Review

Advances in the Study of the Antiatherogenic Function and Novel Therapies for HDL

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Abstract: The hypothesis that raising high-density lipoprotein cholesterol (HDL-C) levels could improve the risk for cardiovascular disease (CVD) is facing challenges. There is multitudinous clear clinical evidence that the latest failures of HDL-C-raising drugs show no clear association with risks for CVD. At the genetic level, recent research indicates that steady-state HDL-C concentrations may provide limited information regarding the potential antiatherogenic functions of HDL. It is evident that the newer strategies may replace therapeutic approaches to simply raise plasma HDL-C levels. There is an urgent need to identify an efficient biomarker that accurately predicts the increased risk of atherosclerosis (AS) in patients and that may be used for exploring newer therapeutic targets. Studies from recent decades show that the composition, structure and function of circulating HDL are closely associated with high cardiovascular risk. A vast amount of data demonstrates that the most important mechanism through which HDL antagonizes AS involves the reverse cholesterol transport (RCT) process. Clinical trials of drugs that specifically target HDL have so far proven disappointing, so it is necessary to carry out review on the HDL therapeutics.

1. Introduction

Hyperlipidemia, a risk factor for atherosclerosis (AS), is a serious consequence for people who have experienced coronary heart disease, stroke, and artery stenosis disease. AS, which is the leading cause of cardiovascular disease (CVD), is responsible for 50% of all mortality in many developed countries [1]. A persistent increase in circulating low-density lipoprotein cholesterol (LDL-C) levels in the body is one of the most important causes for the initiation and progression of AS [2]. It has been shown in epidemiological studies and clinical trials that LDL-C levels are directly related to the rate at which CVD events occur [2,3]. There are abundant antilipemic agents (Table 1) on the market, but the meta-analysis of intervention trials has shown that a per mmol/L decrease in LDL-C is associated with an approximate 22% reduction of CVD events and a 10% reduction of all-cause mortality [3]. There is a wealth of evidence showing that statins that play a beneficial role in lowering LDL-C levels and are efficient in preventing first cardiovascular events. However, a large residual disease burden remains, even in patients treated with a high dose of statins and other CVD risk-modifying interventions [1]. Furthermore, treatment with statins may lead to a significant increase of muscle toxicity and liver transaminase, and may not be suitable for all CVDs. Investigators are eagerly searching for novel therapeutic targets. Because high-density lipoprotein cholesterol (HDL-C) levels, a predictor of major cardiovascular events in patients, are inversely associated with risk for CVD [2,4], strategies for increasing HDL levels have been explored as a new approach for combatting CVD, which may overcome the significant residual cardiovascular risk remaining after treatment with statins [4]. However, a recent genetic analysis failed to show a causal association between genetically raised plasma HDL cholesterol levels and risk for myocardial infarction [5]. In addition, nicotinic acid and fibrates currently are used to increase HDL-C levels, both of which have some weaknesses (e.g., uricosuria, increased glucose tolerance and flushing for nicotinic acid and problematic pharmacokinetic interactions for fibrates) that impose restrictions on their use [6]. Moreover, raising HDL-cholesterol by the cholesteryl-ester transfer protein (CETP) inhibitor did not play its expected role in protection from CVD [7,8]. The goals of previous HDL therapy are currently being reassessed due to numerous difficulties validating the hypothesis. Therefore, HDL structure, composition and function are the focus of ongoing research efforts, as they might provide more valuable information than steady-state HDL-cholesterol levels [9]. From the study of the structure and composition of HDL, novel strategies for the treatment of hyperlipemia-induced AS are being developed. Apolipoprotein A-I (apoA-I) is the major structural protein component of HDL particles [10], and it has been shown to play a pivotal role in reverse cholesterol transport (RCT) [11]. ApoA-I has also been shown to exert direct anti-inflammatory effects [12]. Stimulating increased synthesis of endogenous apoA-I may be a promising approach. Regarding the function of HDL, the atheroprotective activities of HDL particles are attributed to their central role in anti-inflammatory, antithrombotic, and antioxidant processes and their ability to improve endothelial function [13,14]. In addition, RCT is currently understood as the physiological process by which cholesterol in peripheral tissues is transported by HDL to the liver for excretion in the bile and feces [15]. This process is complex and beneficial, and it has been known as a widely accepted mechanism for the protective effect of HDL.

Table 1. Drugs of anti-hyperlipidemia in the current market. In recent years many cholesterol-lowering drugs are commonly used on the market, which mainly includes stains, fibrates, nicotinic acids, cholesterol absorption inhibitor and polyene unsaturated fatty acids.

Classification	Drug	Mechanism	TC	TG	VLDL	LDL	HDL	Advantage	Disadvantage
Stains	Lovastatin	As inhibition of HMG CoA reductase, reduce cholesterol synthesis				↓		The advantage of these drugs is a low incidence of adverse reaction, and can be suitable for a variety of hypercholesterolemia except hypertriglyceridemia , is a lipid-lowering drug with rapid development in recent years	Gastrointestinal symptoms and rash, and the residual risk
	Atorvastatin		↓	↓		↓	↑		
Fibrates	Gemfibrozil	The drug can increase Lp(a) Lipase activity to remove VLDL, TG; Thus reducing VLDL and TG, TC and LDL can also be reduced		↓	↓	↓	↑	These drugs do not cause the increase of diabetic insulin resistance or affect the control of blood sugar, therefore this kind of drugs is the first choice for treating the diabetic patients with hyperlipidemia	Gastrointestinal reactions, allergic reaction, due the drugs increase the concentration of cholesterol in bile, it may cause gallstones, occasional eyesight obstacle and hematological abnormalities
	Fenofibrate			↓					
Nicotinic Acids	Niacin; Inositol Aluminum	The drug can prevent fat decomposition, prevent free fatty acid formation, inhibit synthesis of TG and secretion of VLDL in liver	↓	↓		↓	↑	Cheap, and it is the only lipid-lowering drug can also reduce risk and mortality of cardiovascular disease	It is not suitable for diabetes patients, overdose adverse reactions (toxic to the liver, high blood sugar) has a high incidence common adverse reactions are skin flushing, itching, rash
	Aluminum Nicotinate					↓			
Cholesterol Absorption Inhibitor	Ezetimibe	The drug can combine with bile acid to block the bile acid absorption; Prompte the translation of the cholesterol into bile in the gallbladder, then bile binding to drug is eliminated from the body	↓ ↓	↓		↓	↑	This kind of medicine is recognized as TC lowering drugs, when treats together with statins, the risk of accidental heart disease related to decrease the occurrence of 50% or more	The common adverse reactions are mild nausea and abdominal distension, constipation, therefore, it is not suitable for intestinal diseases and intractable constipation patients
Polyene Unsaturated Fatty Acids	Duoxikang	This drug can combine with total cholesterol to be ester; Then promotes the degradation of bile acid excreted along with the bile, decreases plasma total cholesterol concentration	↓	↓ ↓		↓		These drugs in combination with statins can reduce the level of TG, and play an effective role in the prevention and treatment of coronary heart disease	This kind of medicaments is easy to be oxidized to atherogenic substance, has inhibitory effect on platelet aggregation, so it needs to be used with caution
	Ecosapeatanolic acid								

This paper is aimed at looking for a better HDL biomarker to predict AS precisely and explaining how HDL functions in detail, then providing an overview of novel therapies that target HDL to enhance HDL's ability to reduce residual cardiovascular risk in the population. Finally, we carry out an assessment of a natural drug that has been linked to HDL, which represents a unique field in the treatment of AS that warrants exploration.

2. HDL and AS

AS can be considered to be a form of chronic inflammation, beginning with increased endothelial permeability of monocytes under the influence of adhesion molecules [16]. It promotes endocytosis of oxidized LDL into the arterial wall and intima, which can be devoured by intimal macrophages to become foam cells. This is a key process in the development of atherosclerotic plaque. Along with an increase in macrophages and foam cells, the plaque becomes unstable. This progression can ultimately lead to the development of complex lesions, or plaques, that protrude into the arterial lumen. Plaque rupture and thrombosis result in the acute clinical complications of myocardial infarction and stroke [17,18]. Cardiovascular diseases are the leading cause of death and illness in developed countries, with AS being the most important contributor [13]. In recent years, there has been growing interest in finding a cardiovascular biomarker that provides prognostic and predictive information to act as a tool to influence treatment strategies [19]. The use of HDL-related indexes as biomarkers is undergoing predictive tests, and they represent the greatest promise of this technology and the shortest and most effective path to furthering our understanding.

2.1. HDL as the Biomarker for AS

2.1.1. HDL-C

Because trends relating high plasma levels of HDL-C and decreased incidence of CVD endpoints were observed in prospective epidemiological studies conducted in several countries many years ago, HDL-C levels have served as a significant predictor for relieving AS in the clinic. There have been several attempts to increase HDL-C levels using pharmacological intervention [3,17]. HDL-C levels have been reported to increase upon chronic administration of fibrates as agonists of the peroxisome proliferator activated receptor α (PPARα) in animals and in humans. Niacin is the first antidyslipidemic agent identified that has been available for patients to raise HDL-C. However, recent data from a Global Health outcomes (AIM-HIGH) trial with niacin did not show any significant improvement in the cardiovascular risk over statins, which are commonly used drugs for lipid management [20,21]. Recently a few attempts have been made to inhibit CETP to raise the levels of HDL-C. Torcetrapib, a cholesterol ester transferase inhibitor, reliably increased HDL (without countervailing effects on LDL) but ultimately increased cardiovascular mortality. Dalcetrapib, a new CETP inhibitor, also has unintended effects, and the outcomes are the same as those for torcetrapib [7,8]. As a result, CETP inhibitors continue to face safety hurdles, and no significant clinical benefits have resulted from these pharmacological regimens. A recent report suggested that the sole increase of HDL-C in humans would not necessarily improve the rate of cardiovascular events (all-cause mortality, coronary heart disease mortality, non-fatal myocardial infarction, and stroke) [22–25]. In justification for the use of

statins in prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER), on-treatment HDL-C was not predictive of residual risk among statin-treated individuals, whereas HDL-C was predictive among those taking placebo [26]. Similarly, on-treatment apoA-I and triglycerides were not predictive of residual risk [27]. For these reasons, the use of the HDL-C biomarker as a surrogate end point remains a difficult and distant goal.

2.1.2. HDL Particle Size

Dyslipidemia may influence enzymes and transfer proteins needed for lipoprotein particle remodeling. HDL particle size may differ in the number of molecules of apo and free cholesterol, esterified cholesterol, and phospholipids content on the lipoprotein surface [28,29] due to the changes in activity of Lecithin-cholesterol acyltransferase (LCAT), CETP, phospholipid transfer protein (PLTP) plasma transfer proteins, and enzymes (e.g., lipoprotein lipase (LPL), hepatic lipase (HL)) in the process. Calculated indices and the evaluation of lipoprotein particle size have been widely used to predict cardiovascular risk. An evaluation of the association between HDL particle size and risk of incident coronary artery disease (CHD) in apparently healthy volunteers indicated that decreased HDL particle size is associated with an adverse cardiometabolic risk profile [30]. Small HDL particle size was also associated with an increased CHD risk, which resulted from the different lipid transfer ability [31]. Another study found that the high TG-low HDL cholesterol dyslipidemia, which is found in viscerally obese subjects and characterized by hyperinsulinemia, was strongly correlated with reduced HDL particle size [32]. However, the latest finding is not consistent with previous conclusions. The HDL particle size (nm) values were not different between the dyslipidemia, normolipidemic and dyslipdemic groups without treatment with lipid-lowering drugs [33]. In addition, several studies using GGE-measured HDL size have reported that patients with CHD tend to have smaller HDL particles and that large HDL particles may protect against the development of AS. There are also other studies that show that only small HDL particles are atheroprotective, this is supported by the analysis of HDL subfraction data and carotid artery disease (CAAD) [34]. It is found that among HDL-C, HDL_2, and HDL_3, HDL_3 best predicts CAAD risk, and the remaining phenotypes do not add significant predictive power [34]. Although public opinions about HDL size are divergent, the propagation rate and maximal diene formation during total HDL oxidation correlated significantly with HDL mean particle size [30,32]. It is clear that proper HDL particle size may have advantages in the reduction of CVD events. In the future, we believe that HDL particle size will play an essential role in predicting the risk of AS.

2.1.3. HDL Particles Concentration (HDL-P)

Given the extreme heterogeneity of HDL, measuring the content of HDL particles will, at best, only partially reflect the potential role of HDL in cardiovascular risk assessment and therapeutic drug development. In this regard, the HDL-P may be a better marker of residual vascular risk after potent statin therapy in the JUPITER trial than chemically measured HDL-C or apoA-I [35]. HDL-P may be a promising metric of HDL that is more independent of other metabolic and lipoprotein risk factors than HDL cholesterol or HDL size [36]. In the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk study, HDL-P was inversely associated with CVD, consistent with the above-mentioned observations [37]. However, in the Women's Health Study, HDL-P was not associated

with incident CVD events among healthy low-risk women in contrast with inverse associations seen for HDL size and HDL-C [38]. Moreover, an emerging outcome from a determinant of residual risk among statin-treated individuals suggests that overall HDL-P is not as important as the sub-type of HDL-P, which contains the lysosphingolipid sphingosine-1-phosphate (S1P) [36]. This conclusion is supported by the evidence that it is the S1P content of HDL, as opposed to the HDL particle itself, which is responsible for the beneficial antiatherothrombotic, anti-inflammatory, antioxidant, antiglycation, and profibrinolytic activities of these lipoproteins [36]. At present, two techniques, nuclear magnetic resonance (NMR) and ion mobility analysis (IMA) have been described for quantifying HDL-P in human plasma. However, the final HDL-P yield determined using these two methods differ by up to >5-fold. With the progress of science and technology, the accurate and reasonable use of ^{1}H NMR [39] and application of termed calibrated ion mobility analysis (calibrated IMA) [40] will greatly determine the feasibility of HDL-P as biomarker.

2.1.4. Other Biomarkers

The HDL-associated Apolipoprotein M (apoM) plays a role in the anti-atherogenic function in a variety of atherosclerotic models, making it interesting to investigate whether apoM is a predictor of AS [41–44]. With the discovery of apoM as an important carrier of S1P in HDL particles, it forms a new basis for investigations of apoM biology. An improved understanding of the role of the apoM/S1P axis in relation to AS may unravel new avenues for treatment or use of biomarkers for disease or risk evaluation [45].

The expression of serum amyloid A (SAA) protein was greater in the patients with AS, and it can be explained by high serum levels of SAA-predicted AS [45–49]. By means of two-dimensional electrophoresis (2-DE) coupled with mass spectrometry (MS) analysis on plasma-purified VLDL, LDL and HDL fractions from patients undergoing carotid endarterectomy, increased levels of acute-phase SAA (AP SAA) in all lipoprotein fractions helped to identify AP SAA as a potential marker of advanced carotid AS [50]. Studies in both mice and humans suggest that proinflammatory HDL may be a novel biomarker for increased risk of AS in patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) [51]. Moreover, proinflammatory HDL-associated hemoglobin (Hb) was also found to be differentially associated with HDL from coronary heart disease patients compared with healthy controls [52,53]. Hb contributes to the proinflammatory nature of HDL in mouse and human models of AS and may serve as a novel biomarker for AS [52–54].

In addition, HDL has a wide range of functions, some of which are independent of its cholesterol content. Tests for HDL function as a biomarker may be useful for diagnosing the risk for patients with CHD. As a result, we elaborate the functions of HDL in the next section with the purpose of identifying the best biomarker of AS.

2.2. HDL in Anti-Atherosclerosis

HDLs have several well-documented functions with the potential to protect against AS. These functions include an ability to promote the efflux of cholesterol from macrophages in the artery wall, inhibit the oxidative modification of LDLs, inhibit vascular inflammation, inhibit thrombosis, promote endothelial repairing, promote angiogenesis, enhance endothelial function, improve diabetic control, and inhibit

hematopoietic stem cell proliferation. Some of these functions have been mechanistically linked to the well-known ability of HDLs to induce the activation of cellular cholesterol efflux pathways, whereas many other functions of HDLs are independent of the effects of HDLs on cellular cholesterol homeostasis. Below, we will discuss the most relevant functions of HDLs for AS more specifically (Figure 1).
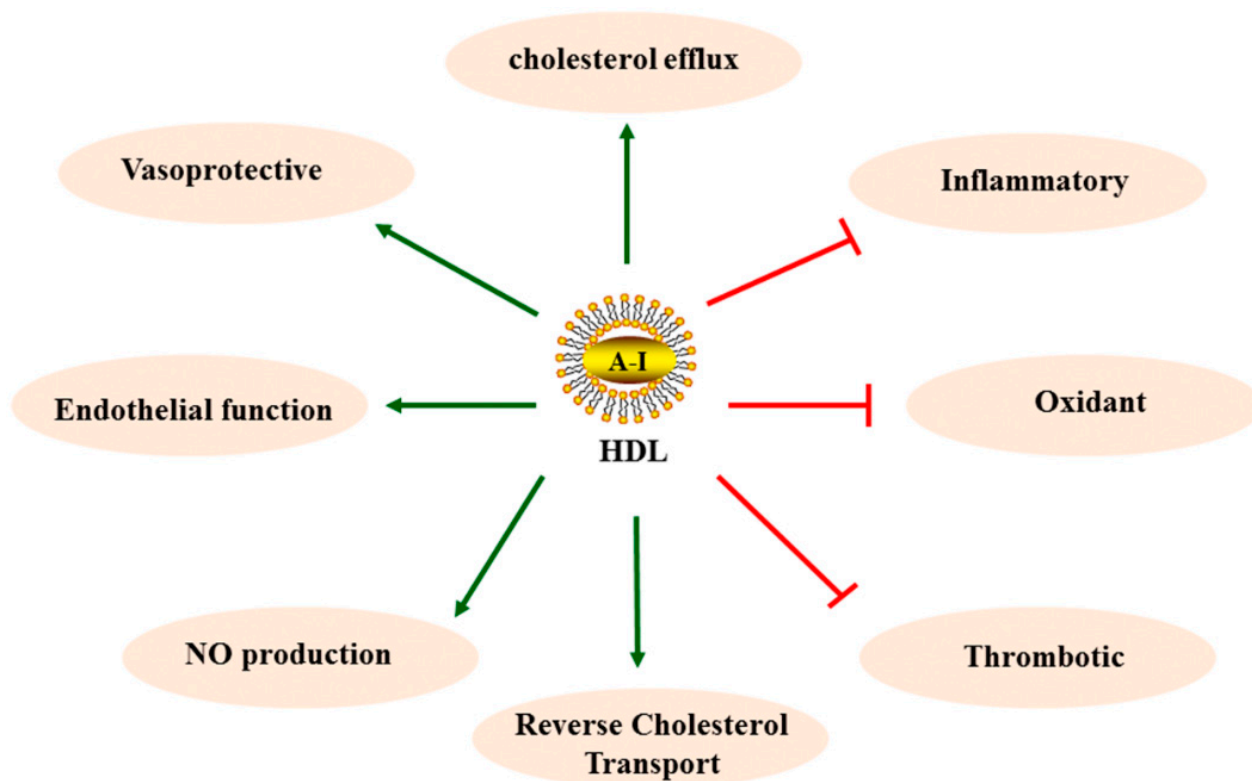

Figure 1. HDL antiatherosclerotic functions. ⬅ (promotion), ⊣ (inhibition).

2.2.1. Reverse Cholesterol Transport

In many studies, we found that the ability of HDL to promote cholesterol efflux from macrophages was strongly and inversely associated with both subclinical AS and obstructive coronary artery disease [55,56]. The efflux of cholesterol from a variety of cell types, including macrophages, to HDLs in the extracellular space is mediated by two distinct processes. One is the efflux of cholesterol induced by a specific cellular transporter [57–62], and the other is passive aqueous diffusion of cholesterol from cell membranes to HDLs [63,64]. Then, excess cholesterol from peripheral tissues will be transported back to the liver for excretion in the bile and ultimately the feces by HDL via a process called RCT. We herein would like to describe the importance of RCT in detail, and therefore, the multiplex RCT pathway has been described in the following four parts (Figure 2) [65].
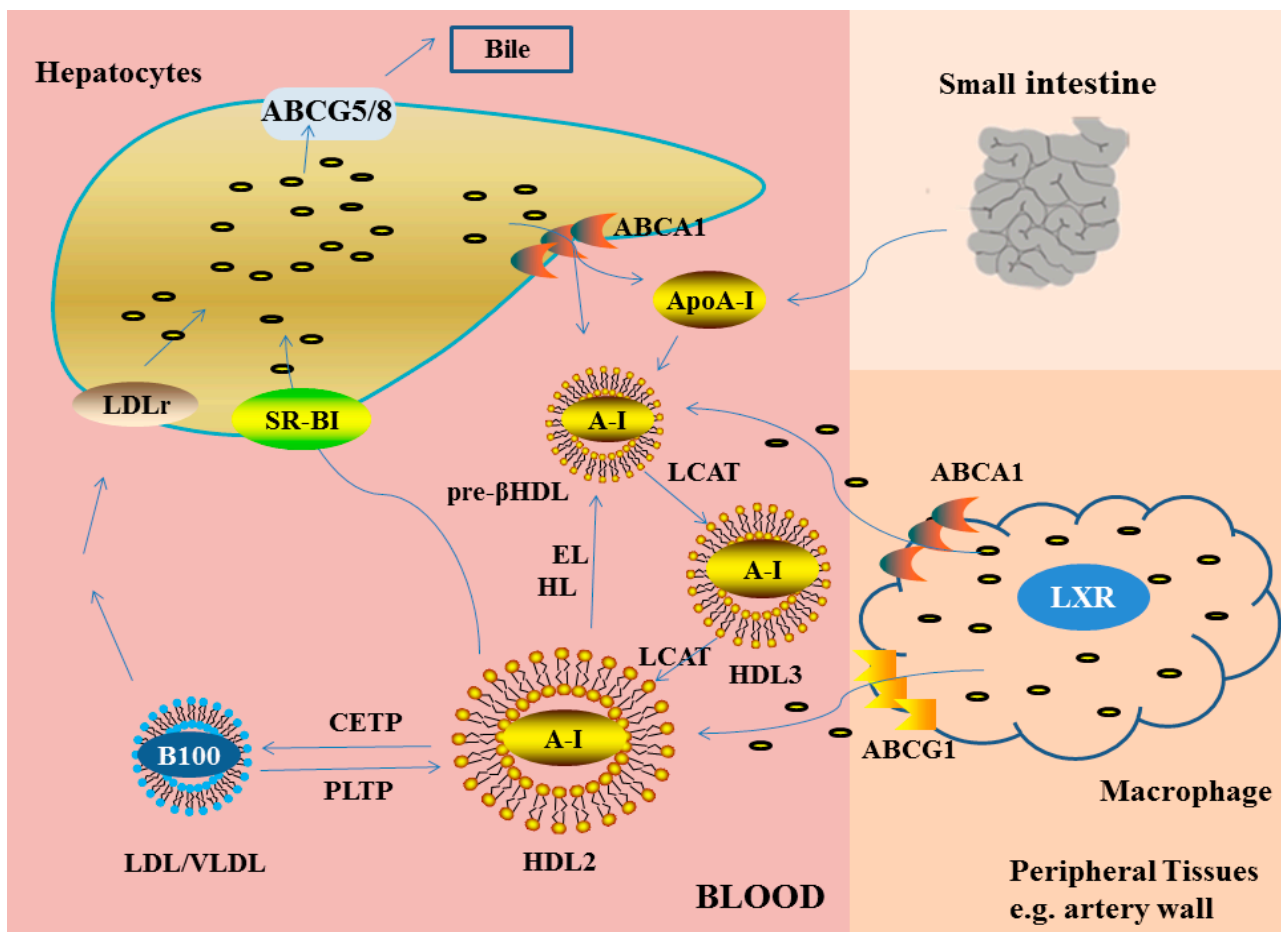
Figure 2. HDL in reverse cholesterol transport pathway. Lipid-poor apoA-I also promotes the efflux of free cholesterol from macrophages via ABCA1. LCAT esterifies free cholesterol to cholesteryl esters to form mature HDL, which promotes cholesterol efflux from macrophages via the ABCG1 transporter, as well as from other peripheral tissues by processes not fully defined. In macrophages, both ABCA1 and ABCG1 are regulated by Liver X receptors (LXR). Mature HDL can transfer its cholesterol to the liver directly via scavenger receptor class B type I (SR-BI) or indirectly via CETP-mediated transfer to ApoB-containing lipoproteins, with subsequent uptake by the liver via the LDL-r. Hepatic cholesterol can be excreted directly into the bile as cholesterol or after conversion to bile acids and, unless reabsorbed by the intestine, is ultimately excreted in the feces. HL, EL, and PLTP, play an indispensable role in remodeling HDL, thus, the RCT pathway is dependent on interaction with them [9].

Part One: The Formation of Nascent HDL

Lipid-free or lipid-poor apoA-I secreted in the liver can mediate cellular efflux of both cholesterol and phospholipids from macrophages via the ATP-binding cassette transporter A1 (ABCA1) and gather them on the surface of pre-β HDL, resulting in the rapid lipidation of apoA-I to generate mature α HDL, which is called nascent HDL particles [66,67]. Once loss of two ABCA1 genes occurs in the body, such as in the case of Tangier disease, the result is abnormally low levels of serum HDL-C, an acceleration of the accumulation of cholesterol in peripheral tissues, and the formation of premature

AS [68]. The mature HDL particles can then serve as acceptors of cholesterol provided by ATP-binding cassette transporter G1 (ABCG1) [69] or SR-BI [70]. ABCG1 is another member of the ATP-binding cassette family that plays a critical role in the efflux of cellular phospholipid (PL) and free cholesterol (FC) to mature HDL, but not pre-β HDL. By intraperitoneal injection of mice with [^{3}H]-cholesterol-labeled J774 macrophages with either increased or reduced ABCG1 expression and primary macrophages lacking ABCG1 expression, followed by measurement of the macrophage-derived [^{3}H]-cholesterol levels in plasma and feces [71], it was shown that macrophages lacking ABCG1 cause damage and secretion of FC and excessive cholesterol accumulation in macrophages. Therefore, ABCG1 mediates cholesterol efflux [72]. In an investigation of extracellular cholesterol microdomains that form during the enrichment of macrophages with cholesterol, extracellular cholesterol microdomains did not develop when ABCG1-deficient mouse bone marrow-derived macrophages were enriched with cholesterol [69]. Many studies have demonstrated that ABCA1 and ABCG1 play a role in many aspects of cholesterol efflux from macrophages [69,71–73].

Liver X receptors (LXR), the members of the steroid nuclear receptor superfamily, are oxysterol-activated transcription factors that, after heterodimerization with the 9-*cis*-retinoic acid receptor (RXR), bind to specific LXR response elements (LXREs), thus regulating the expression of target genes involved in intra- and extracellular lipid metabolism [74]. In part by modulating cholesterol efflux from macrophages to apoA-I and HDL, LXRs induce the direct target genes ABCA1 and ABCG1/ABCG4 to promote reverse cholesterol transport. The oxidation of steroids from FC can activate LXR and regulate gene expression of ABCA1 and ABCG1 to strengthen the peripheral tissue cholesterol secretion. Meanwhile, the LXRs are also readily oxidized by PPARα. PPARαcontrols lipid and glucose metabolism in several tissues and cell types including liver, heart, kidneys, adipose tissue and macrophages. PPARα-activation suppresses chylomicron and increases HDL production by enterocytes [75,76]. In addition, its agonists promote secretion of macrophage cholesterol via stimulating expression of ABCA1 and LXR to increase reverse cholesterol transport [77].

Part Two: The Process of Cholesterol Esterified by LCAT

LCAT, a lipoprotein-associated enzyme, is a key player in the RCT pathway. LCAT has two different catalytic activities that account for its ability to esterify cholesterol. One is phospholipase A2 activity, and the other is its transesterification activity. It requires apoA-I and, to a lesser degree, other apolipoproteins, which most likely activate LCAT by modifying the presentation of its substrates, namely, phospholipids and cholesterol, on the surface of lipoproteins [78]. After FC efflux to pre-β HDL (the nascent, discoidal-shaped HDL), cholesterol in HDLs may be esterified by the enzymatic activity of LCAT. The LCAT reaction occurs in two steps. After binding to a lipoprotein, LCAT cleaves the fatty acid in the sn-2 position of phosphatidylcholine and transfers it onto a serine residue. Next, the fatty acid is transesterified to the 3-β-hydroxyl group on the A-ring of cholesterol to form cholesterol ester. Cholesteryl esters formed by LCAT partition, which are more hydrophobic than free cholesterol, are transferred from the surface of lipoproteins to the hydrophobic core. This process converts pre-β HDL to HDL2 and HDL3 particles, which are the major HDL species found in plasma and which represent larger, spherical-shaped α-migrating forms of HDL. LCAT is important in the process of RCT by generating a gradient of free cholesterol from cells

to HDL [79]. This effect of LCAT prevents the back exchange of cholesterol by passive diffusion from HDL to peripheral cells and thus is believed to promote net removal of cholesterol from peripheral cells to HDL. Without ongoing esterification of cholesterol, the capacity of HDL to remove and bind additional cholesterol would eventually be diminished. Two lipases, endothelial lipase (EL) and HL, are the complete opposite of LCAT in HDL metabolism. HL and EL are members of the triglyceride lipase family, which also includes LPL [80,81]. EL has high phospholipase A1 activity and remodels HDL into small particles, whereas HL is more effective in hydrolyzing triglycerides [82]. Although HL causes a remodeling of HDL into smaller particles, it also promotes the release of lipid-poor apoA-I [83]. The combined functions of HL and EL have a significant effect on plasma HDL-C levels [84–87]. HL- and EL-deficient mice were studied to demonstrate that the magnitude of macrophage-derived [^{3}H] cholesterol in feces was increased, which promoted macrophage-to-feces RCT, and its ability to protect against LDL oxidation was enhanced [88].

Part Three: The Exchange of CE (Cholesteryl Esters) Mediated by CETP

CETP is a hydrophobic glycoprotein that is synthesized in several tissues but mainly in the liver. It facilitates the exchange of cholesteryl esters and triglycerides between HDL and apoB-containing particles (LDL, IDL, VLDL) and represents a major branching point for RCT [7,8]. This results in the recycling of cholesterol and an increasing attenuation in blood circulation, with the potential to go back into the artery wall. Two major observations were made using many novel methods, such as innovative X-ray crystallographic, electron microscopic (EM), and bioinformatics observations: (i) CETP connects with or forms bridges between two lipoproteins, e.g., HDL and LDL, with resultant neutral lipid transfer; and (ii) CETP appears to contain a hydrophobic tunnel along its entire long axis capable of neutral lipid transfer. These observations may clearly explain the relationships between CETP interactions with lipoproteins and the lipid transfer processes [89]. Most CEs derived from LCAT do not return to the liver via the HDL SR-B1 pathway but, rather, through more atherogenic pathways. CETP mediates the transfer of most CE from HDL to VLDL or to other more atherogenic intermediate-density lipoproteins and remnants, and the transfer of triglycerides from VLDL-1 to HDL results in larger, relatively triglyceride-enriched LDL species [90]. Transfer of CE from HDL directly to LDL by CETP could also be antiatherogenic if the LDL is cleared by the liver LDL receptor. Another transfer protein in this part of RCT, PLTP, transfers phospholipids between VLDL and HDL [91]. PLTP is one of the main modulators of plasma HDL size, composition and function [92] and one of the major modulators of HDL metabolism in plasma [93]. The level of HDL or HDL production is dramatically decreased in PLTP$^{-/-}$ mice [94,95], and PLTP deficiency attenuates plaque accumulation in different atherosclerotic models. This indicates that PLTP plays an important role in atherogenesis, and its function goes well beyond that of transferring phospholipids between lipoprotein particles [96,97].

Part Four: Catabolism of HDL Cholesterol in Biliary Pathway

After efflux, cholesterol in HDLs may be esterified by the enzymatic activity of LCAT whereupon HDLs can deliver the excess cholesterol from peripheral cells back to the liver in in distinct ways: HDL cholesteryl esters, but not the protein component of HDLs, are selectively taken up into the liver

via SR-BI. Ultimately, cholesterol is excreted from the liver into the bile, either directly as free cholesterol or after conversion into bile acids, and eliminated from the body via the feces. In humans, HDL-C can be metabolized by the liver via another pathway: CETP exchanges of HDL CE for triglycerides in apoB-containing lipoproteins, followed by hepatic uptake mediated by LDL-r. An LDL-r deficiency in mice substantially decreases selective HDL CE uptake by liver and adrenals. Thus, LDL-r expression has a substantial impact on HDL metabolism in mice [98].

Via the classic RCT pathway, excessive cholesterol collected from peripheral tissues, which is delivered back to the liver, is followed by biliary secretion and elimination via the feces. In addition to the traditional RCT-mediated biliary pathway, in the last few years, direct trans-intestinal excretion of plasma-derived cholesterol (TICE) was shown to contribute substantially to fecal neutral sterol (FNS) excretion in mice, describing the transport of cholesterol from blood to the intestinal lumen directly via enterocytes. The TICE pathway was called a nonhepatobiliary-related route, which has been shown to have a high degree of correlation with the main contributors Niemann-Pick disease, type C1/2 (NPC1/2), ABCG5/G8, LDL-r, and LXR [99–101]. The application of PPAR δ agonist and LXR agonists, have been shown to stimulate the process of TICE [102]. In the RCT pathway, HDL plays an important role. In contrast, there is evidence from animal experiments that HDL plays an essential role in TICE [103].

2.2.2. Antioxidant Properties of High-Density Lipoprotein

LDL is one of the main causes of AS. Oxidation of LDL yields a more pro-atherogenic particle, and numerous studies have found that HDLs are capable of impeding oxidative changes in LDL. HDL exhibits potent antioxidant activity, which may arise from synergy in the inactivation of oxidized LDL lipids by enzymatic and nonenzymatic mechanisms, in part reflecting distinct intrinsic physicochemical properties [104]. The anti-oxidative properties of HDL critically involve HDL-associated enzymes, such as paraoxonase 1 (PON1), lipoprotein-associated phospholipase A2 (Lp-PLA2), and LCAT, which have been reported to hydrolyze oxidized phospholipids into lyso-phosphatidylcholine [105–109]. In addition, HDL carries glutathione selenoperoxidase, which can reduce lipid peroxide (LOOH) to the corresponding hydroxides and thereby detoxify them [110]. ApoA-I can remove oxidized lipids from LDL, suggesting that HDL can function as an acceptor of oxidized lipids. In cell culture experiments, apoA-I removes lipids from LDL and thereby makes LDL resistant to vascular cell-mediated oxidation and prevents oxidized LDL-induced monocyte adherence and chemotaxis [108]. Other HDL apolipoproteins, such as apoA-II, apoA-IV, apoE, and apoJ, also function as antioxidants *in vitro*.

2.2.3. HDL in the Endothelium

Traditionally, the endothelium has been considered to be an inert component of the vessel wall. Injury to the endothelium results in deleterious alterations of endothelial physiology, also referred to as endothelial dysfunction, which represents a key early step in the development of an atherosclerotic lesion and is implicated in the malignant development that follows. It has become evident that HDL from healthy subjects can exert direct potential atheroprotective effects on endothelial cells and can positively affect several endothelial functions in the regulation of vascular tone, inflammation [111]

and endothelial oxidant stress [112], which is associated with activation of NO synthesis by HDL. NO is an endothelium-derived signaling molecule that activates guanylate cyclase in vascular smooth muscle cells (SMCs) to induce relaxation, which is generated by a constitutive eNOS. Notably, HDLs also beneficially affect the vasculature by promoting endothelial cell survival. In endothelial cells, HDLs still maintain anti-thrombotic functions, mainly inhibiting thrombin-induced tissue factor, mediating the extrinsic coagulation pathway, and stimulating the activation of the anticoagulant proteins C and S.

2.2.4. Anti-inflammatory Properties of HDL

HDL and apoA I are believed to protect against the development of AS, in part, which is closely related to their anti-inflammatory function. The anti-inflammatory properties of HDL encompass suppression of macrophage inflammatory cytokine production and inhibition of the expression of endothelial cell adhesion molecules that promote the entry of monocytes and neutrophils into arteries. HDLs potently block murine experimental endotoxinemia, indicating that HDLs bind lipopolysaccharides (LPS), which leads to lower systemic proinflammatory cytokine levels and improved survival rates [113]. Up-regulation of adhesion molecules (E-selectin, ICAM-1, and VCAM-1) and secretion of pro-inflammatory mediators (monocyte chemoattractant 1, MCP-1) lead to the activation of the vascular endothelium and induce AS.

3. Novel Therapies with HDL as the Key Components

3.1. Novel Pharmacotherapeutic Strategies in Increasing HDL

3.1.1. Infusions of Special HDL

One approach to increase the serum levels of HDL is to infuse reconstituted HDL (rHDL) or recombinant HDL particles into the circulation rather than by increasing HDL indirectly by modulating HDL metabolism. CSL-111 is a reconstituted HDL-particle comprising both human apoA-1 and soybean phosphatidylcholine. CSL-112 is being evaluated in phase II trials to improve the weakness of CSL-111. There is a benefit of CSL-111/CSL-112 infusions to increase HDL-C levels and up-regulation of cellular cholesterol efflux in patients with acute coronary syndrome (ACS). Some trials have demonstrated that infusions of recombinant HDL-containing apoA-I Milano and CER-001 significantly reduce or stabilize mean plaque atheroma volume and still exert greater anti-inflammatory effects in LDL-r$^{-/-}$ mice [114–116]. However, most clinical studies have demonstrated that rapid clearance of CER-001 leads to a requirement for repeated administration due to the inability to achieve effective plasma concentrations [117]. Pegylation of apoA-I in rHDL markedly increases its plasma half-life and enhances its antiatherogenic properties *in vivo* [118].

3.1.2. Autologous Delipidated HDL

Another novel approach to HDL therapeutics is to raise the levels of HDL particles by intravenous infusion with the use of autologous delipidated HDL. Preclinical evaluation of selective delipidated HDL in a nonhuman primate model of dyslipidemia achieved a significant 6.9% reduction in aortic

atheroma volume, as assessed by intravenous ultrasound (IVUS) [119]. In a small clinical study in which 28 patients with ACS received five weekly infusions of delipidated HDL, the decreased total atheroma volume by 5.2% from baseline may be associated with selectively increased preβ-HDL [120].

3.2. Novel Pharmacotherapeutic Strategies in Four Steps of RCT

3.2.1. First Part of RCT

ApoA-I Mimetic Peptides

ApoA-I mimetics, which are short synthetic peptides, mimic the amphipathic a-helix of apoA-I. Because the first apoA-I mimetic consisted of 18 amino acids, additional improved peptides were generated by increasing the number of phenylalanine residues on the hydrophobic face (referred to as 2F, 3F, 4F, 5F, 6F, and 7F) of the polypeptide. Two different types of apoA-I mimetic peptide 4F were used in clinical trials, including L-4F (the 4F peptide synthesized from L-amino acids) and D-4F (the 4F peptide synthesized from all D-amino acids). Compared with high plasma L-4F levels not improving HDL anti-inflammatory function, a single dose of D-4F was found to have a significant effect on the inflammatory index of HDL with modest oral bioavailability [10]. ABCA1-independent lipid efflux played a major role in this aspect of RCT. Recently, 5A, an asymmetric bihelical peptide based on 2F, had increased ABCA1-dependent cholesterol efflux and decreased hemolysis compared with its parent compound [121]. Furthermore, ATI-5261 synthetic peptide, like apoA-I mimetic peptides, successfully enhances cholesterol efflux from macrophages and reduces aortic AS, which exerts its effects similar to that of the role of HDL in reverse cholesterol transport in mice. Reservelogix-208 (RVX-208), an apoA-I upregulator that increases endogenous synthesis of apoA-I, has a unique mechanism of action related to epigenetically influencing the accessibility of apoA-I gene by the transcription machinery. Encouragingly, there is a progressive discovery that nanolipid particles containing multivalent peptides (HDL-like nanoparticles) promoted efficient cellular cholesterol efflux and were functionally superior to those derived from monomeric apoA-I mimetic peptides [122].

Regulators of ABCA1 and ABCG1

LXR and miR-33 (similars: miR-758, miR-26 and miR-106b) have been demonstrated to regulate macrophages ABCA1 and ABCG1. The activation of the former can upregulate the expression of two genes, which promote macrophage cholesterol efflux and augment intestinal HDL generation, while the opposite is true of the latter. In the animal experiments, LXR agonists exhibit a significant decrease of atherosclerotic plaque formation and exert anti-inflammatory characteristics [123]. The negative effects caused by hepatic LXR agonists, such as a fatty liver, will be overcome by more intestine-specific LXRα agonists that are still under preclinical investigation [124]. As an alternative to LXR agonism, gene- silencing approaches involving microRNA 33 are being explored to control gene expression at the post-transcriptional level [125]. An antisense oligonucleotide has been developed that effectively silences miR-33 (miRNA-33a/b) and increases ABCA1 and ABCG1 expression in macrophages. Studies in nonhuman primates showed that antisense oligonucleotides to miRNA-33a/b that induce ABCA1 expression as well as the expression of other proteins involved in fatty acid metabolism result

in a 50% increase in HDL-C and a decrease in VLDL [126]. Recent studies in PPARγ-activated macrophages demonstrated that treatment with miR-613 leads to inhibition of cholesterol efflux by downregulating LXRα and ABCA1 [127]. This study sheds new insights into the possibility that the miR-613 inhibitor may serve as a novel therapy for the treatment of cholesterol metabolism diseases. Similarly, PPAR agonists also increase ABCA1 gene expression and target the LXR gene. PPARα (K-877), PPARγ (INT131) and PPARα/δ (GFT505) modulators play pivotal roles in ABCA1 gene expression and apoA-I secretion, and increase HDL-C in patients with hyperlipidemia [126,127]. Growing evidence suggests that MBX-8025, a promising PPARδ agonist targeting the ABCA1 gene and LXRα gene, with a greater binding potency, is under development. Additional dual PPARα/γ and PPARβ/δ agonists that are under development may play an important role in RCT pathway [78].

3.2.2. Second Part of RCT

LCAT Agonists

LCAT plays a major role in this part of RCT, and mediates the esterification of free cholesterol located at the surface of lipoprotein particles. Several drug development approaches have recently been initiated for modulating LCAT activity. Recombinant human LCAT (rhLCAT) efficiently promotes the process of RCT, which induces a marked increase in HDL-C levels and the maturation of small preb-HDL into α-migrating particles in LCAT-deficient plasma [128]. Currently, recombinant LCAT (ACP-501) in Phase 1 clinical trials was shown to rapidly and substantially elevate the formation of cholesteryl esters, representing the promotion of RCT. It is reported that Apo AI-derived peptides present the ability to modulate LCAT activity, with a significant increase in 3H-cholesteryl ester production in a higher proportion at 0.3 mg/mL [129]. An early apoA-I mimetic peptide (ETC-642) promotes LCAT activation and has entered clinical development [130].

3.2.3. Third Part of RCT

CETP Inhibitor

Anacetrapib. The Determining Efficacy and Tolerability of Anacetrapib has shown a 138% increase in HDL-C, a 40% reduction in LDL-C, and a 36% decrease in Lp(a) of patients with CAD. This results from the ability of anacetrapib to inhibit both heterotypic and homotypic CE transfer. A large phase III study, Randomized EValuationof the Effects of Anacetrapib Through Lipid-modification (REVEAL), will be completed in January 2017 [131].

Evacetrapib. Evacetrapib has a similar structure and mechanism of action as torcetrapib but, based on IC_{50} values, appears to be a more potent CETP inhibitor than torcetrapib or anacetrapib [132]. It is the most recently developed CETP-inhibitor currently undergoing a phase III CVD outcome trial. In a phase II study, high doses of evacetrapib do not elevate blood pressure in rats and do not induce aldosterone or cortisol biosynthesis in a human adrenal cortical carcinoma cell line [133]. The Assessment of Clinical Effects of Cholesteryl Ester Transfer Protein Inhibition With Evacetrapib in Patients at a High-Risk for Vascular Outcomes (ACCELERATE) phase III CVD outcome trial is recruiting.

BAY 60-5521/TA-8995. BAY 60-5521 and TA-8995 are two potential inhibitors of CETP. The clinical results suggest that these two agents are safe and well tolerated, with effective inhibition of CETP activity and increased high HDL-C [134,135].

CETP ASO. Antisense oligonucleotide inhibitor of CETP (CETP ASO) is associated with reductions in CETP mRNA and also shows an enhanced effect on macrophage RCT. It is reported that the CETP ASO does not act through binding and inactivating CETP associated with the HDL particle. Instead, it specifically targets and degrades CETP mRNA. In hyperlipidemic, CETP transgenic, LDL-r$^{-/-}$ mice, CETP ASO provided comparable reductions in HDL cholesterol, decreases in CETP activity with an enhanced effect on macrophage RCT, and results in less accumulation of aortic cholesterol [136]. CETP ASO could produce a unique therapeutic profile, distinct from the current CETP drugs being evaluated in late-stage clinical trials.

3.2.4. Fourth Part of RCT

SR-BI Activators

SR-BI, the HDL receptor, is expressed on hepatocytes and facilitates selective absorption of cholesterol ester from HDL. The discovery and development of traditional drugs that can modulate SR-BI expression and/or activity are being investigated through high-throughput screening of chemical compound libraries. Endogenous or exogenous agents (such as natural products) and the detailed high-resolution molecular structure of SR-BI protein, the regulators of SR-BI activity, should provide new insights for the progress of non-genetic therapies to modulate HDL metabolism *in vivo*.

As an alternative to traditional drugs, gene therapeutics are being extensively explored. In general, miRNA-125a and miRNA-455 can bind to specific sites in the 3′ UTR of SR-BI mRNA and act as endogenous attenuators of SR-BI protein expression [137]. It is clear that activation of miRNA-125a and miRNA-455 shows a negative relationship with SR BI-mediated selective HDL uptake and SR-BI-supported steroid hormone synthesis. Therefore, inhibition of miRNA-125a and miRNA-455 may enhance SR-BI expression to alter the course of the atherogenic sequence by increasing plasma HDL-C flux. Furthermore, in a recent report, the level of SR-BI expression was repressed by miR-185, miR-96, and miR-223, individually, in HepG2 cells [138]. Inhibitions of these miRNAs were associated with increased SR-BI expression and reduced AS by facilitating RCT and cholesterol removal from the body.

3.3. Natural Drugs Associated with HDL at the Future Stage

Nature is an irrefutable source of inspiration for the modern man in many aspects. The observation and understanding of nature have allowed the development of new materials, new sources of energies, new drugs, *etc.* Specifically, natural products provide a great contribution to the development of new agents for the treatment of hyperlipidemic diseases. Compared with chemical drugs or other synthetically created products, natural products could more closely mimic physiological processes and may be more effective in treatment of cardiovascular diseases. There are many natural drugs for the treatment of AS associated with HDL, such as polysaccharide, sesamin, anthocyanins, 24(*S*)-Saringosterol and

many others. Chitosan and its related products, which are polysaccharides, are used to enhance natural products and may represent promising strategies in the near future.

3.3.1. Chitosan

Chitosan (CTS), the only positive alkaline polysaccharide extracted from shrimp or crab shells and exhibiting biocompatibility and a nontoxic nature, has the potential for hypolipidemic and weight loss activities. In 1980, the first article demonstrating that proper supplementation of chitosan to the diet seemed to be effective in lowering plasma cholesterol in rats was published [139]. And the meta-analysis by Baker *et al.*, based on six randomized placebo-controlled clinical trials in hypercholesterolemic patients has showed that chitosan only induced a decrease in total cholesterol level but without any significant changes in LDL-cholesterol, HDL-cholesterol or triglyceride concentrations [140]. Since then, the research of CTS in treatment of hyperlipemia has been extensively undertaken. It is reported that because CTS is a biodegradable carbohydrate polymer, it has been prepared in a variety of dosages for use in delivery systems in previous studies [141–143], and these new preparations were found to have a more effective capability of improving hyperlipidemia in rats [144,145].

3.3.2. Chitosan Oligosaccharides

Chitosan oligosaccharides (COS) are depolymerized products of chitosan by either chemical or enzymatic hydrolysis. COS is a polymer of glucosamine with a number of bioactive properties. Recent studies have suggested that COS inhibits adipogenesis and promotes RCT through altered expression of a number of key regulators of lipid metabolism, including leptin [146], SR-BI and CYP7A1. It is reported that COS plays a positive role in the RCT pathway *in vivo* and stimulates hepatic LDL-r expression, inducing the LDL cholesterol lowering. Furthermore, chitosan oligosaccharides have anti-inflammatory functions similar to that of HDL, which downregulates the expression of E-selectin, ICAM-1 and IL-8 induced by LPS in endothelial cells through blockade of p38 MAPK and PI3K/Akt signaling pathways [147,148].

4. Discussion

HDL particles have various effects *in vitro* and *in vivo* that may protect arteries from chemical or biological hazards or facilitate repair of injuries. Nevertheless, HDL has not yet been successfully exploited for therapy. One possible reason may be the complexity of HDL particles, resulting in the physiological heterogeneity that contributes to the antiatherogenic functions of HDL. Moreover, for more than 50 years, HDL-C has been known as an independent clinical biomarker of cardiovascular risk. However, the failures of drugs to increase HDL-C drive scientists to search for new biomarkers to replace it, such as HDL functions, HDL size, and HDL-P. Among these, HDL functions will be a reliable diagnostic biomarker for the identification, personalized treatment stratification, and monitoring of patients at increased cardiovascular risk. The functionality of HDLs has two important roles. First, it promotes reverse cholesterol transport; second, it modulates inflammation and oxidation. HDL-based interventions in promoting the RCT process may be more promising.

Meanwhile, some natural products with functions similar to that of HDL also play unique roles in the RCT process and will enter a new era in treatment of CVD in the near future (Table 2).

Table 2. Overview of Classes of HDL-based therapies. HDL-based therapies are an innovative approach against atherosclerosis. In this review, they can be summarized as the following three categories: strategies increasing HDL, strategies of RCT in four steps and natural drugs. ●: primary; ♦: secondary.

Strategies Increasing HDL		Strategies of RCT in Four Steps		Natural Drugs	
● Infusions of special HDL	♦ rHDL: CSL-111 CER-001 CSL-112	**● First part of RCT** ApoA-I mimetic peptides; ApoA-I upregulator LXR agonists; Mir-33; PPAR modulators	**● Second part of RCT** LCAT Agonists; ♦ rLCAT: ACP-501	● Polysaccharide:	Chitosan; Chitosan oligosaccharides
				● Anthocyanins	
● Autologous delipidated HDL	♦ CETP inhibitor:	**● Third part of RCT** Anacetrapib; Evacetrapib; BAY 60-5521; TA-8995; CETP ASO	**● Fourth part of RCT** ♦ SR-BI activators: Traditional drugs; Gene therapeutics	● Sesamin	
				● 24(*S*)-Saringosterol	
				● Others	

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Conflicts of Interests

The authors declare no conflict of interests.

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