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Commentary

A Translational Medicine perspective of the development of torcetrapib: Does the failure of torcetrapib development cast a shadow on future development of lipid modifying agents, HDL elevation strategies or CETP as a viable molecular target for atherosclerosis? A case study of the use of biomarkers and Translational Medicine in atherosclerosis drug discovery and development

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ABSTRACT

Although the relationship between HDL (high density lipoprotein) function and cardiovascular (CV) risk has been extensively explored, the premise that HDL elevation is linked to reduced CV risks and that high HDL cholesterol (HDL-C) might be a potential surrogate biomarker for reduced CV risk remains controversial. Substantial genetic, molecular, biochemical and preclinical evidence have raised the hope that HDL-C elevation via CETP inhibition might generate clinical benefits. However, four large-scale clinical trials with the CETP inhibitor torcetrapib failed to demonstrate benefits on CV clinical outcomes. Likewise, biomarkers that were supposed to predict vascular risk reduction provided disappointing results. The sad tale of torcetrapib development emphasizes the need for a paradigm shift from the conventional drug development mode to a biomarker-based Translational Medicine (TMed) strategy. Emergence of further CETP inhibitors encourage continued development of such compounds for cardiovascular risk management. However, there is a need to adopt biomarker-driven TMed strategies in target validation, target–compound interaction, pharmacodynamic activities, disease modification and patient selection to guide future drug development efforts. This commentary analyzes the issues surrounding the demise of torcetrapib and proposes a TMed-based road map towards successful development of new CETP inhibitors.

1. Introduction

The central importance of lipids and lipoproteins in atherogenesis and its clinical manifestations, myocardial infarction and sudden coronary death, has been accepted by pharmaceutical regulatory entities as reflected by in their willingness to register drugs that lower total and low density lipoprotein cholesterol (LDL-C) for treatment of certain patient populations prior to establishing morbidity and mortality benefits in large outcome studies [1–3]. The status of LDL-C as a "surrogate biomarker" for clinical benefit has allowed the development of LDL lowering drugs such as statins and Ezetimibe and their marketing over the past decade. Other lipids and lipoproteins such as triglyceride, HDL cholesterol and even fatty acids have been argued to play a pathophysiological role in atherosclerosis and metabolic syndrome and efforts to invent, develop and market agents that modify synthesis, absorption and metabolism of these lipids have yielded promising therapeutic candidates. However, the "license" to register agents that modify lipid fractions other than LDL has not been as yet granted by regulatory agencies (FDA/EMEA). The proposition that permitted regulatory agencies to take the stance

Abbreviations: TMed, Translational Medicine; MOA, mechanism of action.

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they did in respect to total cholesterol/LDL-C lowering registration is founded on the wealth of clinical evidence identifying LDL as a causative agent in atherogenesis.

Over the years there has been an accumulation of evidence that other plasma lipid components such as HDL cholesterol, triglycerides, fatty acids and specific subfractions of lipoproteins are also independent risk factors for atherosclerosis. In particular, the consistent association of low HDL cholesterol with increased incidence of cardiovascular events [4] has stimulated intense and innovative research aimed at understanding the genetic and biochemical basis of HDL metabolic regulation, its functions and the biochemical pharmacology of the HDL-C modifying agents [5]. A signal success was the identification of cholesteryl ester transfer protein (CETP) as a major regulatory enzyme system that governs plasma HDL levels. The discovery of compounds that potently and specifically inhibit the enzyme [6,7] generate substantial increases in plasma HDL-C [6,7] prompted the suggestion from some quarters that HDL elevation alone (by analogy with LDL lowering) could serve as sufficient evidence for registration of CETP inhibitors for treatment of cardiovascular risk associated with low HDL-C. The proponents of this argument pointed to a robust body of evidence including: (1) epidemiology that supports low HDL-C as an independent risk factor for CVD; (2) clinical evidence demonstrating that HDL-C or apolipoprotein A-I concentration predicts coronary events independent of LDL-C levels even in those on-statin therapy; (3) animal data showing the benefits of HDL manipulation and (4) molecular and biochemical evidence on the role of HDL in cholesterol efflux, possibly from the cells of atherosclerotic plaque [5]. In spite of this apparently coherent set of findings, regulatory agencies hesitated to grant plasma HDL-C elevation "surrogate biomarker" status and to allow torcetrapib to be registered for secondary prevention of CVD risk. Instead, outcome studies were requested to supplement the epidemiological and mechanistic data. The ensuing classical development program for torcetrapib registration was ultimately not successful and we offer it as an exemplar of what might have been achieved with a Translational Medicine (TMed)-based approach.

The negative outcome of the torcetrapib development program has had far reaching repercussions that cast doubt on the fundamental belief held by many in the scientific and medical community concerning that HDL was undoubtedly a 'negative' causal factor for CVD. Further, if CETP inhibition does not lead to reduced risk of atherosclerosis then the basis of the "reverse cholesterol" hypothesis must be questioned. The torcetrapib 'meltdown' has raised serious other questions regarding atherosclerosis disease biomarkers such as carotid artery intima-media thickness (cIMT) or other vascular imaging variables as predictors of CVD events. The purpose of the discussion which follows is not to negate the potential value of CETP as a target for HDL elevation strategies, nor to question the fundamental role of HDL in CVD risk. Rather, it aims to highlight the need for a systematic, evidence-based TMed approach in embarking on drug discovery and development in areas where there is insufficient understanding of target biology and the etiology of the disease under study.

2. Is low HDL a causal cardiovascular risk factor?

HDL structure, function and metabolism have been extensively explored since the discovery of the inverse relationship between circulating HDL-C levels and coronary heart disease (CHD) in the Framingham cohort [4]. This lipoprotein remains a strong negative predictor of CHD risk even when other lipid fractions show little association with risk, such as in the elderly [8]. A potential beneficial role of HDL raising therapy has been observed in many clinical trials (Table 1a). For example, pharmacological manipulation or infusion of reconstituted HDL/recombinant apoA-I (the major apolipoprotein component of HDL) offers therapeutic potential for coronary atherosclerosis or acute coronary syndrome [9–22]; findings that are echoed in preclinical animal models. The most convincing atheroprotective evidence comes from these preclinical studies in which infusion of HDL/recombinant HDL or overexpression of apoA-I, leads to significant reduction of atherosclerotic lesions [23,24].

Understanding how HDL is atheroprotective is critical for translation of the concept of HDL raising therapy into a pharmacological basis to treat humans. A variety of mechanisms have been postulated of which the best established is the pathway of "reverse cholesterol transport (RCT)" that is the metabolic route whereby excess tissue cholesterol is returned to the liver for excretion. HDL plays a critical role in facilitating cholesterol efflux from peripheral tissues and cholesterol loaded macrophages

Table 1a
Summary of beneficial role of HDL-based trials.

Study	Cohorts	Duration	Treatment	Outcomes	Mechanism	Refs.
Nissen et al.	47	5 weeks	ApoA-I_{Milano} vs. placebo	ApoA-I_{Milano} triggered regression in coronary atherogenesis	HDL-specific	[9]
ERASE	145	1 month	Reconstituted HDL vs. placebo	Reduced atheroma volume; decreased plaque characterization index and coronary score	HDL-specific	[10]
Coronary Drug Project	8341	15 years	Niacin/clofibrate vs. placebo	Reduced mortality rate in niacin arm	Niacin	[11]
Stockholm Ischemic Heart Disease Study	555	5 years	Niacin + clofibrate vs. placebo	Reduced mortality rate in niacin + clofibrate arm	Niacin	[12]
CLAS I	162	2 years	Niacin + clofibrate vs. placebo	Significant lesion regression	Niacin	[13]
CLAS II	162	4 years	Niacin + clofibrate vs. placebo	Significant lesion regression	Niacin	[14]
UCSF-SCOR	72	2 years	Niacin + colestipol ± lovastatin vs. placebo	Coronary atherogenesis regression	Niacin	[15]
ARBITER-2	167	1 year	Extended release-niacin + stable statin vs. placebo + stable statin	Reduced carotid IMT progression rate in non-insulin resistant cohorts in ER niacin arm	Niacin	[16]
ARBITER-3	130	2 years	Extended release-niacin + stable statin vs. placebo + stable statin	Advanced net regression in carotid IMT compared to ARBITER-2	Niacin	[17]
Helsinki Heart Study	4081	5 years	Gemfibrozil vs. placebo	Reduced CHD incidence gemfibrozil arm	Fibrates	[18]
VA-HIT	2531	5 years	Gemfibrozil vs. placebo	Reduced CHD death rate and non-fatal MI in gemfibrozil arm	Fibrates	[19]
Frick et al.	395	3 years	Gemfibrozil vs. placebo	Reduced progression of native and vein-graft atherosclerosis	Fibrates	[20]
BECAIT	92	5 years	Benzafibrate vs. placebo	Reduced coronary event rate and coronary atherosclerosis progression in benzafibrate arm	Fibrates	[21]
DAIS	731	3 years	Micronised fenofibrate vs. placebo	Reduced coronary atherosclerosis progression	Fibrates	[22]

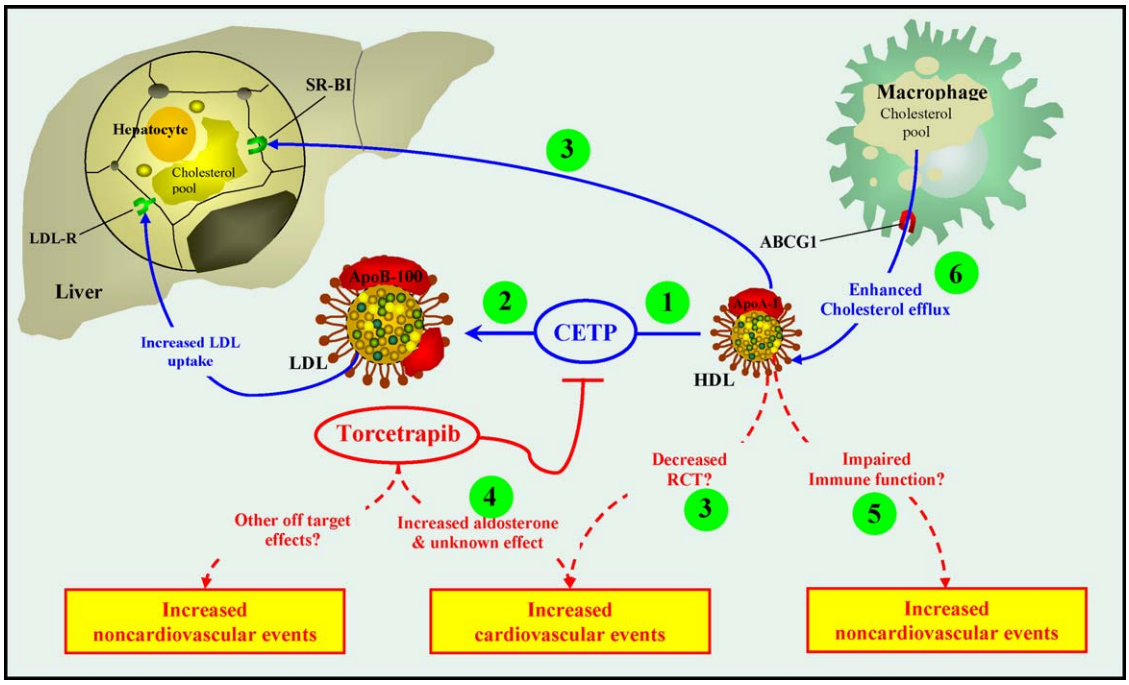


Fig. 1. Illustration of potential mechanisms of action (MOA) of torcetrapib. (1) Torcetrapib inhibits CETP and blocks cholesteryl esters transfer from apoA-I-containing lipoprotein (HDL) to apoB100-containing lipoprotein (LDL), giving rises to increased HDL concentrations as well as enlarged HDL particles and (2) decreased LDL concentrations; (3) CETP inhibition leads to reduced HDL cholesterol return to the liver via binding to scavenger receptor, class B, type I (SB-RI) in the process of cholesterol transport (RCT), which may result in increased cardiovascular events; (4) Torcetrapib has demonstrated "off-target" adverse effects elevating aldosterone and blood pressure, which could be responsible for increasing cardiovascular risks; (5) CETP inhibition may be associated with increased non-cardiovascular risks (e.g., tumor formation and impaired immune function); (6) CETP inhibition results in enhanced cholesterol efflux from macrophages via ABCG1. Steps 1–6 do not represent the temporal sequence of events.

through interaction of apolipoprotein A1 with ATP-binding cassette transporter A1 (ABCA1) on the cell surface or by the direct transfer of cellular cholesterol to the lipoprotein through the action of ABCG1. These transporters facilitate the passage of cholesterol from the cell to HDL where it is esterified by LCAT. The HDL cholesteryl ester so formed can be transferred to the liver for excretion via scavenger receptor-BI (SR-BI) (Fig. 1) or be exchanged into chylomicrons, VLDL or LDL by the agency of CETP. This pathway has the potential to prevent or ameliorate cholesterol deposition in arterial wall [25].

Other anti-atherosclerotic functions have been reported for the lipoprotein class. HDL possesses the capacity to reduce the atherogenicity of LDL [26], possibly through the action of HDL associated enzymes (e.g., paraoxonase, etc.) which mediate detoxification of oxidized-LDL [27] or non-enzymatic de-hydro-peroxidation. Further possible mechanisms include inhibition of oxidized LDL-induced transduction signals (e.g., cell surface adhesion molecule expression in endothelial cells) [28], anti-inflammation, anti-thrombotic and vascular protection properties [28–30]. However, the clinic relevance of these proposed mechanisms has not yet been established.

The HDL in human plasma is highly heterogeneous but the functional significance of its subfractions remains unclear. It can be classified to two subpopulations according to apolipoprotein composition: apolipoprotein A-I (apoA-I)-containing HDL and apoA-I/apoA-II-containing HDLs [31]. ApoA-I appears to account for most of the atheroprotective function of HDL while the second most abundant apolipoprotein, ApoA-II, has been reported to be neutral or even pro-atherogenic [32]. The phospholipid content of HDL has been reported to influence its cholesterol efflux ability, while minor lipids found in HDL such as lysosphingolipids, sphingosine 1 phosphate, sphingosylphosphorylcholine and lyso-sulfatide are bioactive lipids mediating anti-inflammatory effects exerted by HDL [33]. It should be noted that systemic inflammation may trigger conversion of "functional" HDLs to "dysfunctional" HDLs characterized by impaired ability to promote cholesterol efflux via reactive oxygen species-mediated ApoA-I modification [27].

HDL subfractions can also be distinguished by size and charge. Two-dimensional gel electrophoresis separates several categories of lipoprotein particles. HDL levels of the α-1 and preα-3 particles have been shown to exhibit an inverse association with CHD prevalence after adjusting for established CHD risk factors in Framingham Offspring study [34] and statins (with the exception of fluvastatin) have been found to perturb the HDL subpopulation profile by increasing the concentrations of large, cholesterol-rich LpA-I, α-1 and preα-1 HDL subpopulations [35]. Conceivably, the properties of HDL as a density class reflect the aggregate effects from all of its components. It remains to be determined which component possesses a particular anti-atherogenic function and how changes in subfraction profile should be interpreted.

Metabolic regulation plays an essential role in modulating HDL levels. The fractional catabolic rate of apoA-I and apoA-II is strongly inversely correlated with HDL-C levels [36], while the fractional catabolic rate as well as production rate of apoA-I are directly correlated with body mass index (BMI) in men [37]. In overweight-obese subjects, hypercatabolism of apoA-I is paralleled by an increased production of apoA-I, with HDL apoA-I production rate being the stronger determinant of apoA-I concentration [38]. Plasma HDL-C levels and metabolism are also influenced strongly by chylomicron/VLDL concentration [31]. Certain intervention affects HDL kinetics more than steady state plasma level [39].

3. Should HDL-C be considered as a viable surrogate biomarker for drug registration prior to clinical outcome study?

Although substantial genetic, preclinical, molecular and bio-chemical and clinical evidence support the hypothesis that HDL

elevation is linked to reduced CVD risk and HDL-C might be a potential surrogate biomarker for reduced CVD risk, it should be noted that the subject is still controversial. For instance, an increase in concentration of HDL-C may not be necessarily associated with an atheroprotective function [40]. The mechanisms linking low HDL-C concentrations to accelerated atherogenesis are complex, involve multiple pathways that require further elucidation. To date, no evidence has been provided that elevation of HDL alone through pharmacological approaches is associated with reduced risk of a coronary event, which is key to prove the HDL hypothesis [41]. Therefore, general acceptance of HDL-C as a surrogate biomarker awaits the production of compelling evidence to validate a causal link between a rise in HDL specifically and prevention of CHD.

HDL-C is one of the major lipid components of HDL, and different populations of HDL have very diverse cholesterol content and different particles vary potentially in their ability to reduce cardiovascular risks. In a recent posthoc analysis of two prospective studies (IDEAL: Incremental Decrease in End Points through Aggressive Lipid Lowering; EPIC: European Prospective Investigation into Cancer and Nutrition) looking at the relationship of HDL-C, HDL particle size (measured by NMR) and apoA-I levels with prevalence of CVD [42], higher HDL-C (IDEAL) and HDL particles (EPIC) proved significant major cardiac risk predictors after adjustment for apoA-I and apoB levels. In contrast, apoA-I appeared to be negatively associated with major cardiovascular events in both studies. Contrasting results were reported in the Veterans Affairs HDL Intervention Trial, which suggested that the levels of large HDL particles (α-1 and α-2) were significant negative risk factors, whereas the levels of small particle (α-3) were a positive risk factor for new CHD events [43]. Further large scale population studies as well as development of standardized measurement of HDL subpopulations are needed to elucidate whether specific subpopulations of HDL are related to cardiac risk, and more importantly, whether such variation in such subpopulation of HDL confer protective effects. Thus, future HDL elevating drug trials should always include measurement of plasma apoA-I, apoA-II and subfraction concentrations and biomarkers of the quality and function of HDL.

In summary, the hypothesis that a specific drug-induced elevation in HDL results in reduced CHD risk is still unproven. Our attention should not only be focused on the magnitude of the rise in HDL concentrations or particle number, but also on the properties of the lipoprotein on treatment. Given the natural history of atherosclerosis as it progresses from fatty streak lesions to advance, complex lesions, it is important to elucidate when HDL raising therapy is best applied. It is possible that the most beneficial outcome might emerge either in response to an early treatment or that HDL raising interventions are most effective in stabilizing "vulnerable lesions" that may be about to rupture.

4. Is CETP a viable target for pharmacotherapy?

CETP, a glycoprotein secreted from liver, binds mainly to HDL in the circulation. CETP facilitates the equimolar transfer of neutral lipids, including cholesteryl ester (CE) and triglyceride between all lipoprotein particles. Of particular importance for the present discussion is the ability of CETP to facilitate the exchange of CE from HDL (ApoA-I containing particles) to apoB containing lipoproteins, such as chylomicrons, very low density lipoprotein (VLDL) and LDL (Fig. 1) and the reciprocal transfer of triglyceride from apoB particles to HDL.

CETP was first recognized as a HDL-C modulator when association between mutations in human *CETP* gene and elevated levels of HDL-C was identified [44,45]. Subsequent genetic studies revealed that the *CETP* locus is one of the important genes regulating HDL particle levels [46], and the link was established firmly when it was shown that pharmacological CETP inhibition by JTT-705 leads to elevated levels of HDL-C [47]. In addition to its effects on HDL levels, CETP also plays a role in modulating HDL particle size and composition by affecting the metabolism and interconversion of different HDL subclasses [48]. CETP overexpression in mice caused the appearance of smaller HDL, lower apoA-I levels and a diminished abundance of apoE-rich HDL [48]. Conversely, partial loss of function CETP mutations in humans results in increased ratio of larger HDL_2 to smaller HDL_3 and increased plasma concentrations of HDL_2 and apoA-I. Pharmacological studies using CETP inhibitors in rabbit and human have yielded similar findings.

Further delineation of the link between CETP and HDL function has come from study of "loss of function" mutations in the CETP gene. These genetic variants result in low HDL_2 in plasma and a 2∼3-fold increased ability of HDL to promote net cholesterol efflux compared with "wildtype" HDL_2, possibly through increased expression of the ABCG1-dependent pathway [49]. CETP inhibition may exert a positive impact on cholesterol efflux as shown in studies by Rye and Barter [50]. However, it has also been suggested that CETP inhibition may reduce the *in vivo* regeneration of lipid poor apoA-I thus potentially decreasing ABCA1-mediated cholesterol efflux from cells [51]. The overall effects of CETP inhibition on reverse cholesterol transport as well as other HDL functions remain unclear.

Evidence supporting the hypothesis that CETP inhibition might be beneficial for preventing ischemic cardiovascular disease is indicative rather than overwhelming. Manipulation of CETP activity in preclinical studies has yielded both pro- and anti-atherogenesis results (Table 1b). Overexpression of human CETP in apoE or LDL-R knockout and apoE*3-Leiden mice promoted atherogenesis [52,53], and this was also the case with non-human primate CETP overexpression in cholesterol-fed C57BL/6 mice [54]. In a bone marrow transplantation study, an increase in macrophage CETP levels was sufficient to enhance atherosclerosis progression [55]. Overexpression of CETP was associated with higher risk of myocardial infarction in Dahl salt-sensitive hypertensive rats [56], while pharmacological inhibition of CETP (using torcetrapib and JTT-705) resulted in a 60–70% reduction in aortic atherosclerotic lesions in cholesterol-fed white New Zealand or Japanese rabbits [6,7]. Likewise, inhibition of CETP activity by vaccination with a peptide derived from CETP curbed atherogenesis [57].

Conversely, there are reports that CETP can act as an anti-atherosclerotic agent. Expression of human CETP in human apoC3- or lecithin:cholesterol acyltransferase-transgenic mice significantly reduced mean aortic lesion area [58,59]. Human CETP induction reduced diet-induced atherosclerosis in male db/db and ovariectomized mice [60]. Therefore, in the preclinical setting, experiments in model systems indicate that metabolic context, genetic background and epigenetic factors may all play a role in modulating the impact of CETP on atherogenesis.

Genetic evidence supporting the association between *CETP* deficiency and cardiovascular disease is not consistent despite the fact that HDL-C levels are strongly determined by *CETP* mutations (Table 1b). HDL-2 from homozygous CETP deficient subjects has enhanced ability to promote cholesterol efflux from foam cells [49]. Although subjects with certain CETP variants have elevated HDL-C and decreased CHD, other CETP mutation carriers have increased risk of CHD [40]. The nature of the relationship between *CETP* deficiency and CHD appears to be dependent on the prevailing HDL level; thus in male heterozygotes with low or moderately increased HDL-C levels (1.0∼1.54 mmol/L) a higher risk for CHD was noted, compared with men with similar HDL levels without *CETP* mutations. In contrast, men with or without

Table 1b
Summary of pro- and anti-atherogenesis results in response to CETP inhibition.

Study	Species	Model/trial	Outcomes	Refs.
Pro-atherogenesis	Mouse	Overexpression of human CETP in apoE or LDL-R knockouts	Decreased HDL-C levels; increased atherosclerotic lesion area	[52]
	Mouse	Human CETP expression in human apoE*3-Leiden mouse	Increased atherosclerotic lesions	[53]
	Mouse	Non-human primate CETP overexpression in C57BL/6 mice on high cholesterol diet	Increased atherosclerotic lesions	[54]
	Mouse	Bone marrow transplantation in LDL-R knockouts	Bone marrow derived cells from CETP human transgenic mice induced serum CETP activity, CETP mass, atherosclerotic lesions and reduced HDL-C levels	[55]
	Rat	Human CETP transgene in Dahl rat	Severe combined hyperlipidemia, atherosclerotic lesions, myocardial infarctions and decreased survival	[56]
	Rabbit	CETP inhibition (JTT-705) in White Japanese rabbits	Increased HDL-C and decreased aortic atherosclerotic lesions	[6]
	Rabbit	CETP inhibition (torcetrapib) in New Zealand rabbits	Increased HDL-C and decreased aortic atherosclerotic lesions	[7]
	Rabbit	CETP inhibition (vaccined-CETP) in New Zealand White rabbits	Increased HDL-C and decreased aortic atherosclerotic lesions	[57]
	Human	Honolulu Heart Study in 3469 men	CETP gene mutations were associated with increased HDL-C levels and no increased risk of CHD (HDL-C > 1.54 mmol/L)	[40]
	Human	Cholesterol efflux capacity study in subjects with homozygous deficiency of CETP	HDL-2 from homozygous CETP deficient subjects has enhanced ability to promote cholesterol efflux from foam cells in an ABCG1-dependent pathway	[49]
Anti-atherogenesis	Mouse	Human CETP expression in human apoC3 transgenic mouse	Decreased aortic atherosclerotic lesions in CETP/apoC3 mice	[58]
	Mouse	Human CETP expression in human LCAT transgenic mouse	Decreased aortic atherosclerotic lesions in CETP/LCAT mice	[59]
	Mouse	Overiactomized CETP transgenic mouse on LDL-R knockout background	Reduced mean aortic lesion area in overiactomized CETP transgenic mouse	[60]
	Human	Honolulu Heart Study in 3469 men	CETP gene mutations were associated with increased risk of CHD (HDL-C < 1.54 mmol/L)	[40]

CETP gene defects but with markedly elevated levels of HDL-C (>1.54 mmol/L) had less risk of CHD [40].

Whether variation in plasma CETP activity or CETP mass is associated with altered CHD risk remains largely unclear at present due to the limited data available. In healthy subjects, plasma CETP activity is positively related to both cIMT and coronary calcification [61]. High CETP concentration was associated with either reduced CHD risk or increased prevalence of CHD in patients [61]. Therefore, the utility of plasma CETP activity or mass as a potential biomarker warrants is still a matter of active investigation.

5. What is the take home message from the torcetrapib trials?

Statins are a well established pharmacologic strategy to reduce cardiovascular morbidity and mortality [3]. However, even on maximal statin therapy, patients have substantial residual risk of CVD, especially individuals with low HDL-C. The torcetrapib trials were designed to test the impact of raising HDL on such residual risk and therefore provided a direct test of the "HDL hypothesis". To date, four large-scale clinical trials evaluating the efficacy of torcetrapib on cardiovascular morbidity and mortality as well as surrogate biomarkers including vascular plaque imaging biomarkers have been completed (Table 2a). The overall results of these studies were negative, disappointing to protagonists of the benefits of CETP inhibition, expensive for the sponsoring company, but highly informative for the pharmaceutical industry as to how drugs should be developed in the future.

The Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events (ILLUMINATE) trial was a randomized, multi-center, double-blind study [62]. Upon reaching target goal of LDL-C less than 100 mg/dl, 15067 cohorts received atorvastatin monotherapy or combined torcetrapib (60 mg/day)

Table 2a
Summary of torcetrapib trials.

Study	Cohorts	Duration	Treatment	Clinical outcomes	Changes in biomarkers	Refs.
ILLUMINATE	15067 (high CV risk)	550-day	Atorvastatin vs. torcetrapib + atorvastatin	CV events: 26%↑, CV death: 40%↑, non-CV death: 100%↑, SBP: 5.4 mmHg↑	HDL-C: 33% ↑, LDL-C: 16% ↓, serum aldosterone ↑, serum Na^+ ↑, serum K^+ ↓	[62]
ILLUSTRATE	1188 (CHD)	24-month	Atorvastatin vs. torcetrapib + atorvastatin	SBP: 4.6 mmHg ↑	HDL-C: 61%↑, LDL-C: 20%↓, % atheroma volume (primary measure): no change, change in atheroma volume (2nd measure): ↓, serum Na^+↑, serum K^+↓	[63,64]
RADIANCE 1	904 (FH)	24-month	Atorvastatin vs. torcetrapib + atorvastatin	SBP: 2.8 mmHg ↑	HDL-C: 56%↑, LDL-C: 20%↓ increase in cIMT (primary measure): no change, change/year in cIMT (2nd measure): ↑, serum Na^+↑, serum K^+↓	[65,67]
RADIANCE 2	752 (mixed dyslipidemia)	22-month	Atorvastatin vs. torcetrapib + atorvastatin	SBP: 5.4 mmHg ↑	HDL-C: 63%↑, LDL-C: 18%↓, increase in cIMT (primary measure): no change, change/year in cIMT (2nd measure): ↑, serum Na^+↑, serum K^+↓	[66,67]

and atorvastatin respectively. The trial was prematurely terminated based on interim data indicating increased CVD hazard (hazard ratio, 1.25; 95% confidence interval, 1.09–1.44; $P = 0.001$) in the torcetrapib plus statin arm. In the study, patients on torcetrapib exhibited approximately 26% increase in major cardiovascular events (death from CHD, non-fatal myocardial infarction, stroke and hospitalization for unstable angina), 40% increase in cardiovascular related death and 100% increase in non-cardiovascular mortality. Although torcetrapib therapy did lead to alteration in lipid profiles (~16% decrease in LDL-C, 33% increase in HDL-C, 74% increase in HDL_2 and 26% increase in HDL_3), these changes did not translate into any benefit in clinical endpoints. In addition, torcetrapib administration was associated with elevated systolic blood pressure (5.4 ± 13.2 mmHg in torcetrapib treatment arm vs. 0.9 ± 11.5 mmHg in placebo group, $P < 0.001$), augmented levels of aldosterone and electrolytes changes indicative of mineralocorticoid excess. These "off-target" effects of torcetrapib might have contributed to the overall adverse clinical outcome of the trial. Overall, the results from ILLUMINATE trial indicated that in spite of torcetrapib-induced HDL-C elevation and LDL-C reduction, clinical outcomes were negative.

The Investigation of Lipid Level Management Using Coronary Atherosclerosis by CETP Inhibition and HDL Elevation (ILLUSTRATE) trial was a prospective study designed to evaluate the additive impact of torcetrapib on top of atorvastatin on surrogate imaging biomarkers of atherogenesis in CHD patients [63,64]. Torcetrapib (60 mg/day) raised HDL-C by 61% and lowered LDL-C by 20%, but the primary vascular biomarker-percent atheroma volume showed no decrease, although in torcetrapib-treated patients, an inverse relationship was observed between change in HDL-C and percentage atheroma volume ($r = -0.17$, $P < 0.001$). Participants with regression had greater increases in HDL-C ($62.9 \pm 37.4\%$ vs. $54.0 \pm 39.1\%$, $P = 0.002$). Similar to the ILLUMINATE trial, those in the torcetrapib arm displayed an increase in systolic blood pressure (a least-square mean increase of 4.6 mmHg (95% confidence interval, 3.7–5.6; $P < 0.001$), raised serum sodium (0.44 ± 0.14 mmol/L, $P = 0.02$) and lowered serum potassium (0.11 ± 0.02 mmol/L, $P < 0.0001$). Thus, ILLUSTRATE confirmed the lack of efficacy in the torcetrapib-based HDL therapy on coronary atherosclerosis progression, and the safety concerns over electrolyte disturbances and elevation of blood pressure.

Two more torcetrapib trials aimed to study the effect of torcetrapib on carotid atherogenesis also need to be mentioned. The Rating Atherosclerotic Disease changes by Imaging with A New CETP Inhibitor (RADIANCE 1) and RADIANCE 2 trials focused on the impact of torcetrapib on atherosclerosis progression in atorvastatin-treated patients from familial hypercholesterolemia (FH) and mixed dyslipidemia populations respectively [65–67]; endpoints were evaluated using ultrasound to measure IMT. Again, despite a ~60% increase in HDL-C and 20% reduction in LDL-C, there was neutral impact of torcetrapib on the progression or regression of atherosclerosis. During the studies, average SBP increased 2.8 mmHg (95% confidence interval, 1.9–3.7; $P < 0.001$) and 5.4 mmHg (95% confidence interval, 4.3–6.4, $P < 0.0001$) in the combined treatment group as compared to the atorvastatin alone group, respectively. It should be noted that RADIANCE 2 trial had to be halted due to identification of excess mortality in the torcetrapib plus atorvastatin arm post 18-month follow-up.

Taken together, these four trials gave a definitive negative answer (at the estimated cost of ~$0.8 billion) to the question as to the safety and efficacy of torcetrapib and the development of this compound was halted. Whether or not other CETP inhibitors will display similar properties is a hot topic. There may yet be utility in this therapeutic modality if the adverse, possibly off-target effects can be eliminated in other molecules. Potential mechanisms of action (MOA) of torcetrapib on cardiovascular and non-cardiovascular risks are summarized [63,68,69] (Fig. 1 and Table 2b).

6. TMed perspectives of torcetrapib trials: the price to pay when TMed principles are "short cut"

The failure of the torcetrapib trials has raised substantial doubts about the concept of CETP inhibitor-based therapy and even the entire "HDL therapy" hypothesis. Does CETP still represent a valid target for anti-atherosclerosis therapy? Are torcetrapib's "off-target" effects a "couplet of failure"? Were an appropriate range of pharmacodynamic efficacy and safety biomarkers implemented for outcome prediction in the clinical trials? Perhaps the best way to address these issues is to review what is known of the discovery and development of torcetrapib from the TMed angle since one key objective of TMed is to minimize the greater risk of failure in the drug development process [70] (Fig. 2).

6.1. Target validation: is CETP a validated therapeutic target for CVD?

A large body of human genetic, preclinical, pharmacological and early clinical evidence supports a potentially beneficial role of CETP inhibition in reducing risks of CVD [6,7,45,52]. However, uncertainty remains in CETP target validation in both human genetics [40,45] and preclinical animal studies [59]. CETP inhibitors were developed since they can be used to achieve a favorable lipid profile and therefore test the HDL hypothesis. Torcetrapib demonstrated dramatic effects against atherogenesis in preclinical studies but this did not translate into clear benefit in clinical trials. Addressing this kind of discrepancy, i.e., a failure to translate animal model observations into the human situation is one of the pillars of target validation in TMed. What could have been done differently had TMed-based approaches been applied during CETP target validation? In retrospect, loose ends remained in both preclinics and clinics. For instance, preclinical target validation studies only demonstrated that CETP inhibition prevented the onset of atherosclerosis progression; a comprehensive regression

Table 2b
Potential MOAs of torcetrapib on cardiovascular and non-cardiovascular risk management.

Influences on CV risk	Mechanism of action		Potential CV outcomes	Refs.
Beneficial	Inhibits CETP	Elevate HDL-C levels Decrease LDL-C levels	Expect to reduce CV risks	[62]
Detrimental	Binds to CETP and interferes with HDL non-RCT related functions	Interfere with HDL anti-oxidative properties Interfere with HDL anti-inflammatory properties	Increase non-CV death	[62,68]
	Molecule-specific "off-target" effects	Lead to elevated levels of aldosterone *in vivo*	Increase CV events Increase CV death Elevate SBP	[62,69]
		Induce aldosterone production in adreno cells *in vitro*	Increase serum aldosterone concentrations, Raise serum Na^+ levels Lower serum K^+ levels	

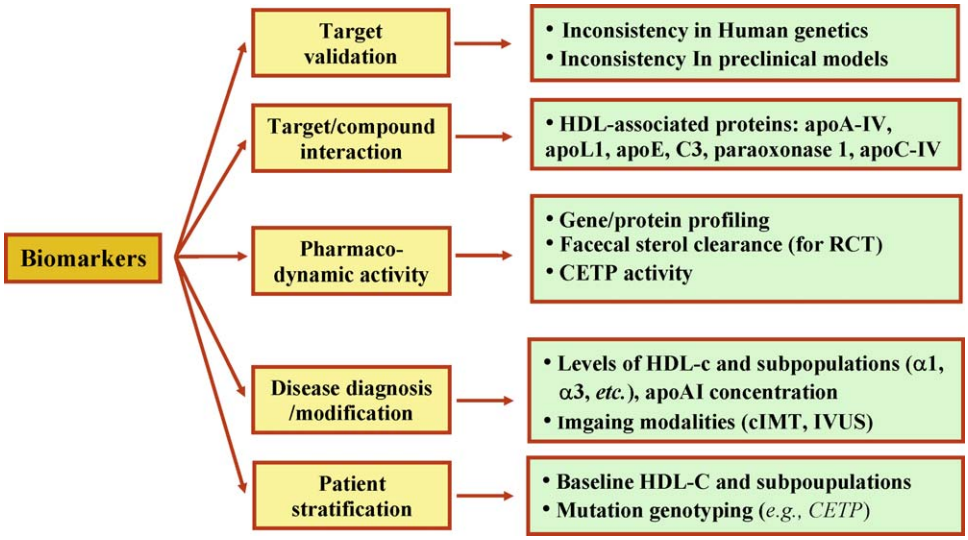


Fig. 2. Potential biomarkers for future CETP inhibition trials.

study with treatment modalities that reflected those used in the clinical trials appears to be the key missing piece of evidence. Similarly, the efficacy of torcetrapib as monotherapy was tested in rabbits while the torcetrapib trials were carried out in combination with statins (based on marketing strategy for broadest indication at registration). Whether torcetrapib in combination with statins is able to reduce the residual risk beyond statins therapy remained unexplored in the preclinical studies. Likewise, torcetrapib monotherapy in clinical trials has yet to be evaluated. We propose the concept that pharmacology-based, patient-centered TMed approaches being employed for further CETP target validation (e.g., ideally, it would be nice to collect efficacy and safety of combined therapy of CETP inhibitors plus statins in the preclinical testing phase and CETP inhibitor monotherapy evaluated in proof of concept clinical trials). However, on the other hand, it should be noted that in reality there are no "gold standard" preclinical model of atherosclerotic lesion regression since challenges remain in developing such models mimicking human atherosclerotic disease biology and pathology, especially in the context of complexity of the disease advancement, species difference, lipid metabolism, drug pharmacokinetics and pharmacodynamics. Therefore, it was probably beyond the sponsor's ability to generate meaningful data in the scenario of torcetrapib trials.

6.2. Compound–target interaction: is torcetrapib a "clean" CETP inhibitor?

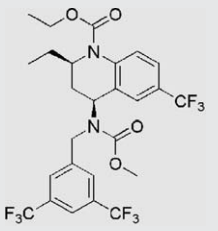
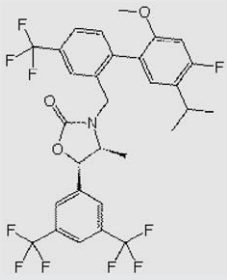
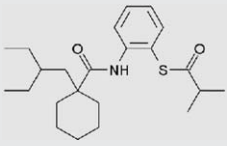
The failure of torcetrapib raised essential TMed questions of compound–target interaction: that is whether the interaction between torcetrapib and CETP interfered with HDL function? Were the adverse events observed the result of CETP inhibition or an "off-target" side effect? Have appropriate biomarker studies been performed to monitor such an interaction?

Additional to RCT (the primary function that HDL mediates to protect against atherosclerosis), HDL is also associated with non-RCT functions such as anti-thrombotic, anti-inflammatory responses and maintains endothelial function which has been well documented. HDL is anti-thrombogenic since it reverses LDL-induced inhibition of fibrinolysis, inhibits tissue factors (Va, VIIIa and X), enhances the anticoagulant activities of protein S and C. HDL/ABCG1 cascade is responsible for reducing inflammatory cytokines and chemokines secretion [71]. Depending on the content of PAF acetylhydrolase, HDL takes up oxidized phospholipids from other lipoproteins and promotes dendritic cells migration. HDL and ABCG1 maintain endothelial function through promoting efflux of cholesterol and 7-oxysterols and preserving active eNOS dimer levels [25]. Therefore, dysfunction HDL could most likely lead to pro-thrombotic, pro-inflammatory state and impair endothelial function which triggers the subsequent cardiovascular hazardous events documented in ILLUMINATE trial. The interaction between torcetrapib and CETP results in a large CETP–HDL–torcetrapib complex, which raised the speculation of non-productive or even disrupted HDL function in RCT and other non-RCT-related functions. Direct evidence linking torcetrapib and HDL functions remains to be actively and closely examined, especially in the patients from the torcetrapib trials. The results of such studies will not only answer the question whether HDL-raising therapy is indeed the effective strategy to curb cardiovascular disease but also highlight the high priority of integration of TMed-driven approaches in future trials.

A comprehensive examination of the impact of torcetrapib on HDL-associated proteins using gene ontology approach would have identified compound–target interaction biomarkers reflecting modulated HDL function. For instance, shotgun proteomics analysis on HDL_3 has revealed that HDL from CAD patients is indeed enriched in specific proteins, e.g., apoA-IV, apoL1, apoE, C3, paraoxonase 1, apoC-IV, etc. The abundance of these HDL-associated proteins in response to torcetrapib remains to be investigated.

The adverse event of hypertension has not been seen in trials with other CETP inhibitors, e.g., JTT-705 and anacetrapib (MK0859) [72,73]. Nor have subjects with genetic deficiency of CETP been observed to have an elevation in blood pressure. These findings provided circumstantial proof that the torcetrapib molecule rather than the class of CETP inhibitors might be responsible for this major adverse effect. Although the adverse effect on blood pressure emerged in preclinical studies and early phase of the clinical trial programme, neither the mechanism of torcetrapib-induced increment in blood pressure nor the effect of the drug on appropriate biomarkers have been carefully examined. If this had been undertaken in preclinical work and in small scale human studies then a red flag would have been raised prior to the expensive and danger of an large outcome study. A head-to-head comparison of torcetrapib vs. alternative CETP inhibitors in preclinical settings could have provided valuable information on the potential mechanisms underlying the torcetrapib "off-target" side effect and identified a panel of biomarkers to be monitored in clinical trials. Recently Forrest et al. demonstrated that the torcetrapib

Table 2c
Summary of leading CETP inhibitors in development.

Compound	Company	Status	Class	Properties			
				Molecular formula	Molecular mass (g/mol)	CAS number	Structure
Torcetrapib	Pfizer	Phase III (discontinued)	CETP inhibitor	$C_{26}H_{25}F_9N_2O_4$	600.473	262352-17-0	
Anacetrapib	Merck	Phase II	CETP inhibitor	$C_{30}H_{25}F_{10}NO_3$	637.51	875446-37-0	
JTT-705 (R1658)	Japan Tobacco (Roche)	Phase II	CETP inhibitor	$C_{23}H_{35}NO_2S$	389.5945	N/A	

induced acute increase in blood pressure is CETP- and adrenal steroid-independent but relies on the presence of an intact adrenal gland [69]. Future studies are necessary to identify adrenal gland-oriented biomarkers (e.g., the cultured primary adrenal cell-based hormone release assay) and to understand how well these biomarkers are associated with the clinical picture and hence have utility as indices of the propensity of a drug to cause blood pressure elevation. Selected candidate biomarker needs to be "added back" to the *in vivo* adrenalectomy model to gauge their individual impact on blood pressure modulation. Whether the candidates remain biomarkers for "off-target" effect prediction warrants further confirmation in superior alternative preclinical models to rule out species difference.

Properties of torcetrapib, anacetrapib and JTT-705 are briefly summarized (Table 2c). Anacetrapib and JTT-705 are currently positioned as the new hope for CETP inhibition with superior efficacy and no side effect of blood pressure elevation in preclinical and phase I and II trials [69,72,73]. It is crucial to integrate the once overlooked biomarker approaches in future CETP inhibition studies.

6.3. Pharmacodynamic (PD) efficacy and safety activities: will PD biomarkers show the way?

The failure in torcetrapib trials is a case study highlighting the need for integrating TMed-oriented PD biomarker research into pharmaco-epidemiology. In the case of torcetrapib, indications of aldosterone/sodium excess, a possible contributing factor to the elevated BP, might just be the tip of the iceberg considering the broad deleterious effects of excess aldosterone secretion in vascular remodeling, cardiac fibrosis, hypertrophy, stroke and heart failure [74]. Unfortunately, genetics and proteomics-based quantitative biomarkers were not explored at all, which limited the power to interpret the adverse events in an outcome trial. PD efficacy and safety biomarkers, a center piece of successful TMed, were segregated from these torcetrapib clinical trials, thus the safety measurements as well as the efficacy of torcetrapib could not be predicted. Future biomarker-driven efforts need to provide thorough understanding of PD efficacy and safety of the compounds. Since the lipids PD readout in response to torcetrapib treatment might not be separable from the "off-target" side effect, biomarkers for reverse cholesterol transport (RCT), e.g., fecal sterol secretion need to be incorporated into future CETP inhibition trials. In addition, biomarkers of non-cardiovascular events of in response to CETP inhibition should also be closely monitored.

6.4. Disease biomarker: did imaging modalities play a role to predict efficacy and alarm safety issues?

Although imaging modalities (cIMT and IVUS) were built into the torcetrapib clinical data package as surrogate endpoints, they were positioned in a confirmatory role and so had little chance to be used as predictors of efficacy or to reveal potential safety concerns (correlation of imaging to outcome would have been helped greatly for the HDL hypothesis if there had not been a necessary premature termination of ILLUMINATE). That said, the readouts from the cIMT studies appeared to be consistent with the clinical outcomes: both primary and secondary efficacy measures indicated a negative impact of the drug on atherosclerosis progression in the carotid artery [65,66], which expanded the

validity of cIMT as an essential imaging biomarker to predict vascular outcomes in interventional trials. However, the IVUS results (a single secondary measure in coronary artery) indicated a trend to reduction in normalized atheroma volume in coronary artery following torcetrapib treatment, which is apparently paradoxical to the clinical outcomes [63]. These discordant findings may cast shadow on the plausibility of IVUS-based atheroma volume as potential surrogate biomarker for cardiovascular risk assessment. It should be noted that, interestingly, intraplaque composition change (e.g., necrotic core volume) occurred without significant treatment difference in total atheroma volume in a recent lipoprotein-associated phospholipase ($Lp\text{-}PLA_2$) inhibitor study, suggesting necrotic core expansion might be an additional biomarker to explore in future CETP inhibition trials [75].

6.5. Missing steps in patient stratification: have baseline HDL-C concentrations and genotyping biomarkers been integrated for patient selection/exclusion?

It has been proposed that HDL-C concentrations might potentially allow stratification of the patient populations with perhaps the most responsiveness to HDL raising therapy being found in those with low HDL-C levels. Recent data from the ILLUSTRATE trial [64] revealed an inverse relationship between change in HDL-C and percentage atheroma volume, i.e., subpopulations of participants who had greater increases in HDL-C in response to torcetrapib treatment exhibited atheroma volume regression. Thus, although the majority of torcetrapib-treated patients demonstrated no regression of coronary atherosclerosis, this posthoc analysis suggests that regression was present in selected patients at the highest on treatment HDL-C levels. If correct these data support the idea that biomarker-based patient stratification should be a part of early proof of concept studies. In this example, a better outcome may have resulted if the subjects were selected on the basis of a low baseline HDL-C/maximum change in HDL-C levels. Change in HDL-C levels might become an independent biomarker to predict the effect of treatment on atherosclerosis progression/regression in future trials. Sub-groups of patients with "loss of function" mutations in *CETP* gene, although minor in number, should have been identified via genotyping and excluded from the trial since they are the non-responders to CETP inhibitor. In addition, differences among individuals in the response to torcetrapib would be expected to influence drug efficacy as well as their susceptibility to cardiovascular complications. The variability of response within and between subjects (e.g., degree of CETP inhibition and subsequent changes in HDL-C, cardiovascular outcome, etc.) upon torcetrapib treatment warrants further examination. The mechanisms underlying the genetic sources of variances, such as candidate gene polymorphisms detections need to be further investigated. The actual degree of CETP inhibition by the torcetrapib relates to both chemical properties of the drug and to factors within an individual that modulate drug response. These sources of variability might be exploited to identify patients uniquely susceptible to benefit or at developing risk of cardiovascular complications.

It should be noted that Ezetimibe could be regarded as a "LDL version" mirroring the RADIANCE 1/torcetrapib trial in the context of patient population, surrogate imaging end points and neutral results. The authors believe it is important to provide TMed perspective of Ezetimibe in parallel to torcetrapib trials. Thus, target validation for Ezetimibe has been well characterized. Ezetimibe is fractionally absorbed systemically and the impact on reduced ABCA1 expression could be secondary to changes in intracellular cholesterol, thus it might be unlikely that an "off-target" effect is responsible for the neutral end readouts. cIMT has been accepted as proven disease biomarker for atherosclerosis. Based on results from multiple lipid-therapy trials, a prolonged treatment (only 2 years for ENHANCE trial) is suggested for future trial to be powerful enough to differentiate changes in cIMT in combined therapy vs. monotherapy. Two steps of patients stratification is suggested in a proof of concept (POC) study prior to phase III trial. First, patients without statins pre-treatment (e.g., 19% in ENHANCE trial) or with cIMT great than 0.7 need to be selected for entry; second, high baseline LDL-C/maximum reduction in LDL-C levels warrants integration as selection biomarkers to magnify treatment effect early in the drug development process.

7. Conclusion

The demise of torcetrapib raises the need of a paradigm shift from the conventional drug development mode to a biomarker-based TMed strategy. This concept involves biomarker-driven research in target validation, target–compound interaction, PD activities, disease modification and patient selection. Integration of genetic, biochemical, and physiological biomarkers across preclinical and clinical stages along the principles of TMed might have avoided this failure. Despite the disappointing torcetrapib experience, CETP-focused therapy may still hold potential promises in translating into clinical benefits through HDL manipulation. The emergence of anacetrapib without hypertensive side effects should encourage the pharmaceutical industry to renew the efforts to explore its potential medical benefits. If such attempts will be made, we hope that TMed guided strategies and studies will be incorporated.

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