Cholesteryl Ester Transfer Protein (CETP) Inhibitors: Is There Life After Torcetrapib?

Hemanth Neeli, MD[a,b], Daniel J. Rader, MD[b,*]

KEYWORDS

• CETP • CETP inhibitors • Torcetrabip • HDL • Illuminate

Statins have revolutionized the prevention and treatment of coronary heart disease (CHD) for the last 15 years by targeting LDL-c (low density lipoprotein cholesterol), one of the major risk factors for CHD. Recent guidelines have recommended achieving even lower LDL-c levels especially in the high-risk patients[1] as this translates into improved clinical outcomes. Despite tremendous progress made in the management of CHD, a significant number of fatal and nonfatal CHD events still occur [2,3] that entail the need to target other modifiable risk factors for CHD including low HDL-c (high density lipoprotein cholesterol). Low HDL-c is an independent risk factor for CHD even in patients who have low LDL-c levels.[4] Large-scale population studies have demonstrated a strong independent inverse relationship between the HDL-c level and the risk of CHD,[5] which has propelled efforts to find novel therapies to increase HDL-c levels. One mg/dl increase in HDL-c lowers CHD risk by 2% in men and 3% in women.[6] Data from the landmark Framingham Heart Study showed that for a given level of LDL-c, the risk of CHD increases 10-fold as the HDL-c varies from high to low. Conversely, for a fixed level of HDL-c, the risk increases three-fold as LDL-c varies from low to high.

HDL METABOLISM AND MECHANISMS OF ATHEROPROTECTION

HDL particles and their major protein apolipoprotein A-I (apoA-I) are synthesized and secreted by both the liver and intestine. After apoA-I is secreted, it acquires phospholipids and unesterified cholesterol from the same organs via the transporter ATP binding cassette A1 (ABCA1) forming the discoid nascent HDL particle.[7,8] HDL and apoA-I promote a key process called ''reverse cholesterol transport'' (RCT) in which they serve as acceptors for cholesterol from peripheral tissues and then transport the cholesterol back to liver for excretion into bile eventually. Lipid-loaded macrophages in the arterial wall efflux free cholesterol to nascent HDL via ABCA1 and to mature HDL via ATP-binding cassette G1 (ABCG1) respectively.[9,10] Much of the free cholesterol is esterified by the enzyme lecithin:cholesterol acyl transferease (LCAT) forming spherical mature HDL. HDL returns cholesterol to the liver by at least two major pathways. One pathway is via scavenger receptor BI (SR-BI) in the liver, which mediates the ''selective uptake'' of cholesterol from HDL. This is an important pathway in rodents. The second pathway involves the transfer of cholesteryl ester (CE) facilitated by the cholesteryl ester transfer protein (CETP) to apoB containing lipoproteins that are eventually taken up by the liver via the LDL receptor. This is an important pathway for the hepatic uptake of HDL cholesterol in humans.[11]

CURRENT THERAPIES TARGETING HDL-C

Life style changes like daily exercise,[12] weight loss,[13] moderate consumption of alcohol,[14] and

[a] Section of Hospital Medicine, Temple University Hospital, 1316 West Ontario Street, Philadelphia, PA 19140, USA
[b] Institute for Translational Medicine and Therapeutics, University of Pennsylvania School of Medicine, 654 BRB II/III, 421 Curie Blvd, Philadelphia, PA 19104, USA
* Corresponding author.
E-mail address: rader@mail.med.upenn.edu (D.J. Rader).

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smoking cessation[15] can raise HDL-c levels. Moderate intensity exercise for more than 12 weeks raises HDL-c by 4.6%; weight loss of 1 kg increases HDL-c by 1%; moderate consumption of alcohol (30 grams per day) increases HDL-c by 8.3%. The National Cholesterol Education Program Adult Treatment Panel III update specifies that drug therapies to increase HDL-c may have a role in high-risk patients after their LDL-c and non-HDL-c level goals are met.[1] Statins can raise HDL-c by 6%–12%,[16–18] which may be in part through decreased CETP expression.[19]

Nicotinic acid (niacin) is the most effective drug currently available to raise HDL-c levels. A meta-analysis of all randomized trials with nicotinic acid have shown that it increases HDL-c by an average of 16%.[20] Niacin significantly decreased recurrent myocardial infarction (MI) and total mortality in the Coronary Drug Project (CDP).[21] Simvastatin-niacin combination in CHD patients who have low HDL-c and normal LDL-c has shown angiographic regression of the atherosclerotic plaque accompanied by 26% increase in HDL-c in the HDL-Atherosclerosis Treatment Study (HATS).[22] In the Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol (ARBITER) 2 trial, addition of extended-release niacin to statin therapy in patients who have CHD and moderately low HDL-c levels increased HDL-c by 21% and showed a trend towards decrease in the progression of atherosclerosis (as measured by change in carotid intima-media thickness [IMT]).[23] The Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides and Impact on Global Health Outcomes (AIM-HIGH) and Heart Protection Study 2 Treatment of HDL to Reduce the Incidence of Vascular Events (HPS2-THRIVE) will determine the long-term clinical benefit of extended release niacin when added to statin therapy.

Fibrates can increase HDL-c levels modestly. Meta-analysis of all randomized controlled trials with fibrates including the CDP, Bezafibrate Infarction Prevention (BIP) trial, Veterans Affairs HDL-c Intervention trial (VA-HIT)[24] and Helsinki Heart Study[25] have shown that fibrates increase HDL-c by an average of 10%.[20] However, the Fenofibrate Intervention and Endpoint Lowering in Diabetes (FIELD) trial, which randomized type II diabetic patients with and without CHD to micronized fenofibrate and placebo showed only a 1% increase in HDL-c.[26] Although several clinical trials with fibrates have shown efficacy regarding reduction in cardiovascular events, the FIELD trial did not achieve its primary endpoint, possibly due in part to statin drop-in,[26] and the benefits of fibrates are not proven to be related to HDL raising. The Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial,[27] an ongoing large clinical trial will determine if HDL-c raised by fenofibrate translates into improved clinical outcomes beyond the benefits offered by statins in a cohort of diabetes patients. Given the limitations and inconsistent outcomes of the available therapies that raise HDL-c, there is a growing need to develop novel therapies aimed at increasing HDL-c.

EVOLUTION OF CETP AS A NOVEL TARGET

CETP is a plasma protein that facilitates transfer of CE from HDL particles to apoB- containing particles in exchange for triglycerides.[28] CETP emerged as a potential target when it was found that a subset of the Japanese population with genetic deficiency of CETP had markedly elevated HDL-c.[29] The human CETP gene has been mapped to chromosome 16 and spans 25,000 base pairs including 16 exons.[29] Two CETP gene mutations that cause CETP deficiency are more common than the others: a G to A substitution in the 5′ splice donor site of intron 14 (Int14+1 G→A) and a missense mutation of exon 15 (D442G).[30,31] Homozygotes and compound heterozygotes for these loss-of-function mutations have little to no detectable CETP activity in the plasma and very high levels of HDL-c.[32] However, the association of CETP deficiency with coronary disease remains unclear.[33–35]

The human CETP gene is highly polymorphic and several single nucleotide polymorphism (SNP) have been characterized.[36,37] The TaqIB polymorphism is the most extensively studied in relation to its association with HDL-c level, CETP mass and even CHD risk. It accounts for about 6% of the variance in CETP levels.[38] The most common genotype B1B1 is associated with higher CETP mass/activity and lower HDL-c in various population studies.[37,39] The effects of the TaqIB polymorphism on the lipid parameters are influenced by gender, alcohol consumption, insulin levels, and body mass index.[37,40,41] The B2B2 genotype is associated with lower CETP, higher HDL-c, and in some studies a decreased CHD risk.[39,42] In the REGRESS (Regression Growth Evaluation Statin Study) Study[43] pravastatin slowed the progression of coronary atherosclerosis to a greater extent in individuals with the B1B1 than the B2B2 genotype.

Klerkx and colleagues[38] described a haplotype model with promoter mutations −629 C/A, −971 G/A and −2708 G/A in a cohort of CHD patients that explained the variation in CETP activity better than TaqIB. Later the same group showed in

REGRESS cohort that there is functional interaction between –629C/A, –971G/A and –1337C/T polymorphisms and CETP concentration.[44] McCaskie and colleagues[45] evaluated the effect of −629 C/A, −2708 G/A, and TaqIB in two community-based populations and a cohort of CHD patients in Australia; they found that the common haplotype AAB2 was consistently associated with decreased CETP activity and elevated HDL-c levels, but it was not associated with CHD risk. In a genetic substudy of PREVEND, Borggreve and colleagues[46] demonstrated that −629A, TaqIB B2 allele carriers had increased risk of CHD despite higher HDL-c levels. A recent meta-analysis of 132 studies involving approximately 200,000 subjects showed that the common CETP variants TaqIB, I405V and –629C/A are associated with modest decrease in CETP activity, modest increase in HDL-c levels, and weakly with reduced CHD risk.[47] Thus, although this issue has not been definitively settled, the weight of evidence suggests that genetic variants that reduce CETP activity and increase HDL-C are associated with modest protection against CHD.

Studies have also addressed the relationship between plasma CETP mass or activity and atherosclerosis and cardiovascular events. A small case-control study showed that patients who have MI and stroke were found to have higher CETP mass than healthy controls controlled for CETP gene mutations.[48] Elevated CETP mass is associated with atherogenic lipid profile[49] and rapid progression of angiographic coronary lesions[50] and carotid IMT.[49] A nested case-control study of the prospective EPIC-Norfolk study showed increased incidence of coronary events in subjects with elevated CETP mass.[51] However, a nested case-control study of Prevention of Renal and Vascular End-stage Disease (PREVENT) study showed the contrary.[52] The milieu in which CETP functions might modify its own function, explaining the above discrepant results. In the EPIC- Norfolk study, higher CETP mass increased the incidence of CHD only when the triglycerides were high, while in the PREVEND study higher CETP mass was associated with lower risk of CHD in the setting of low triglyceride levels.

Measures of CETP activity might be more informative than CETP mass, but the assays for CETP activity are cumbersome and not standardized. Only a few studies have looked into the CETP activity and its correlation to CHD. In patients who underwent diagnostic coronary angiography, higher CETP activity was found in patients who have significant CHD than those without significant CHD.[53] In another small study, diabetic patients had a higher CETP activity than nondiabetic controls, and the CETP activity was positively correlated to carotid IMT in both diabetic and control groups.[54] An observational study of all patients who have first MI showed that higher CETP activity was associated with earlier presentation of MI than lower CETP activity.[55] Thus the CETP mass and activity data, although not completely consistent, also generally support the concept that higher plasma levels of CETP are associated with increased cardiovascular risk.

CLINICAL EXPERIENCE WITH THE CETP INHIBITOR TORCETRAPIB

Torcetrapib, the most extensively studied CETP inhibitor to-date, selectively inhibits plasma CETP by causing a nonproductive complex between CETP and HDL.[56] Several early clinical trials were conducted to define the dose and change in lipid parameters, the kinetics of the drug and dynamics of HDL metabolism. Torcetrapib at 120 mg twice daily increased HDL cholesterol by 91% and decreased LDL cholesterol by 42% in healthy subjects.[57] In subjects with low HDL-c levels, torcetrapib increased HDL-c and decreased LDL-c levels, either when administered as monotherapy or combined with a statin.[58] Torcetrapib was shown to increase apoA-I concentrations modestly by decreasing the catabolism of apoA-I.[59] In a phase 2 trial, 162 subjects with below average HDL-c were randomized to torcetrapib 10, 30, 60, or 90 mg/day or placebo.[60] There was a dose-dependent increase in HDL-c from 9% to 55%. In another phase 2 multicenter, randomized, double-blinded trial, 174 patients who have low HDL-c either on statins or with LDL-c > 130 mg/dL who would be eligible for statins according to NCEP ATP III guidelines were randomized to varying doses of torcetrapib or placebo for 8 weeks after a run-in period with atorvastatin.[61] There was a dose-dependent increase in HDL-c from 8% to 40%, with additional decrease in LDL-c beyond that achieved with atorvastatin alone. In the same trial, torcetrapib/atorvastatin combination was shown to increase the large HDL2 subfractions and decrease the small LDL subfractions while increasing both HDL and LDL particle size.[62] In patients who have heterozygous familial hypercholesterolemia (HeFH) who lack LDL receptor, torcetrapib/atorvastatin combination not only raised HDL-c but also decreased LDL-c significantly when compared to atorvastatin alone.[63] In patients who have hypertriglyceridemia, torcetrapib/atorvastatin combination had an enhanced LDL lowering effect.[63] When fenofibrate and ezetimibe were administered with torcetrapib and atorvastatin in healthy subjects, there was

no change in the pharmacokinetics of torcetrapib.[64,65]

Torcetrapib at the 60 mg dose was studied in three phase III atherosclerosis imaging trials. The Rating Atherosclerotic Disease Change by Imaging with a New CETP Inhibitor (RADIANCE) 1 and 2 trials were designed to determine the effect of torcetrapib on atherosclerosis progression in patients with HeFH and mixed dyslipidemia respectively.[66,67] After an initial run-in phase with atorvastatin, 850 patients were randomized to receive atorvastatin/torcetrapib combination or atorvastatin monotherapy. At the end of 2 years, despite significant rise in HDL-c and reduction in LDL-c levels, there was no difference in the progression of carotid IMT among the intervention and control groups in both RADIANCE 1 and 2.

The Investigation of Lipid Level Management Using Coronary Ultrasound to Assess Reduction of Atherosclerosis by CETP Inhibition and HDL Elevation (ILLUSTRATE) trial studied the effect of torcetrapib on coronary atherosclerosis progression. Patients who have CHD were pretreated with atorvastatin to reach an LDL goal of $\leq$of 100 mg/dL and then randomized to torcetrapib/atorvastatin or atorvastatin monotherapy.[63] All patients underwent intravascular ultrasonography (IVUS) at baseline and 77% of them had a repeat IVUS after 2 years. At the end of 2 years, there was no significant difference in the percent atheroma volume between the two groups. Posthoc analysis showed an inverse relationship between change in HDL-c level and both total atheroma volume and percent atheroma volume.[68]

Torcetrapib was also studied in a large phase III clinical outcome trial, the Investigation of Lipid Level Management to understand its Impact in Atherosclerotic Events (ILLUMINATE) trial.[69] ILLUMINATE randomized 15,067 patients who have CHD or CHD equivalents to torcetrapib/atorvastatin or atorvastatin after a run-in period with atorvastatin to reach an LDL goal of $\leq$100 mg/dL. The trial was terminated on December 2, 2006 because of increased mortality in the torcetrapib arm and the clinical development of torcetrapib was terminated. Interim analysis at 12 months showed a 72% increase in HDL-c and 25% decrease in LDL-c in due to torcetrapib. There were more cardiovascular (49 versus 35) and noncardiovascular (40 versus 20) deaths in the torcetrapib group than the atorvastain group. Cancer (24 versus 14) and infections (9 versus 0) were the most common cause of noncardiovascular death. There were significant increases in systolic blood pressure of 5.4 mm Hg, increases in serum sodium and bicarbonate levels, and decreases in serum potassium in the torcetrapib group. Posthoc analysis showed that the aldosterone levels in the torcetrapib arm were significantly elevated at 3 months when compared to the atorvastatin-only group despite being similar at the beginning of the trial. Cardiovascular events were inversely proportional to the increase in HDL-c above the median.

It is likely that off-target effects of torcetrapib contributed to the adverse outcome in ILLUMINATE.[70] All phase III imaging trials of torcetrapib also showed a significant increase in systolic blood pressure in the torcetrapib arm (2.8 mm Hg, 5.1 mm Hg, 4.6 mm Hg increase in RADIANCE 1, 2 and ILLUSTRATE respectively). In rats (both normotensive and spontaneously hypertensive models), which naturally lack CETP, torcetrapib increased blood pressure dose-dependently with a concomitant increase in gene expression of renin angiotensin system (RAS) and endothelin-1 from the adrenal glands as well as aorta (**Fig. 1**).[71] Torcetrapib caused an acute elevation in blood

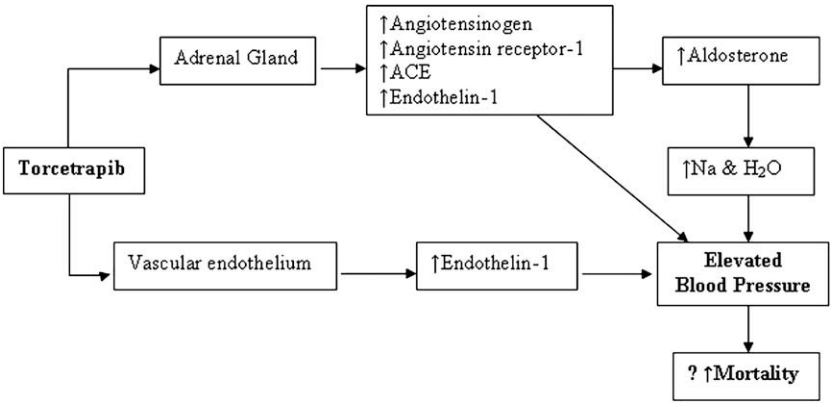

Fig. 1. Off-target effects of torcetrapib. ACE, angiotensin converting enzyme.

pressure through its interaction with adrenal glands in rats, rabbits and nonhuman primates despite the use of various receptor-blocking agents to counteract the increase in blood pressure.[72] Structural analogs of torcetrapib devoid of CETP inhibitory function still raised blood pressure in animal models suggesting that the blood pressure effect is not through CETP inhibition.[73] These studies suggest that the elevation in the blood pressure in the ILLUMINATE trial is molecule specific and not class specific. It is unclear whether the mechanisms leading to the blood pressure accounted for the adverse cardiovascular events in ILLUMINATE, but it is likely that off-target effects of torcetrapib at least contributed to the increased cardiovascular risk in this trial.[70]

POTENTIAL ADVERSE EFFECTS VERSUS BENEFITS OF CETP INHIBITION

Despite substantial increases in HDL-c levels, the impact of CETP inhibition with torcetrapib on atherosclerosis and CV events is disappointing.[63,67,69] As noted above, the off-target effects of torcetrapib undoubtedly played a role in the disappointing outcomes. However, this experience has focused increased attention on whether the mechanism of CETP inhibition is beneficial or could even be adverse despite increasing HDL-c levels. Specifically, it has been suggested that CETP inhibition might impair RCT by generating HDL particles that are less competent at promoting macrophage cholesterol efflux and/or by reducing the return of HDL-derived cholesterol to the liver for biliary excretion. Although earlier data suggested that HDL from CETP deficient subjects could be defective in promoting cholesterol efflux, more recent data suggests that CETP deficient HDL is as good or even better at promoting efflux, particularly via the ABCG1 pathway.[74] Furthermore, HDL from torcetrapib-treated subjects (120 mg daily) was also shown to increase macrophage cholesterol efflux primarily through ABCG1,[75] possibly enhanced by increased content of LCAT and apoE. Thus the concept that CETP inhibition impairs cholesterol efflux is not supported by experimental data; however, promotion of cholesterol efflux may require adequate increases in HDL-c levels.

The issue of whether CETP inhibition could impair RCT by slowing the return of HDL-derived cholesterol to the liver is more complex. It appears that in healthy normolipidemic humans, most HDL cholesteryl ester (CE) is shuttled back to the liver after transfer to apoB-containing lipoproteins via the CETP pathway.[11] Indeed, in wild-type mice, introduction of CETP expression promoted RCT from macrophages to feces, although in LDL receptor–deficient mice, CETP expression had the opposite effect,[76] suggesting that the effect of CETP inhibition on RCT may depend on the efficiency of hepatic uptake of apoB-containing lipoproteins. Torcetrapib did not change fecal cholesterol excretion or biomarkers of cholesterol or bile acid synthesis,[59] but these are crude markers of RCT and the effect of CETP inhibition on RCT in humans has yet to be definitively tested.

In addition to promoting RCT, HDL has several other properties including anti-inflammatory, antioxidant and antithrombotic properties, which confer added atheroprotection. The anti-inflammatory properties include inhibition of expression of adhesion molecules in endothelial cells, thereby decreasing monocyte recruitment in the arterial wall.[77] By its antioxidant property, it inhibits the oxidation of LDL.[77] HDL improves the milieu of endothelial cells by increased production of nitric oxide (NO) through up regulation of endothelial NO synthase and decreased apoptosis through SRB1 initiated signaling.[78,79] HDL also demonstrates antithrombotic effects by a variety of mechanisms including activation of prostacycline synthesis, attenuation of tissue factor expression, and decreased thrombin generation.[78]

CETP inhibition also has favorable effects on apoB-containing lipoproteins. As reviewed above, torcetrapib consistently reduced LDL-c levels by 20%–25%. Torcetrapib decreased the VLDL, IDL, and LDL apoB-100 pools by increasing the clearance of apoB-100.[80] In the presence of atorvastatin, torcetrapib decreased VLDL apoB-100 by increasing clearance and LDL apoB-100 by decreasing its production.[80] Torcetrapib, significantly reduces the postprandial formation and accumulation of atherogenic triglyceride-rich subspecies including chylomicrons and VLDL-1, as well as reduces their core CE content.[81] Although most animal studies have suggested that CETP inhibition decreases the progression of atherosclerosis,[73,82,83] a recent study failed to show an effect beyond that achieved by atorvastatin alone.[84]

CETP INHIBITORS IN CLINICAL DEVELOPMENT

JJT-705 (Japan Tobacco, Tokyo, Japan), also called the RO4607381, was the first CETP inhibitor to report human phase I trials. JTT-705 has been shown to ubiquitously inhibit CETP activity in rabbits, hamsters, and marmosets while increasing HDL-c levels and decreased the ratio of non-HDL-c to HDL-c.[85] Inhibition of CETP activity by

JTT-705 in rabbits not only increased the quantity of HDL, but also favorably affected the size distribution of HDL subpopulations and increased apo-E levels as well as the enzyme activity of paraoxonase and platelet-activating factor acetylhydrolase in HDL.[86] In a phase II trial for 4 weeks, JJT-705, administered at a dose of 900 mg, increased HDL-c by 34% and decreased CETP activity by 37% in healthy subjects with mild hyperlipidemia without any significant adverse effects.[87] In a small, randomized, placebo-controlled study, type II hyperlipidemic patients on pravastatin were randomized to JJT-705 or placebo. At the end of 4 weeks, the drug was well tolerated with statins and there was 28% increase in HDL-c level and 30% decrease in CETP activity.[88] In-depth analysis of 5 phase II trials of RO4607381/JTT-705 that include patients who have type II dyslipidemia, CHD or CHD risk equivalents randomized to RO4607381/JTT-705 or placebo in combination with different statins have shown a favorable safety profile of the drug with no differences in the cardiac or vascular adverse effects among the active and placebo arm.[89,90] In preclinical studies, and in contrast with torcetrapib, RO4607381/JTT-705 had no impact on blood pressure or RAS gene expression.[71]

Anacetrapib is another CETP inhibitor in clinical development. Two phase I randomized double blinded trials were conducted: one to study the pharmacokinetics and pharmacodynamics of anacetrapib in patients who have dyslipidemia; and the other, to study the effect of anacetrapib on ambulatory blood pressure in healthy individuals.[91] In the first, 50 patients with LDL-c between 100–190 mg/dL underwent a 2–4 week washout of their lipid lowering therapy followed by a 2-week diet run-in period prior to randomization. They were randomized to receive one of four doses of anacetrapib — 10, 40, 150, and 300 mg — or placebo, administered once a day for 28 days with a meal. At the end of 4 weeks, there was a dose-dependent increase in HDL-c from 41% in the 10 mg group to 129% in the 300 mg group, an increase in apoA-1 from 24% to 41%, and a dose-dependent decrease in LDL-c from 5% to 38%. An ambulatory blood pressure study used a crossover design in which 22 healthy subjects were randomized to 150 mg of anacetrapib or matching placebo for 10 days followed by a 14-day washout period before the crossover treatment began. Continuous 24-hour ambulatory blood pressure was measured on day −1 and last day of the treatment periods. At the end of the trial, there was no difference in the systolic or diastolic pressure between the treatment and placebo group. In both of the studies, the drug was well tolerated with no differences in adverse effects among the study and placebo group. In preclinical studies, and in contrast with torcetrapib, anacetrapib did not cause elevation in blood pressure or plasma levels of adrenal corticosteroids.[72]

SUMMARY

Though the torcetrapib experience was a major blow to CETP inhibition and indeed the entire field of HDL-targeted therapeutics, it was not fatal. The off-target effects of torcetrapib appear to be substantial and may have overridden any potential cardiovascular benefit. Despite continued uncertainty regarding the cardiovascular implications of genetic CETP deficiency and pharmacologic CETP inhibition, there remain reasons to believe in the mechanism and the possibility that clean CETP inhibitors will not only improve plasma lipids, but that they will reduce cardiovascular risk. However, there will be substantial scrutiny of the CETP inhibitors in clinical development and the only hope of registration is a positive clinical endpoint trial with acceptable safety. Currently, there is still life after torcetrapib; future developments will determine just how long that life will last.

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