-C levels.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

- Goff DC Jr, Bertoni AG, Kramer H, et al. Dyslipidemia prevalence, treatment, and control in the Multi-Ethnic Study of Atherosclerosis (MESA): gender, ethnicity, and coronary artery calcium. *Circulation* 2006;113:647-56
- Johansen H, Nair C. The changing face of heart disease and stroke in Canada 2000. In: Wielgosz A, Arango M, Carew M, Ferguson M, Johansen H, Nair C, et al., editors. *Health Outcomes*. Ottawa, Canada: Heart and Stroke Foundation of Canada; 1999. p. 65-85
- Rosamond W, Flegal K, Furie K, et al. Heart disease and stroke statistics – 2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* 2008;117:e25-146
- Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* 1994;344:1383-9
- Castelli WP, Anderson K, Wilson PW, et al. Lipids and risk of coronary heart disease. The Framingham Study. *Ann Epidemiol* 1992;2:23-8
- Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002;106:3143-421
- Rader DJ. Molecular regulation of HDL metabolism and function: implications for novel therapies. *J Clin Invest* 2006;116:3090-100
- Castelli WP, Garrison RJ, Wilson PW, et al. Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study. *JAMA* 1986;256:2835-8
- Abbott RD, Wilson PW, Kannel WB, et al. High density lipoprotein cholesterol, total cholesterol screening, and myocardial infarction. The Framingham Study. *Arteriosclerosis* 1988;8:207-11
- Wilson PW, Abbott RD, Castelli WP. High density lipoprotein cholesterol and mortality. The Framingham Heart Study. *Arteriosclerosis* 1988;8:737-41
- Joy T, Hegele RA. Is raising HDL a futile strategy for atheroprotection? *Nat Rev Drug Discov* 2008;7:143-55
- Ginsberg HN, Zhang YL, Hernandez-Ono A. Regulation of plasma triglycerides in insulin resistance and diabetes. *Arch Med Res* 2005;36:232-40
- Hussain MM, Fatma S, Pan X, et al. Intestinal lipoprotein assembly. *Curr Opin Lipidol* 2005;16:281-5
- Shelness GS, Sellers JA. Very-low-density lipoprotein assembly and secretion. *Curr Opin Lipidol* 2001;12:151-7
- Vance DE, Vance JE. *Biochemistry of lipids, lipoproteins and membranes*. 4th edition. Amsterdam: Elsevier Science; 2002
- Karpe F, Steiner G, Uffelman K, et al. Postprandial lipoproteins and progression of coronary atherosclerosis. *Atherosclerosis* 1994;106:83-97
- Bansal S, Buring JE, Rifai N, et al. Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. *JAMA* 2007;298:309-16
- Nordestgaard BG, Benn M, Schnohr P, et al. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA* 2007;298:299-308
- Mensah GA, Brown DW. An overview of cardiovascular disease burden in the United States. *Health Aff (Millwood)* 2007;26:38-48
- Pipeline insight: Antidyslipidemics – The power of two. Market Research.com, 2005. Available from: <http://www.marketresearch.com/search/results.asp?sid=97553088-405814861-501265549&query=antidyslipidemic&vendid=72&publisher=Datamonitor> [Last accessed 26 February 2008]
- Ford I, Murray H, Packard CJ, et al. Long-term follow-up of the West of

Emerging antidiabetic drugs

- Scotland Coronary Prevention Study. *N Engl J Med* 2007;357:1477-86
22. Shepherd J, Cobbe SM, Ford I, et al. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. *N Engl J Med* 1995;333:1301-7
 23. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial. *Lancet* 2002;360:7-22
 24. Cannon CP, Braunwald E, McCabe CH, et al. Intensive versus moderate lipid lowering with statins after acute coronary syndromes. *N Engl J Med* 2004;350:1495-504
 25. Superko HR, King S 3rd. Lipid management to reduce cardiovascular risk: a new strategy is required. *Circulation* 2008;117:560-8; discussion 68
 26. Mays ME, Dujovne CA. Pleiotropic effects: should statins be considered an essential component in the treatment of dyslipidemia? *Curr Atheroscler Rep* 2008;10:45-52
 27. Balk EM, Lau J, Goudas LC, et al. Effects of statins on nonlipid serum markers associated with cardiovascular disease: a systematic review. *Ann Intern Med* 2003;139:670-82
 28. LaRosa JC, Grundy SM, Waters DD, et al. Intensive lipid lowering with atorvastatin in patients with stable coronary disease. *N Engl J Med* 2005;352:1425-35
 29. Girman CJ, Rhodes T, Mercuri M, et al. The metabolic syndrome and risk of major coronary events in the Scandinavian Simvastatin Survival Study (4S) and the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS). *Am J Cardiol* 2004;93:136-41
 30. Baigent C, Keech A, Kearney PM, et al. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet* 2005;366:1267-78
 - **A landmark meta-analysis that showed a 12% reduction in all-cause mortality for each 1 mmol/l of reduction in plasma LDL cholesterol concentration.**
 31. Ahn SC. Neuromuscular complications of statins. *Phys Med Rehabil Clin North Am* 2008;19:47-59
 32. Sueta CA, Massing MW, Chowdhury M, et al. Undertreatment of hyperlipidemia in patients with coronary artery disease and heart failure. *J Card Fail* 2003;9:36-41
 33. Hoeg JM. Pharmacologic and surgical treatment of dyslipidemic children and adolescents. *Ann NY Acad Sci* 1991;623:275-84
 34. Melian EB, Plosker GL. Colesevelam. *Am J Cardiovasc Drugs* 2001;1:141-6; discussion 47-8
 35. Leren TP, Hjerjmann I, Berg K, et al. Effects of lovastatin alone and in combination with cholestyramine on serum lipids and apolipoproteins in heterozygotes for familial hypercholesterolemia. *Atherosclerosis* 1988;73:135-41
 36. Vega GL, Grundy SM. Treatment of primary moderate hypercholesterolemia with lovastatin (mevinolin) and colestipol. *JAMA* 1987;257:33-8
 37. Einarsson K, Ericsson S, Ewerth S, et al. Bile acid sequestrants: mechanisms of action on bile acid and cholesterol metabolism. *Eur J Clin Pharmacol* 1991;40(Suppl 1):S53-8
 38. Bays HE, Davidson M, Jones MR, et al. Effects of colesvelam hydrochloride on low-density lipoprotein cholesterol and high-sensitivity C-reactive protein when added to statins in patients with hypercholesterolemia. *Am J Cardiol* 2006;97:1198-205
 39. Chapman MJ. Fibrates in 2003: therapeutic action in atherogenic dyslipidaemia and future perspectives. *Atherosclerosis* 2003;171:1-13
 40. Valasek MA, Clarke SL, Repa JJ. Fenofibrate reduces intestinal cholesterol absorption via PPAR-alpha-dependent modulation of NPC1L1 expression in mouse. *J Lipid Res* 2007;48:2725-35
 41. Davidson MH, Armani A, McKenney JM, et al. Safety considerations with fibrate therapy. *Am J Cardiol* 2007;99:3C-18C
 42. Bottorff MB. Statin safety and drug interactions: clinical implications. *Am J Cardiol* 2006;97:27C-31C
 43. Frick MH, Elo O, Haapa K, et al. Helsinki Heart Study: primary-prevention trial with gemfibrozil in middle-aged men with dyslipidemia. Safety of treatment, changes in risk factors, and incidence of coronary heart disease. *N Engl J Med* 1987;317:1237-45
 44. Rubins HB, Robins SJ, Collins D, et al. Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol. Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial Study Group. *N Engl J Med* 1999;341:410-8
 45. Secondary prevention by raising HDL cholesterol and reducing triglycerides in patients with coronary artery disease: the Bezafibrate Infarction Prevention (BIP) study. *Circulation* 2000;102:21-7
 46. Keech A, Simes RJ, Barter P, et al. Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. *Lancet* 2005;366:1849-61
 47. Saha SA, Kizhakepunnur LG, Bahekar A, et al. The role of fibrates in the prevention of cardiovascular disease – a pooled meta-analysis of long-term randomized placebo-controlled clinical trials. *Am Heart J* 2007;154:943-53
 48. Davis HR, Veltri EP. Zetia: inhibition of Niemann-Pick C1 Like 1 (NPC1L1) to reduce intestinal cholesterol absorption and treat hyperlipidemia. *J Atheroscler Thromb* 2007;14:99-108
 49. Knopp RH, Gitter H, Truitt T, et al. Effects of ezetimibe, a new cholesterol absorption inhibitor, on plasma lipids in patients with primary hypercholesterolemia. *Eur Heart J* 2003;24:729-41
 50. Dujovne CA, Erttinger MP, McNeer JF, et al. Efficacy and safety of a potent new selective cholesterol absorption inhibitor, ezetimibe, in patients with primary hypercholesterolemia. *Am J Cardiol* 2002;90:1092-7
 51. Ballantyne CM, Houry J, Notarbartolo A, et al. Effect of ezetimibe coadministered with atorvastatin in 628 patients with primary hypercholesterolemia: a prospective, randomized, double-blind trial. *Circulation* 2003;107:2409-15
 52. McKenney JM, Farnier M, Lo KW, et al. Safety and efficacy of long-term co-administration of fenofibrate and ezetimibe in patients with mixed hyperlipidemia. *J Am Coll Cardiol* 2006;47:1584-7
 53. Florentin M, Liberopoulos EN, Elisaf MS. Ezetimibe-associated adverse effects: what the clinician needs to know. *Int J Clin Pract* 2008;62:88-96

54. Malik S, Kashyap ML. Niacin, lipids, and heart disease. *Curr Cardiol Rep* 2003;5:470-6
55. Tunaru S, Kero J, Schaub A, et al. PUMA-G and HM74 are receptors for nicotinic acid and mediate its anti-lipolytic effect. *Nat Med* 2003;9:352-5
56. Wise A, Foord SM, Fraser NJ, et al. Molecular identification of high and low affinity receptors for nicotinic acid. *J Biol Chem* 2003;278:9869-74
57. Soga T, Kamohara M, Takasaki J, et al. Molecular identification of nicotinic acid receptor. *Biochem Biophys Res Commun* 2003;303:364-9
58. Taylor AJ, Lee HJ, Sullenberger LE. The effect of 24 months of combination statin and extended-release niacin on carotid intima-media thickness: ARBITER 3. *Curr Med Res Opin* 2006;22:2243-50
59. Taylor AJ, Sullenberger LE, Lee HJ, et al. Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol (ARBITER) 2: a double-blind, placebo-controlled study of extended-release niacin on atherosclerosis progression in secondary prevention patients treated with statins. *Circulation* 2004;110:3512-7
- **A study showing the benefit of niacin on altering the progression of a vascular surrogate marker (carotid intima-media thickness).**
60. Clofibrate and niacin in coronary heart disease. *JAMA* 1975;231:360-81
61. Charlton-Menys V, Durrington PN. Squalene synthase inhibitors: clinical pharmacology and cholesterol-lowering potential. *Drugs* 2007;67:11-6
62. Burnett JR, Huff MW. Cholesterol absorption inhibitors as a therapeutic option for hypercholesterolaemia. *Expert Opin Investig Drugs* 2006;15:1337-51
63. Tardif JC, Gregoire J, L'Allier PL, et al. Effects of the acyl coenzyme A:cholesterol acyltransferase inhibitor avasimibe on human atherosclerotic lesions. *Circulation* 2004;110:3372-7
64. Rudel LL, Lee RG, Cockman TL. Acyl coenzyme A: cholesterol acyltransferase types 1 and 2: structure and function in atherosclerosis. *Curr Opin Lipidol* 2001;12:121-7
65. Wetterau JR, Lin MC, Jamil H. Microsomal triglyceride transfer protein. *Biochim Biophys Acta* 1997;1345:136-50
66. Wetterau JR, Aggerbeck LP, Bouma ME, et al. Absence of microsomal triglyceride transfer protein in individuals with abetalipoproteinemia. *Science* 1992;258:999-1001
67. Liao W, Hui TY, Young SG, et al. Blocking microsomal triglyceride transfer protein interferes with apoB secretion without causing retention or stress in the ER. *J Lipid Res* 2003;44:978-85
68. Wetterau JR, Gregg RE, Harrity TW, et al. An MTP inhibitor that normalizes atherogenic lipoprotein levels in WHHL rabbits. *Science* 1998;282:751-4
69. Tall AR. Plasma cholesteryl ester transfer protein. *J Lipid Res* 1993;34:1255-74
70. Koizumi J, Mabuchi H, Yoshimura A, et al. Deficiency of serum cholesteryl-ester transfer activity in patients with familial hyperalphalipoproteinaemia. *Atherosclerosis* 1985;58:175-86
71. Inazu A, Brown ML, Hesler CB, et al. Increased high-density lipoprotein levels caused by a common cholesteryl-ester transfer protein gene mutation. *N Engl J Med* 1990;323:1234-8
72. Morehouse LA, Sugarman ED, Bourassa PA, et al. Inhibition of CETP activity by torcetrapib reduces susceptibility to diet-induced atherosclerosis in New Zealand White rabbits. *J Lipid Res* 2007;48:1263-72
73. Okamoto H, Yonemori F, Wakitani K, et al. A cholesteryl ester transfer protein inhibitor attenuates atherosclerosis in rabbits. *Nature* 2000;406:203-7
74. Nissen SE, Tardif JC, Nicholls SJ, et al. Effect of torcetrapib on the progression of coronary atherosclerosis. *N Engl J Med* 2007;356:1304-16
75. Kastelein JJ, van Leuven SI, Burgess L, et al. Effect of torcetrapib on carotid atherosclerosis in familial hypercholesterolemia. *N Engl J Med* 2007;356:1620-30
76. Bots ML, Vissers FL, Evans GW, et al. Torcetrapib and carotid intima-media thickness in mixed dyslipidaemia (RADIANCE 2 study): a randomised, double-blind trial. *Lancet* 2007;370:153-60
77. Barter PJ, Caulfield M, Eriksson M, et al. Effects of torcetrapib in patients at high risk for coronary events. *N Engl J Med* 2007;357:2109-22
- **A study showing that torcetrapib was not beneficial for reducing cardiovascular events.**
78. Tall AR, Yvan-Charvet L, Wang N. The failure of torcetrapib: was it the molecule or the mechanism? *Arterioscler Thromb Vasc Biol* 2007;27:257-60
79. Kuivenhoven JA, de Grooth GJ, Kawamura H, et al. Effectiveness of inhibition of cholesteryl ester transfer protein by JTT-705 in combination with pravastatin in type II dyslipidemia. *Am J Cardiol* 2005;95:1085-8
80. Segrest JR, Li L, Anantharamaiah GM, et al. Structure and function of apolipoprotein A-I and high-density lipoprotein. *Curr Opin Lipidol* 2000;11:105-15
81. Navab M, Hama SY, Cooke CJ, et al. Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density lipoprotein: step 1. *J Lipid Res* 2000;41:1481-94
82. Plump AS, Scott CJ, Breslow JL. Human apolipoprotein A-I gene expression increases high density lipoprotein and suppresses atherosclerosis in the apolipoprotein E-deficient mouse. *Proc Natl Acad Sci USA* 1994;91:9607-11
83. Garber DW, Datta G, Chaddha M, et al. A new synthetic class A amphipathic peptide analogue protects mice from diet-induced atherosclerosis. *J Lipid Res* 2001;42:545-52
84. Navab M, Anantharamaiah GM, Hama S, et al. Oral administration of an ApoA-I mimetic Peptide synthesized from D-amino acids dramatically reduces atherosclerosis in mice independent of plasma cholesterol. *Circulation* 2002;105:290-2
85. Ou J, Wang J, Xu H, et al. Effects of D-4F on vasodilation and vessel wall thickness in hypercholesterolemic LDL receptor-null and LDL receptor/apolipoprotein A-I double-knockout mice on Western diet. *Circ Res* 2005;97:1190-7
86. Ou J, Ou Z, Jones DW, et al. L-4F, an apolipoprotein A-I mimetic, dramatically improves vasodilation in hypercholesterolemia and sickle cell disease. *Circulation* 2003;107:2337-41
87. Li X, Chyu KY, Faria Neto JR, et al. Differential effects of apolipoprotein A-I-mimetic peptide on evolving and established atherosclerosis in apolipoprotein E-null mice. *Circulation* 2004;110:1701-5

Emerging antidiabetic drugs

88. Franceschini G, Sirtori CR, Capurso A 2nd, et al. A-Milano apoprotein. Decreased high density lipoprotein cholesterol levels with significant lipoprotein modifications and without clinical atherosclerosis in an Italian family. *J Clin Invest* 1980;66:892-900
89. Nissen SE, Tsunoda T, Tuzcu EM, et al. Effect of recombinant ApoA-I Milano on coronary atherosclerosis in patients with acute coronary syndromes: a randomized controlled trial. *JAMA* 2003;290:2292-300
- **Proof-of-concept that apoA-I Milano could affect a vascular surrogate marker (coronary plaque on intravascular ultrasound).**
90. Brown BG, Zhao XQ, Chait A, et al. Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. *N Engl J Med* 2001;345:1583-92
91. Kastelein JJ, Akdim F, Stroes ES, et al. Simvastatin with or without ezetimibe in familial hypercholesterolemia. *N Engl J Med* 2007;356:1620-30
92. Goff DC Jr, Gerstein HC, Ginsberg HN, et al. Prevention of cardiovascular disease in persons with type 2 diabetes mellitus: current knowledge and rationale for the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial. *Am J Cardiol* 2007;99:4i-20i
93. Dias N, Stein CA. Antisense oligonucleotides: basic concepts and mechanisms. *Mol Cancer Ther* 2002;1:347-55
94. Hannon GJ, Rossi JJ. Unlocking the potential of the human genome with RNA interference. *Nature* 2004;431:371-8
95. Lambert G. Unravelling the functional significance of PCSK9. *Curr Opin Lipidol* 2007;18:304-9
96. Kwon HJ, Lagace TA, McNutt MC, et al. Molecular basis for LDL receptor recognition by PCSK9. *Proc Natl Acad Sci USA* 2008
97. Abifadel M, Varret M, Rabes JP, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. *Nat Genet* 2003;34:154-6
98. Cohen JC, Boerwinkle E, Mosley TH Jr, et al. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N Engl J Med* 2006;354:1264-72
99. Zimmermann TS, Lee AC, Akinc A, et al. RNAi-mediated gene silencing in non-human primates. *Nature* 2006;441:111-4
100. Akdim F, Harchaoui KE, Stroes ES, et al. Antisense apolipoprotein B-100 as novel treatment for hypercholesterolemia: focus on ISIS 301012. *Future Lipidol* 2007;2:387-93
101. Kramer W, Corsiero D, Girbig F, et al. Rabbit small intestine does not contain an annexin II/caveolin 1 complex as a target for 2-azetidinone cholesterol absorption inhibitors. *Biochim Biophys Acta* 2006;1758:45-54
102. A study evaluating the safety and efficacy of AVE5530 (4 weeks) in patients with mild to moderate hypercholesterolemia, NCT00440154, 2008. Available from: <http://www.clinicaltrials.gov> [Last accessed 16 March 2008]
103. Cuchel M, Bloedon LI, Szapary PO, et al. Inhibition of microsomal triglyceride transfer protein in familial hypercholesterolemia. *N Engl J Med* 2007;356:148-56
- **A human trial of an inhibitor of microsomal triglyceride transfer protein.**
104. Aggarwal D, West KL, Zern TL, et al. JTT-130, a microsomal triglyceride transfer protein (MTP) inhibitor lowers plasma triglycerides and LDL cholesterol concentrations without increasing hepatic triglycerides in guinea pigs. *BMC Cardiovasc Disord* 2005;5:30
105. Homma Y, Kobayashi T, Yamaguchi H, et al. Specific reduction of plasma large, light low-density lipoprotein by a bile acid sequestering resin, cholebine (MCI-196) in type II hyperlipoproteinemia. *Atherosclerosis* 1997;129:241-8
106. Asami T, Uchiyama M. Treatment of children with familial hypercholesterolemia with colestilan, a newly developed bile acid-binding resin. *Atherosclerosis* 2002;164:381-2
107. Yamakawa T, Takano T, Utsunomiya H, et al. Effect of colestimide therapy for glycemic control in type 2 diabetes mellitus with hypercholesterolemia. *Endocr J* 2007;54:53-8
108. Shneider BL. Intestinal bile acid transport: biology, physiology, and pathophysiology. *J Pediatr Gastroenterol Nutr* 2001;32:407-17
109. Zhang EY, Phelps MA, Cheng C, et al. Modeling of active transport systems. *Adv Drug Deliv Rev* 2002;54:329-54
110. Kitayama K, Nakai D, Kono K, et al. Novel non-systemic inhibitor of ileal apical Na⁺-dependent bile acid transporter reduces serum cholesterol levels in hamsters and monkeys. *Eur J Pharmacol* 2006;539:89-98
111. Wasan KM, Najafi S, Wong J, et al. Assessing plasma lipid levels, body weight, and hepatic and renal toxicity following chronic oral administration of a water-soluble phytostanol compound, FM-VP4, to gerbils. *J Pharm Pharm Sci* 2001;4:228-34
112. Looije NA, Risovic V, Stewart DJ, et al. Disodium ascorbyl phytostanyl phosphates (FM-VP4) reduces plasma cholesterol concentration, body weight and abdominal fat gain within a dietary-induced obese mouse model. *J Pharm Pharm Sci* 2005;8:400-8
113. Lukic T, Wasan KM, Zamfir D, et al. Disodium ascorbyl phytostanyl phosphate reduces plasma cholesterol concentrations and atherosclerotic lesion formation in apolipoprotein E-deficient mice. *Metabolism* 2003;52:425-31
114. Ramaswamy M, Yau E, Wasan KM, et al. Influence of phytostanol phosphoryl ascorbate, FM-VP4, on pancreatic lipase activity and cholesterol accumulation within Caco-2 cells. *J Pharm Pharm Sci* 2002;5:29-38
115. Wasan KM, Yau E, Boulanger KD, et al. Effects of disodium ascorbyl phytostanol phosphates (FM-VP4) on cholesterol accumulation within rat intestinal cells. *AAPS PharmSci* 2003;5:E6
116. Forbes Medi-Tech, Inc., FM-VP4: a new class of cholesterol lowering drugs, 2008. Available from: <http://www.forbesmedi.com/s/FM-VP4.asp> [Last accessed 6 February 2008]
117. Farrell B, Godwin J, Richards S, et al. The United Kingdom transient ischaemic attack (UK-TIA) aspirin trial: final results. *J Neurol Neurosurg Psychiatry* 1991;54:1044-54
118. Lewington S, Clarke R, Qizilbash N, et al. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet* 2002;360:1903-13
119. Krishna R, Anderson MS, Bergman AJ, et al. Effect of the cholesterol ester transfer protein inhibitor, anacetrapib, on lipoproteins in patients with dyslipidaemia

- and on 24-h ambulatory blood pressure in healthy individuals: two double-blind, randomised placebo-controlled phase I studies. *Lancet* 2007;370:1907-14
120. de Grooth GJ, Kuivenhoven JA, Stalenhoef AF, et al. Efficacy and safety of a novel cholesteryl ester transfer protein inhibitor, JTT-705, in humans: a randomized phase II dose-response study. *Circulation* 2002;105:2159-65
 121. Lai E, De Lepeleire I, Crumley TM, et al. Suppression of niacin-induced vasodilation with an antagonist to prostaglandin D2 receptor subtype 1. *Clin Pharmacol Ther* 2007;81:849-57
 122. Treatment of HDL to reduce the incidence of vascular events HPS2-THRIVE. *ClinicalTrials.gov*, 2007. Available from: <http://www.clinicaltrials.gov/ct/show/NCT00461630?order=6> [Last accessed 15 February 2008]
 123. Stamler CJ, Breznan D, Neville TA, et al. Phosphatidylinositol promotes cholesterol transport in vivo. *J Lipid Res* 2000;41:1214-21
 124. Burgess JW, Boucher J, Neville TA, et al. Phosphatidylinositol promotes cholesterol transport and excretion. *J Lipid Res* 2003;44:1355-63
 125. Burgess JW, Neville TA, Rouillard P, et al. Phosphatidylinositol increases HDL-C levels in humans. *J Lipid Res* 2005;46:350-5
 126. Pandey NR, Renwick J, Misquith A, et al. Linoleic acid-enriched phospholipids act through peroxisome proliferator-activated receptors alpha to stimulate hepatic apolipoprotein A-I secretion. *Biochemistry* 2008;47:1579-87
 127. Liponex, Inc. CRD5 Science. Available from: <http://www.liponex.ca/science/crd5.html> [Last accessed 6 February 2008]
 128. Liponex updates CRD5 status and corporate strategy (News: December 13, 2007). Available from: <http://www.liponex.ca/ut/pdf/12.13.07%20Liponex%20Updates%20CRD5%20Status.pdf> [Last accessed 6 February 2008]
 129. Gillard BK, Courtney HS, Massey JB, et al. Serum opacity factor unmasks human plasma high-density lipoprotein instability via selective delipidation and apolipoprotein A-I desorption. *Biochemistry* 2007;46:12968-78
 130. Alnylam presents first ever non-human primate data with an RNAi therapeutic targeting PCSK9 showing significant and durable reductions in LDL cholesterol levels, 2007. Available from: [http://phx.corporate-ir.net/phoenix.zhtml?c=148005&p=irol-newsArticle2&ID=1059699&highlight=D=1059699&highlight=D=1059699](http://phx.corporate-ir.net/phoenix.zhtml?c=148005&p=irol-newsArticle2&ID=1059699&highlight=D=1059699&highlight=D=1059699&highlight=D=1059699) [Last accessed 26 February 2008]
 131. Bristol-Myers Squibb enters cardiovascular disease collaboration with Isis Pharmaceuticals, 2007. Available from: <http://ir.isispharm.com/releasedetail.cfm?ReleaseID=241674> [Last accessed 28 February 2008]
 132. Akdim F, Stroes ES, Kastelein JJ. Antisense apolipoprotein B therapy: where do we stand? *Curr Opin Lipidol* 2007;18:397-400
 133. Kastelein JJ, Wedel MK, Baker BF, et al. Potent reduction of apolipoprotein B and low-density lipoprotein cholesterol by short-term administration of an antisense inhibitor of apolipoprotein B. *Circulation* 2006;114:1729-35
 134. Isis reports positive results of phase 2 study of ISIS 301012 in heterozygous FH patients, 2007. Available from: <http://ir.isispharm.com/releasedetail.cfm?ReleaseID=267339> [Last accessed 3 March 2008]
 135. Isis Pharmaceuticals updates its positive phase 2 data for ISIS 301012 in familial hypercholesterolemia patients, 2007. Available from: <http://ir.isispharm.com/releasedetail.cfm?ReleaseID=244309> [Last accessed 28 February 2008]
 136. Isis reports new data for mipomersen in routine high cholesterol patients and provides cumulative safety summary, 2007. Available from: <http://ir.isispharm.com/releasedetail.cfm?ReleaseID=275455> [Last accessed 28 February 2008]

Affiliation

Rebecca L Pollex, Tisha R Joy & Robert A Hegele[†]
[†]Author for correspondence
 University of Western Ontario,
 Blackburn Cardiovascular Genetics Laboratory,
 Robarts Research Institute,
 100 Perth Drive, Room 406,
 London, Ontario, N6A 5K8 Canada,
 Tel: +1 519 663 3461; Fax: +1 519 663 3037;
 E-mail: hegele@robarts.ca

