CURRENT OPINION

LDL-cholesterol reduction in patients with hypercholesterolemia by modulation of adenosine triphosphate-citrate lyase and adenosine monophosphate-activated protein kinase

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Purpose of review
To review the profile of ETC-1002, as shown in preclinical and clinical studies, including LDL-cholesterol (LDL-C)-lowering activity and beneficial effects on other cardiometabolic risk markers as they relate to the inhibition of adenosine triphosphate-citrate lyase and the activation of adenosine monophosphate-activated protein kinase.

Recent findings
ETC-1002 is an adenosine triphosphate-citrate lyase inhibitor/adenosine monophosphate-activated protein kinase activator currently in Phase 2b clinical development. In seven Phase 1 and Phase 2a clinical studies, ETC-1002 dosed once daily for 2–12 weeks has lowered LDL-C and reduced high-sensitivity C-reactive protein by up to 40%, with neutral to positive effects on glucose levels, blood pressure, and body weight. Importantly, use of ETC-1002 in statin-intolerant patients has shown statin-like lowering of LDL-C without the muscle pain and weakness responsible for discontinuation of statin use by many patients. ETC-1002 has also been shown to produce an incremental benefit, lowering LDL-C as an add-on therapy to a low-dose statin. In over 300 individuals in studies of up to 12 weeks, ETC-1002 has been well tolerated with no serious adverse effects.

Summary
Because adenosine triphosphate-citrate lyase and adenosine monophosphate-activated protein kinase play central roles in regulating lipid and glucose metabolism, pharmacological modulation of these two enzymes could provide an important therapeutic alternative for statin-intolerant patients with hypercholesterolemia.

Keywords
adenosine monophosphate-activated protein kinase, adenosine triphosphate-citrate lyase, ETC-1002 (8-hydroxy-2,2,14,14 tetramethylpentadecanedioic acid), LDL-cholesterol, statin intolerance

INTRODUCTION

Despite recent medical advances in cholesterol management, cardiovascular disease (CVD) remains a leading cause of death and disability [1,2]. As elevated levels of LDL-cholesterol (LDL-C) represent a significant modifiable risk factor for CVD, hypercholesterolemia has become a primary target for lipid-lowering therapies and for reduction of the risk of major adverse cardiac events (MACE) [1,3,4]. Inhibition of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase with statins remains a leading therapeutic strategy to treat hypercholesterolemia and to reduce cardiovascular risk [3]. In addition to LDL-C lowering, statins reduce high-sensitivity C-reactive protein (hsCRP) levels and to limit chronic low-grade inflammation, which often coincides with altered lipid metabolism and represents a hallmark feature of coronary artery disease [5▪,6]. Although the benefits of using statins to effectively control LDL-C and

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KEY POINTS

- Pharmacological modulation of ACL and AMPK is a promising therapeutic strategy to lower LDL-C levels in hypercholesterolemic patients who cannot tolerate, or are unable to fully benefit from, statin therapy.

- ETC-1002 is an oral ACL inhibitor/AMPK activator currently in Phase 2b clinical development for treatment of hypercholesterolemic patients with and without a history of statin intolerance.

- ETC-1002, as monotherapy or in combination with a statin, has effectively reduced LDL-C in hypercholesterolemic patients with and without type 2 diabetes, including those with documented statin intolerance.

- In addition to LDL-C-lowering benefits, ETC-1002 has reduced levels of hsCRP and may have positive effects on blood pressure, glycemic control and body weight.

hsCRP levels and to reduce cardiovascular risk are well documented, a significant subset of patients is unable to take full advantage of statin treatment because of muscle pain or weakness, or the risk of increased blood glucose levels. Approximately 12% of patients on statins discontinue therapy, citing myalgia as the primary reason for discontinuation [7]. More than two million adults in the USA are considered to be statin intolerant. Poor statin adherence can lead to worsening of cardiovascular outcomes and increased risk of MACE [7–9]. Recently, the Food and Drug Administration has issued a warning that statins may increase the risk of elevated blood glucose and new-onset type 2 diabetes [10]. This regulatory warning reinforces the growing need for new therapeutic options to lower LDL-C levels in patients with hypercholesterolemia and diabetes or at risk for developing diabetes.

The present review will focus on adenosine triphosphate-citrate lyase (ACL) and adenosine monophosphate-activated protein kinase (AMPK) as promising targets for the development of novel lipid-lowering therapies designed not only to yield statin-like benefits in hypercholesterolemic patients but also to avoid side-effects associated with statin treatment. In addition to target justification, we will describe the mechanism of action [11,12] and review the most recent clinical data [13,14] for ETC-1002 (8-hydroxy-2,2,14,14 tetramethylpentadecanedioic acid) – the only dual ACL inhibitor/AMPK activator currently in Phase 2 clinical development for the treatment of hypercholesterolemia in patients with and without a history of statin intolerance or at risk of worsening glycemic control [15,16].

ADENOSINE TRIPHOSPHATE-CITRATE LYASE AND ADENOSINE MONOPHOSPHATE-ACTIVATED PROTEIN KINASE: THERAPEUTIC TARGET JUSTIFICATION

ACL is an extra-mitochondrial enzyme that is highly expressed in lipogenic tissues such as liver and adipose [17]. ACL catalyzes the cleavage of mitochondrial-derived citrate to cytosolic acetyl-CoA and oxaloacetate, with acetyl-CoA serving as a common substrate for de-novo cholesterol and fatty acid synthesis [18–23]. Transcription of ACL is controlled by the sterol regulatory element-binding protein-1 (SREBP-1) which is highly responsive to nutritional status through insulin signaling and glucose metabolites [24,25]. In the lipogenic state, ACL expression and cytosolic citrate levels are elevated and serve as a critical link between glycolysis and lipid synthesis and storage. Because of its strategic position in cholesterol biosynthetic pathways, ACL inhibition is considered to be an attractive therapeutic strategy for the reduction of elevated LDL-C levels.

Inhibition of de-novo hepatic cholesterol synthesis by statins, highly potent and selective inhibitors of HMG-CoA reductase, represents an effective therapeutic approach to reduce LDL-C [26–28]. The primary mechanism linking inhibition of hepatic sterol synthesis to reductions in LDL-C involves compensatory upregulation of sterol regulatory element-binding protein-2 (SREBP2)-dependent gene transcription in response to reduced intracellular cholesterol levels [29]. Induction of SREBP-2 activity triggers a transcriptional program aimed to restore intracellular cholesterol via a concurrent increase in cholesterogenic enzymes and upregulation of LDL receptor expression [29]. As a consequence, elevated LDL receptor activity increases the fractional catabolic rate of LDL particles in the blood, thus reducing LDL-C [29]. Similar to the mechanism described for statins, pharmacological inhibition of ACL limits de-novo cholesterol synthesis and increases LDL receptor activity [30]. Unlike statins, however, this occurs without direct inhibition of HMG-CoA. Furthermore, inhibition of ACL also reduces de-novo fatty acid synthesis, resulting in decreased malonyl-CoA levels, and subsequent enhancement of CPT-1-dependent mitochondrial transport of long-chain fatty acids for β-oxidation. This switch from fatty acid synthesis to β-oxidation limits the fatty acid availability for synthesis of cholesteryl esters, triglycerides, and VLDL secretion [20]. Consequently, reduced VLDL production in response to ACL inhibition may even further contribute to LDL-C lowering because VLDL serves

as the main metabolic precursor for LDL particle formation.

AMPK is a heterotrimeric complex of an α-catalytic and βγ-regulatory subunits that modulates lipid and carbohydrate metabolism, immune response, protein synthesis, and cell growth [31]. As a key regulator of energy homeostasis [32,33], AMPK controls cellular metabolism by phosphorylating key enzymes, transcription factors, and coactivators which results in a metabolic shift from anabolic processes, including gluconeogenesis and lipid synthesis, to catabolic processes such as fatty acid β-oxidation [31]. Although acute regulation of lipid metabolism by AMPK is linked to inhibitory phosphorylation of the rate-limiting enzymes of fatty acid and cholesterol synthesis [34–36], the more sustained control of lipid homeostasis by AMPK is mediated via transcriptional program which combines inhibition of SREBP1-dependent fatty acid synthase expression, and upregulation of mitochondrial fatty acid oxidation through the activation of peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α) [37]. Similarly, the long-term regulatory role of AMPK in carbohydrate metabolism involves complex transcriptional control of the gluconeogenic enzymes phosphorenol pyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G-6-Pase) [38–41]. In tandem, these acute and chronic mechanisms are expected to reverse elevated triglyceride storage, increase insulin sensitivity, and improve glycemic control in the liver [42].

As inception and propagation of the immune response is a highly energy-demanding process [43■,44], AMPK activation also limits inflammation by directly suppressing the anabolic events in immune cells and restricting energy availability for protein biosynthesis [43■,44]. This occurs largely through the complex overlapping networks of the sirtuin family of protein deacetylases, PGC1α and PGC1β, as well as mTOR signaling [43■,44,45,46■] and leads to transition into the Th2/M2 anti-inflammatory phenotype [47–49], inhibition of NF-kB, RelA/p65, and MAP kinase signaling, and reduced production of soluble mediators of inflammation by the immune cells [11,46■,50,51].

ETC-1002: MECHANISM OF ACTION

ETC-1002 is a novel, small molecule, dual ACL inhibitor/AMPK activator that beneficially modulates lipid, lipoprotein and carbohydrate metabolism, and inflammation (Fig. 1). ETC-1002 was selected from a library of structural analogues based on its ability to mediate equipotent inhibition of de-novo cholesterol and fatty acid synthesis in primary hepatocytes and favorably modify serum lipid variables in obese female Zucker (fa/fa) rats [52].

In a series of in-vitro and in-vivo follow-up studies aimed at elucidating the underlying
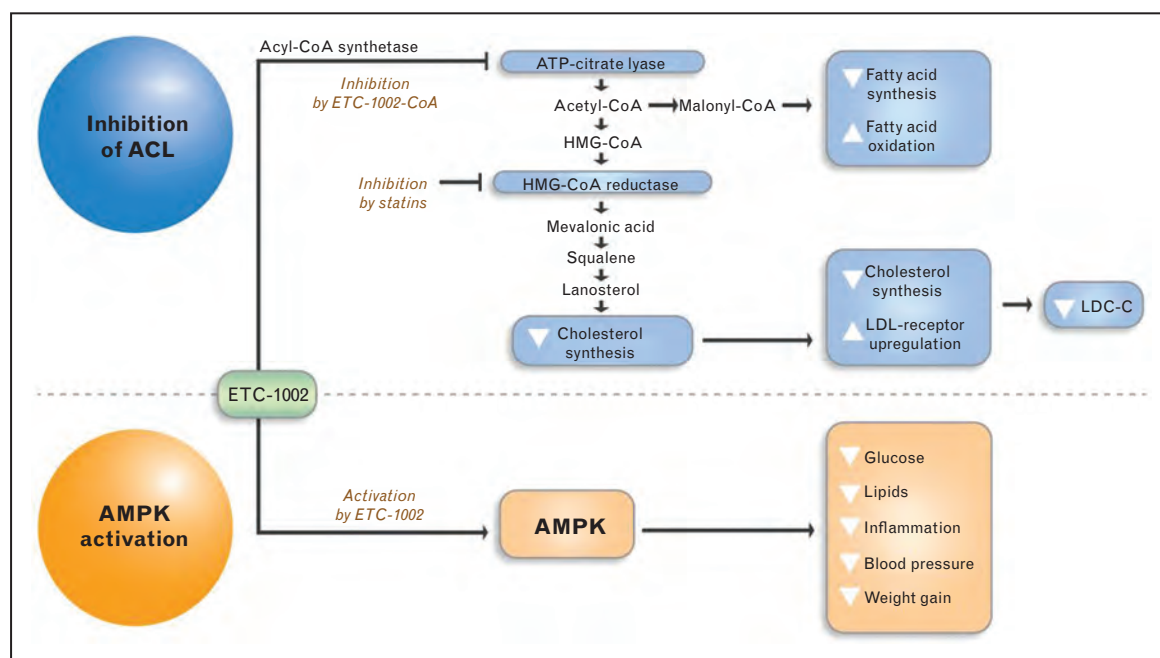

FIGURE 1. ETC-1002 Mechanism of Action. ETC-1002 is an ACL inhibitor/AMPK activator that beneficially modulates lipid, lipoprotein, and carbohydrate metabolism, and inflammation. ETC-1002 reduces LDL-C via inhibition of ACL, an enzyme that is upstream of HMG-CoA reductase in the cholesterol synthesis pathway. Activation of AMPK by ETC-1002 is complementary to ACL inhibition, and mediates beneficial effects on other cardiometabolic risk markers.

molecular mechanisms for equipotent inhibition of lipid synthesis, formation of ETC-1002-Coenzyme A (ETC-1002-CoA) thioester has been identified as a required step for ETC-1002-mediated inhibition of lipid synthesis [12]. Quantitative tracking of multiple metabolic intermediates of lipid synthesis provided further insights to the point of inhibition and identified ACL as a primary target based on reduction in all metabolites downstream of ACL coupled with concomitant increase in ACL substrate – citrate. Consistently, in a cell-free assay, ETC-1002-CoA directly inhibited recombinant human ACL via competitive inhibition for coenzyme A, whereas neither ETC-1002 nor ETC-1002-CoA inhibited partially purified rat HMG-CoA reductase activity [52]. Importantly, ETC-1002 treatment was also associated with a concentration-dependent increase in LDLR activity *in vitro* (Esperion's unpublished data). ETC-1002 was also shown to activate AMPK in a Ca^{2+}/calmodulin-activated protein kinase kinase β (CaMKKβ)-independent and liver kinase β (LKB1)-dependent manner, without inducing detectable changes in adenylate energy charge (AEC). Furthermore, in primary rat hepatocytes, ETC-1002 reduced both glucagon-dependent glucose production as well as the expression of PEPCK and G-6-Pase [12].

In immune cells treated with ETC-1002, increased levels of AMPK phosphorylation coincided with reduced activity of JNK and p38 MAP kinases along with decreased production of proinflammatory cytokines and chemokines [11]. siRNA-mediated gene silencing confirmed that ETC-1002 activates macrophage AMPK and exerts its anti-inflammatory effects via a mechanism dependent on the LKB1/AMPK axis [11]. Consequently, ETC-1002 diminished homing of neutrophils and macrophages into the disease site and decreased adipose mass, IL-6 release as well as macrophage presence in the inflamed tissue [11].

Thus, inhibition of ACL and activation of AMPK by ETC-1002 represent a unique tandem of complementary activities aimed to correct imbalances in lipid and carbohydrate metabolism. Indeed, in preclinical models of hypercholesterolemia, diet-induced and genetic models of obesity/diabetes, high-fat/high-cholesterol-fed models of atherosclerosis, and genetic models of hypertension, use of ETC-1002 has lowered LDL-C, reduced glucose/ insulin levels, decreased body weight gain without altering food intake, reduced the progressive development of atherosclerotic plaques, lowered inflammatory markers linked to atherosclerosis, and reduced blood pressure [11,12]. Subsequent clinical translation of these benefits could potentially enable ETC-1002 as an alternative therapy designed to control LDL-C levels in patients with dyslipidemia and a history of statin intolerance.

ETC-1002: CLINICAL EVALUATION

ETC-1002 is the only, orally available, once-daily dual ACL inhibitor/AMPK activator currently in Phase 2b clinical development. To date, ETC-1002 has been evaluated in seven completed clinical studies (Table 1).

Hypercholesterolemia

In a multicenter, randomized, double-blind, placebo-controlled study of 177 patients with elevated LDL-C

Table 1. Summary of LDL-C lowering by ETC-1002 in seven completed Phase 1 and Phase 2a clinical studies

Study number	Title	LDL-C lowering[a]	Dose range (mg)	Treatment duration
001	Phase 1a single-dose tolerance, $N=18/18$	ND	2.5, 10, 45, 125, 250	Single dose
002, 004	Phase 1b multiple-dose tolerance, $N=77/57$	Up to 36%	20, 60, 100, 120, 140, 180, 220	2 weeks/4 weeks
003	Phase 2a proof of concept in hypercholesterolemic patients, $N=177/133$	Up to 27%	40, 80, 120	12 Weeks
005	Phase 2a proof of concept in patients with hypercholesterolemia and type 2 diabetes, $N=60/30$	43%	80, 120	4 Weeks
006	Phase 2a proof of concept in patients with hypercholesterolemia and a history of statin intolerance, $N=56/37$	32%	60, 120, 180, 240	8 Weeks
007	Phase 2a in patients with hypercholesterolemia added on to 10 mg atorvastatin, $N=58/42$	22%	60, 120, 180, 240	8 Weeks

Total individuals: 446; Treated Individuals: 317.
[a]Average LDL-C % change from baseline.

(130–220 mg/dl), ETC-1002 administered at 40-mg, 80-mg, and 120-mg daily significantly lowered LDL-C levels in a dose-dependent manner by -17.9 ± 2.2, -25.0 ± 2.1, and $-26.6 \pm 2.2\%$, respectively, versus a reduction of $-2.1 \pm 2.2\%$ with placebo (Table 1) [13]. Maximum LDL-C reduction was independent of baseline triglyceride levels, occurred within 2 weeks of treatment, and was maintained for the remaining 10 weeks of the study [13]. LDL-C lowering was accompanied by reductions in non-high-density lipoprotein-cholesterol (non-HDL-C), apoB, and LDL particle number at all doses. A post-hoc analysis revealed reductions in plasma levels of hsCRP of up to 63.5% versus a 7% reduction with placebo in patients with elevated hsCRP levels (≥ 2 mg/l) at baseline [13].

Type 2 diabetes

A single-center, double-blind, placebo-controlled, in-clinic study evaluated the efficacy of ETC-1002 in 60 patients with type 2 diabetes and elevated LDL-C [14]. Patients discontinued all diabetes and lipid-regulating drugs and were randomized to receive ETC-1002 80 mg for 2 weeks followed by 120 mg daily for an additional 2 weeks or placebo for 4 weeks [14]. At the end of the study, LDL-C was reduced by 43% in the ETC-1002 group compared with 11% in the placebo group [14]. Reductions in LDL-C levels occurred across a broad range of baseline LDL-C and triglyceride values. The ETC-1002-treated group also showed significant reductions in non-HDL-C and total cholesterol. In addition, ETC-1002 treatment lowered hsCRP by 41% compared with 11% with placebo [14]. Importantly, ETC-1002 treatment did not result in a worsening of glycemic control. A nonsignificant reduction of all prespecified glycemic markers was observed with ETC-1002 treatment compared with placebo [14]. A post-hoc analysis of a subgroup of patients with mild elevations in blood pressure, showed that treatment with ETC-1002 decreased both systolic and diastolic blood pressure by -2.4 and -7.3 mm Hg, respectively, compared with placebo [14].

Statin intolerance

A proof-of-concept clinical study was designed to evaluate LDL-C lowering by ETC-1002 in patients with hypercholesterolemia and a history of intolerance to two or more statins. A total of 56 patients were evaluated in this study. Three patients in the placebo group withdrew from the study because of muscle-related adverse events, whereas no patients in the ETC-1002 group withdrew for these reasons

Table 2. Safety and tolerability of ETC-1002 in patients with a history of statin intolerance

	ETC-1002	PBO
Adverse event occurrence	70%	79%
Muscle-related AEs	27%	32%
Discontinuation rates	14%	16%
Patients discontinuing because of muscle-related AEs	0	3

AE, adverse event.

(Table 2). ETC-1002 lowered LDL-C by an average of 32% compared with an LDL-C reduction of 3% in the placebo group. Consistent with previous studies, hsCRP was also significantly reduced by 42% after 8 weeks of ETC-1002 therapy (Esperion's unpublished results).

Add-on therapy

A multicenter study was designed to evaluate ETC-1002 for 8 weeks in patients with hypercholesterolemia receiving 10 mg of atorvastatin. In this study, ETC-1002 demonstrated incremental LDL-C lowering of up to 22% when added to atorvastatin and was well tolerated (Esperion's unpublished results).

Safety

ETC-1002 was generally safe and well tolerated in all seven clinical studies. Adverse event rates were comparable between the ETC-1002 and placebo groups (Table 3). No drug-related serious adverse events have been observed in patients treated with ETC-1002 [13,14].

Table 3. ETC-1002 overall clinical safety summary

Number of individuals with drug-related safety findings	ETC-1002 (*N* = 317)
Overview of serious AEs	
Serious AEs	0
Key lab abnormalities (repeated and confirmed)	
ALT/AST > 3 × ULN	1[a]
CK > 5 × ULN	0
Total bilirubin > 2 × ULN	0
Creatinine > 0.5 mg/dl ULN	0

AE, adverse event; ALT, alanine transaminase; AST, aspartate transaminase; CK, creatine kinase; ULN , upper limit normal.
[a]This lab abnormality (ALT/AST 3× ULN in a single patient) was assessed by the Investigator as probably related to study medication.

CONCLUSION

With the recently added regulatory warnings, it has become increasingly clear that a significant subset of patients is unable to take full advantage of statin treatment because of muscle pain or weakness and a risk of increased blood glucose levels. In contrast, modulation of ACL and AMPK offers an innovative alternative to standard-of-care lipid management that may address the growing need for new therapeutic options for hypercholesterolemic patients with and without a history of statin intolerance or at risk of worsening glycemic control.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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