



# Cholesterol Efflux Capacity and Pre-Beta-1 HDL Concentrations Are Increased in Dyslipidemic Patients Treated With Evacetrapib

Stephen J. Nicholls, MD, PhD,\* Giacomo Ruotolo, MD, PhD,† H. Bryan Brewer, MD,‡ John P. Kane, MD, PhD,§ Ming-Dauh Wang, PhD,† Kathryn A. Krueger, MD,† Steven J. Adelman, PhD,|| Steven E. Nissen, MD,¶ Daniel J. Rader, MD#

## ABSTRACT

**BACKGROUND** Potent cholesteryl ester transfer protein (CETP) inhibitors have been shown to substantially increase high-density lipoprotein cholesterol (HDL-C) and apolipoprotein A-I levels as monotherapy and combined with statins. However, data on the effects of this class of drugs on macrophage cholesterol efflux capacity (CEC), a functional assay that characterizes a key step in the process of reverse cholesterol transport, are limited.

**OBJECTIVES** This study assessed the impact of evacetrapib, statins, or combination therapy on CEC.

**METHODS** We analyzed samples from 377 subjects with elevated low-density lipoprotein cholesterol (LDL-C) or low HDL-C levels who were enrolled in a phase 2 trial of evacetrapib. Percent changes from baseline in CEC (total, non-ABCA1-, and ABCA1-specific) and HDL subpopulations were evaluated after 12 weeks of treatment with placebo, statin monotherapy, evacetrapib monotherapy, or evacetrapib combined with statins. Pre-beta-1 HDL levels were quantified by immunofixation and nondenaturing 2-dimensional gel electrophoresis (2DGE).

**RESULTS** Relative to placebo, evacetrapib monotherapy increased dose-dependent total and non-ABCA1-specific CEC up to 34% and 47%, respectively. Evacetrapib monotherapy also increased ABCA1-specific CEC up to 26%. Relative to statin monotherapy, evacetrapib with statins also increased total, non-ABCA1-, and ABCA1-specific CEC by 21%, 27%, and 15%, respectively. In contrast, rosuvastatin and simvastatin significantly reduced total and ABCA1-specific CEC, whereas atorvastatin had no significant effect. Consistent with ABCA1-specific CEC, evacetrapib monotherapy and evacetrapib combined with statins significantly increased pre-beta-1 HDL levels as measured by either method.

**CONCLUSIONS** Evacetrapib, as monotherapy and combined with statins, not only increased total CEC, but also increased ABCA1-specific CEC and pre-beta-1 HDL. The mechanisms by which potent CETP inhibition increases ABCA1-specific CEC and pre-beta-1 HDL require further study. (A Study of LY2484595 in Patients With High LDL-C or Low HDL-C; [NCT01105975](https://doi.org/10.1016/j.jacc.2015.09.013)) (J Am Coll Cardiol 2015;66:2201-10) © 2015 by the American College of Cardiology Foundation. Published by Elsevier. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

From the \*South Australian Health and Medical Research Institute, University of Adelaide, Adelaide, Australia; †Cardiovascular Unit, Eli Lilly and Company, Indianapolis, Indianapolis; ‡MedStar Heart & Vascular Institute, MedStar Health, Washington, DC; §Cardiovascular Research Institute, University of California, San Francisco, California; ||Vascular Strategies, LLC, Plymouth Meeting, Pennsylvania; ¶Cardiovascular Medicine, Cleveland Clinic, Cleveland, Ohio; and the #Cardiovascular Institute, University of Pennsylvania, Philadelphia, Pennsylvania. Dr. Nicholls has received research support from AstraZeneca, Amgen, Anthera, Cerenis, Novartis, Eli Lilly and Company, Atheronova, Resverlogix, and LipoScience; and is a consultant for AstraZeneca, Omthera, CSL Behring, Boehringer Ingelheim, Novartis, Resverlogix, Eli Lilly and Company, Amgen, Sanofi-Regeneron, Merck, Pfizer, Takeda, and Roche. Drs. Ruotolo, Wang, and Krueger are stockholders of Eli Lilly and Company. Dr. Brewer serves as a consultant/speaker for Merck, Pfizer, Roche, Eli Lilly and Company, Amgen, CSL, Cerenis, and The Medicines Company. Dr. Nissen has received research support to perform clinical trials through the Cleveland Clinic Coordinating Center for Clinical Research from Amgen, Pfizer, AstraZeneca, Cerenis, Novartis, Takeda, The Medicines Company, and Eli Lilly and Company; and is a consultant and advisor for these pharmaceutical companies and Novo Nordisk, Boehringer Ingelheim, Bristol-Myers Squibb,

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## ABBREVIATIONS AND ACRONYMS

**2DGE** = nondenaturing  
2-dimensional gel  
electrophoresis

**ABC** = adenosine triphosphate-  
binding cassette transporter

**Apo** = apolipoprotein

**ASCVD** = atherosclerotic  
cardiovascular disease

**CEC** = cholesterol efflux  
capacity

**CETP** = cholesteryl ester  
transfer protein

**HDL-C** = high-density  
lipoprotein cholesterol

**LDL-C** = low-density  
lipoprotein cholesterol

**SR-B1** = scavenger receptor  
class B, type I

**P**otent cholesteryl ester transfer protein (CETP) inhibitors substantially increase high-density lipoprotein cholesterol (HDL-C) levels as monotherapy and in combination with statins (1-4). Levels of HDL-C are known to inversely associate with the prevalence and incidence of atherosclerotic cardiovascular disease (ASCVD) in epidemiological studies, independently of all other major risk factors (5). Randomized controlled trials with HDL-C-raising medications and genetic mechanisms that raise HDL-C levels have suggested that HDL-C may not be causally associated with ASCVD (6-9). However, Mendelian randomization studies have linked common CETP variants associated with low CETP activity, high HDL-C, and reduced LDL-C to a 4% lower risk of myocardial infarction (7).

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Cholesterol efflux from peripheral tissues is a key function of HDL particles, and the first step of reverse cholesterol transport to the liver for biliary secretion (10). Recent studies have shown that cholesterol efflux capacity (CEC) is inversely associated with prevalent ASCVD (11,12) and incident cardiovascular events in a population-based cohort without cardiovascular disease at baseline (11,13). These associations were independent of HDL-C and traditional cardiovascular risk factors, which suggested that the HDL-C level is only a modest biomarker of HDL function, and that CEC may be more closely correlated with cardiovascular outcomes.

Lipid-poor pre-beta-1 HDL particles are believed to be one of the primary acceptors of cholesterol effluxed from macrophages via the adenosine triphosphate-binding cassette transporter A1 (ABCA1) (14). ABCA1 is primarily localized in the plasma membrane of cells, and ABCA1-mediated cholesterol efflux to lipid-poor pre-beta-1 particles is essential for HDL formation. Thus, ABCA1-specific cholesterol efflux is generally correlated with lipid-poor pre-beta-1 HDL, but is not correlated with large HDL particles (15). However, although ABCA1 is a major mediator of macrophage cholesterol efflux, HDL also promotes macrophage cholesterol efflux via ABCG1

and probably other pathways as well (16). ABCG1 is also expressed in vascular endothelium, where it mediates cellular sterol efflux to large HDL particles (17). Thus, ABCA1 and ABCG1 have complementary functions in mediating cholesterol efflux to HDL, and their association with cardiovascular disease is not clear (18). However, data on the regeneration of pre-beta-1 HDL and the role of ABCA1-specific cholesterol efflux following pharmacological CETP inhibition are limited.

In this study, we assessed the changes from baseline in CEC and HDL subclasses after 12 weeks of treatment with evacetrapib monotherapy, statin monotherapy, or evacetrapib in combination with statins in dyslipidemic patients. The relationship among changes in CEC and CETP mass or activity, HDL subclass, HDL-C, and apolipoprotein A-I (apoA-I) concentrations was also assessed. We demonstrate that evacetrapib, at different doses as monotherapy and at the 100 mg/day dose in combination with statins, increased pre-beta-1, alpha-1, and alpha-2 HDL levels, with corresponding increases in both non-ABCA1- and ABCA1-specific CEC.

## METHODS

**STUDY DESIGN.** This was a multicenter, randomized, double-blind, parallel, placebo-controlled trial conducted among 398 patients with elevated low-density lipoprotein cholesterol (LDL-C) or low HDL-C. The details of the study design, conducted from April 2010 to January 2011 in the United States and Europe, have been previously reported (4). The study was conducted in accordance with the Declaration of Helsinki and the Guidelines for Good Clinical Practice. All patients provided informed consent, and the local institutional review committees approved the study.

This study evaluated the effects of 12 weeks of treatment with evacetrapib as monotherapy and in combination with statins. For the monotherapy evaluation, patients were randomly assigned to receive either placebo or evacetrapib at daily doses of 30, 100, or 500 mg. For the combination treatment groups, patients were randomly assigned to receive either placebo or evacetrapib 100 mg, in combination with atorvastatin 20 mg, rosuvastatin 10 mg, or simvastatin 40 mg. Changes from baseline in CEC,

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pre-beta-1 HDL, and HDL subclasses were evaluated after 12 weeks from available samples in 377 subjects.

**STUDY PARTICIPANTS.** Patient inclusion and exclusion criteria have been previously described (4). In brief, this study included patients who were at least 18 years old and were eligible on the basis of meeting low HDL-C or high LDL-C criteria, in the presence of triglyceride levels <400 mg/dl. Patients who met the low HDL-C criteria had an HDL-C level of <45 mg/dl for men or 50 mg/dl for women, with an LDL-C level that met the current National Cholesterol Education Program Adult Treatment Panel III goal. Patients who met the high LDL-C criteria had LDL-C levels between 100 and 190 mg/dl in the presence of 0 or 1 risk factors; LDL-C levels between 100 and 160 mg/dl with at least 2 risk factors and a 10-year coronary risk of <10%; or LDL-C levels between 100 and 130 mg/dl with at least 2 risk factors and a 10-year risk of 10% to 20%, in the presence of any level of HDL-C. Patients were excluded if they had any clinical manifestation of atherosclerotic disease, hypertension (systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg), documented hyperaldosteronism, uncontrolled diabetes (hemoglobin A<sub>1C</sub> ≥8%), or significant liver, kidney, cardiac, or neuromuscular disease.

**STUDY PROCEDURES.** Fasting blood samples were obtained at baseline and week 12 for the measurement of CEC, pre-beta-1 HDL, and HDL subclasses. CEC (total, ABCA1-specific, and non-ABCA1-specific) was quantified in blood samples by depleting patient serum of apoB particles using polyethylene glycol precipitation (11). The intra-assay and interassay coefficients of variation were 8% and 10% for total efflux, and 9% and 15% for ABCA1-specific efflux, respectively (11). Non-ABCA1-specific efflux was calculated as the difference between total and ABCA1-specific efflux. Pre-beta-1 HDL levels were measured in plasma using an immunofixation method validated by the isotope dilution technique by O'Connor et al. (19). Assays were run in duplicate with intra-assay and interassay coefficients of variation of 6% and 7%, respectively (20). HDL subclasses (alpha- and pre-alpha-1, -2, -3, and -4, and pre-beta-1) were also quantitatively determined by nondenaturing 2D gel electrophoresis (2DGE) (21,22); 2DGE samples were only available for 199 subjects at baseline and week 12. The coefficient of variation was <15% for all particles assessed using the nondenaturing 2DGE method.

**STATISTICAL ANALYSIS.** Demographic, baseline, and on-treatment data were summarized using frequencies for categorical variables and mean ± SD or

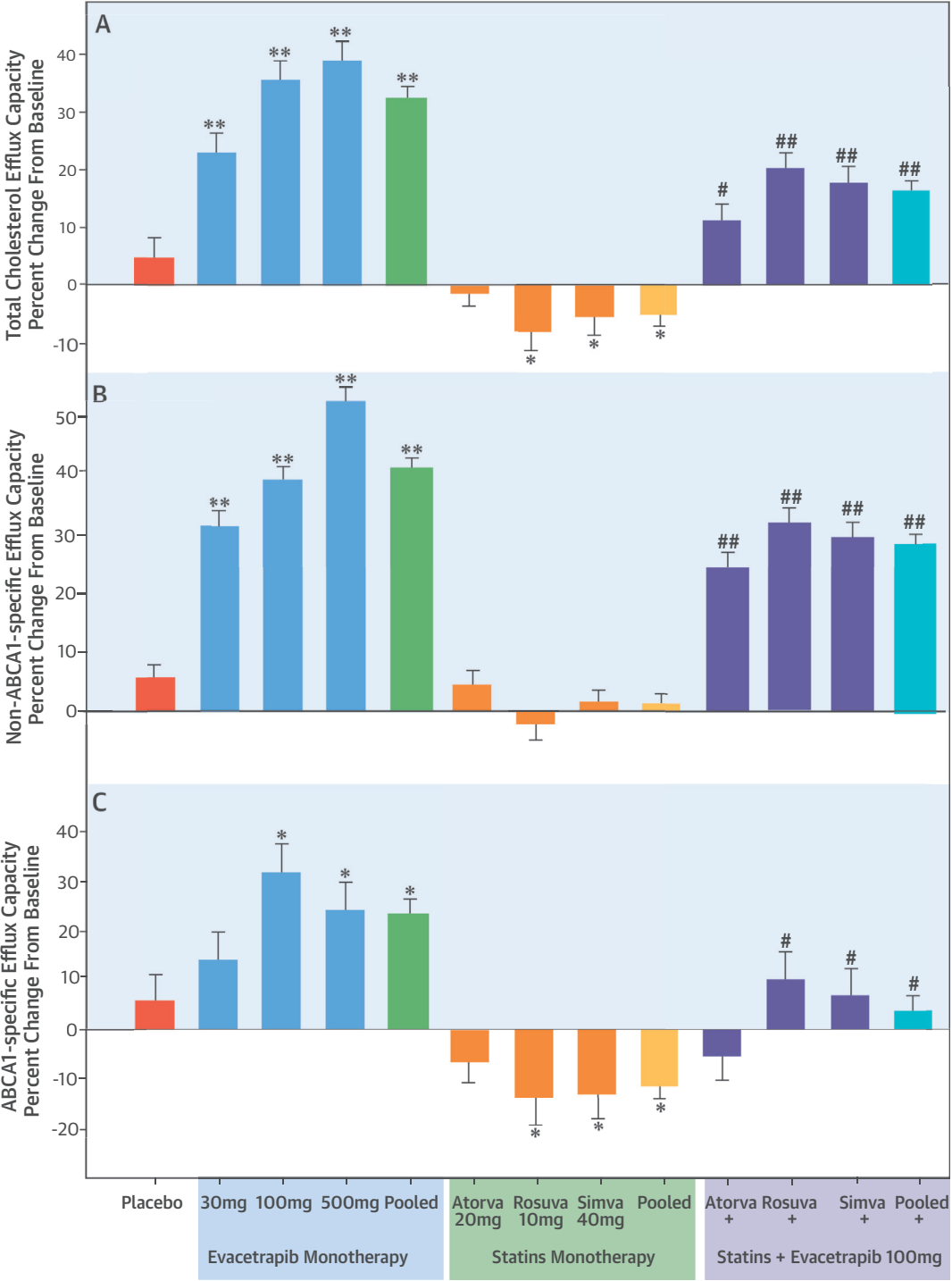
median and interquartile range for continuous variables. For each of the cholesterol efflux and HDL subclass measures, patients in the intention-to-treat population with both baseline and post-baseline values were analyzed using analysis of variance. The response variable of the model used for all analyses was percent change from baseline to week 12 or early discontinuation in the analyzed measure; the explanatory variables were baseline measurement and treatment. Results were reported as least-square means and 95% confidence intervals by treatment. For the evaluation of evacetrapib monotherapy, comparisons were made between individual evacetrapib dosages and placebo, as well as between pooled evacetrapib monotherapy and placebo. The statin combination evaluation reflected comparisons of statins in combination with evacetrapib 100 mg with respective individual statins. Pooled statins in combination with evacetrapib 100 mg were also compared with pooled statins. In addition, individual statins and pooled statins were compared with placebo. Correlations among the investigated measures were assessed by Pearson's correlation coefficients for their baseline levels, their levels at week 12 or early discontinuation, and their changes from baseline to week 12 or early discontinuation. The p values for the comparisons were presented, and statistical significance was indicated at  $p < 0.05$  and  $p < 0.001$ . All analyses were conducted using SAS version 9.2 (SAS Institute, Inc., Cary, North Carolina).

## RESULTS

**SUBJECT BASELINE CHARACTERISTICS.** The overall disposition of patients participating in this study has been previously reported (4). A total of 398 patients were randomized to receive placebo ( $n = 38$ ), statin monotherapy ( $n = 123$ ), evacetrapib monotherapy ( $n = 121$ ), or evacetrapib with statin combination therapy ( $n = 116$ ). Baseline characteristics were similar across all treatment groups. Baseline CEC and levels of HDL subpopulations are presented in [Online Tables 1 and 2](#).

**CHANGES IN CHOLESTEROL EFFLUX CAPACITY.** Percentage changes from baseline to week 12 in total, ABCA1-specific, and non-ABCA1-specific CEC are shown in the [Central Illustration](#) and [Online Tables 3 and 4](#) according to treatment. There was no significant change from baseline in the placebo group. Compared with placebo, evacetrapib monotherapy significantly increased total efflux capacity in a dose-dependent manner, ranging from an 18% increase at the 30-mg dose to a 34% increase at the 500-mg dose. These changes were paralleled by

**CENTRAL ILLUSTRATION Evacetrapib Enhances Total, Non-ABCA1-, and ABCA1-Specific Cholesterol Efflux Capacity, Both in Monotherapy and in Combination With a Statin**



Nicholls, S.J. et al. J Am Coll Cardiol. 2015; 66(20):2201-10.

**(A)** Total, **(B)** non-ABCA1-, and **(C)** ABCA1-specific cholesterol efflux capacity. \* $p < 0.05$ ; \*\* $p < 0.001$  versus placebo; # $p < 0.05$ ; ## $p < 0.001$  versus statin counterpart. Atorva = atorvastatin; Rosuva = rosuvastatin; Simva = simvastatin.

dose-dependent increases in non-ABCA1-specific efflux, which increased by up to 47% at the 500-mg dose. Moreover, evacetrapib monotherapy also significantly increased ABCA1-specific efflux at the 100- and 500-mg doses (17% overall and up to 26% across the 3 doses), but not in a dose-dependent fashion.

Compared with placebo, statin monotherapy (averaged across the 3 statins) decreased total, non-ABCA1-specific, and ABCA1-specific cholesterol efflux by 10%, 4%, and 17%, respectively. However, the 3 statins differed in their effects. Atorvastatin had no statistically significant effect on any of the efflux measures, whereas rosuvastatin and simvastatin both significantly reduced total efflux (–13% and –10%, respectively) and ABCA1-specific efflux (–20% and –19%, respectively).

When evacetrapib 100 mg was combined with statin therapy, efflux capacity significantly increased, but this effect was blunted compared with evacetrapib monotherapy. In a pooled analysis across the 3 statins, evacetrapib combined with statins increased total efflux by 21% (vs. statin monotherapy) compared with an increase of 31% (vs. placebo) with the same dose of evacetrapib as monotherapy. This same pattern was seen for non-ABCA1-specific and ABCA1-specific efflux (increases of 27% and 15% for evacetrapib with statins vs. 33% and 26% for evacetrapib 100 mg monotherapy, respectively). When combined with atorvastatin, evacetrapib had no significant effect on ABCA1-specific efflux.

**PRE-BETA-1 HIGH-DENSITY LIPOPROTEIN.** Percent changes from baseline to week 12 in pre-beta-1 HDL levels are shown in [Figure 1](#) by treatment. Consistent with the efflux data, pooled evacetrapib monotherapy significantly increased pre-beta-1 HDL levels measured by either immunofixation ([Figure 1A](#)) or 2DGE ([Figure 1B](#)) compared with placebo. In contrast, pooled statin monotherapy decreased pre-beta-1 HDL levels ( $p < 0.05$  for 2DGE). Consistent with the efflux data, pooled evacetrapib 100 mg combined with statins significantly increased pre-beta-1 HDL levels by either method ( $p < 0.05$  by immunofixation and  $p < 0.001$  by 2DGE compared with statin monotherapy), but the magnitude of the effect was smaller than that observed with evacetrapib 100 mg as monotherapy.

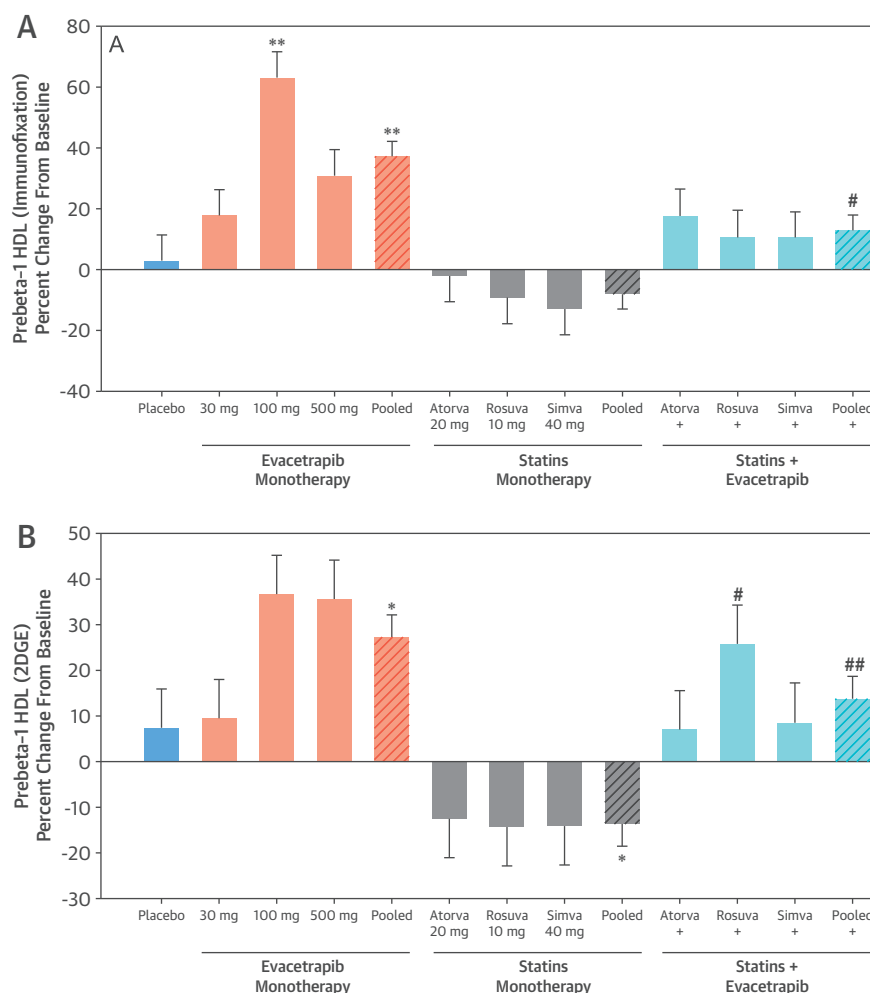
**APOLIPOPROTEIN A-I-CONTAINING HDL SUB-POPULATIONS.** Percent changes from baseline to week 12 in HDL subpopulations are shown in [Figure 2](#) and [Online Tables 5 and 6](#). Evacetrapib monotherapy significantly increased alpha-1 by 208%, pre-alpha-1 by 174%, and pre-beta-1 HDL by 45% compared with

placebo, and these increases were dose-dependent. Evacetrapib monotherapy also increased alpha-2 by 14% and decreased pre-alpha-3 by 24%, but these effects were not dose-dependent. Pooled statin monotherapy had no significant effect on HDL subpopulations, except for a 26% increase in pre-alpha-2 HDL levels compared with placebo. Pooled evacetrapib combined with statins also significantly increased alpha-1 by 164%, pre-alpha-1 by 106%, and pre-beta-2 by 40% compared with pooled statin monotherapy, and these increases were evident for all statins. As seen for evacetrapib monotherapy, an increase in alpha-2 and decrease in pre-alpha-3 were also observed with evacetrapib combined with statins versus statin monotherapy. Alpha-4 HDL levels were also significantly increased with evacetrapib combined with statins.

**CORRELATION ANALYSES.** Correlations between total, non-ABCA1-specific, or ABCA1-specific cholesterol efflux and lipid variables at baseline are shown in [Table 1](#). ApoA-I, apoB, apoE, HDL-C, alpha-1, and pre-beta-1 HDL levels were directly correlated with total cholesterol efflux at baseline. Relatively high ( $>0.5$ ) correlation coefficients were observed for correlations between non-ABCA1-specific cholesterol efflux and apoA-I, HDL-C, or alpha-1 HDL levels, and between ABCA1-specific efflux and pre-beta-1 HDL levels. At baseline, high-sensitivity C-reactive protein and CETP activity inversely correlated with total, ABCA1-, and non-ABCA1-specific efflux. No correlation was observed between CETP mass and cholesterol efflux.

ApoA-I, HDL-C, alpha-1, and pre-beta-1 HDL levels were also significantly correlated with total cholesterol efflux after 12 weeks of treatment, and were found in both pooled treatment groups (evacetrapib monotherapy and evacetrapib plus statins) ([Table 2](#)). Direct correlations between non-ABCA1-specific cholesterol efflux and apoA-I, HDL-C, or alpha-1 HDL levels, and between ABCA1-specific efflux and pre-beta-1 HDL levels were maintained on treatment, and were present in both pooled evacetrapib treatment groups. Non-ABCA1-specific efflux was correlated directly with on-treatment CETP mass and inversely with on-treatment CETP activity in the 2 pooled treatment groups. On-treatment CETP mass or activity was not correlated with ABCA1-specific efflux. On-treatment CETP activity and apoB concentrations were inversely correlated with non-ABCA1-specific efflux in the pooled evacetrapib plus statins group.

Correlations between percent changes from baseline in total, non-ABCA1-specific, or ABCA1-specific

**FIGURE 1** Percent Change in Pre-Beta-1 HDL as Measured by Immunofixation and 2DGE

Percent change in pre-beta-1 high-density lipoprotein (HDL) as measured by **(A)** immunofixation (sample sizes: placebo, n = 35; evacetrapib 30 mg, n = 35; evacetrapib 100 mg, n = 35; evacetrapib 500 mg, n = 34; pooled evacetrapib monotherapy, n = 104; atorvastatin+placebo, n = 35; rosuvastatin+placebo, n = 34; simvastatin+placebo, n = 35; pooled statins, n = 104; atorvastatin+evacetrapib, n = 32; rosuvastatin+evacetrapib, n = 31; simvastatin+evacetrapib, n = 35; pooled statins+evacetrapib, n = 98) and **(B)** nondenaturing 2-D gel electrophoresis (2DGE) (sample sizes: placebo, n = 20; evacetrapib 30 mg, n = 20; evacetrapib 100 mg, n = 20; evacetrapib 500 mg, n = 20; pooled evacetrapib monotherapy, n = 60; atorvastatin+placebo, n = 20; rosuvastatin+placebo, n = 20; simvastatin+placebo, n = 20; pooled statins, n = 60; atorvastatin+evacetrapib, n = 20; rosuvastatin+evacetrapib, n = 20; simvastatin+evacetrapib, n = 19; pooled statins+evacetrapib, n = 59). As shown, evacetrapib monotherapy or in combination with statin increased pre-beta-1 HDL levels assessed either by immunofixation or 2DGE. Results are presented as least-square mean  $\pm$  SE. \* $p < 0.05$ ; \*\* $p < 0.001$  (vs. placebo); # $p < 0.05$ ; ## $p < 0.001$  (vs. statin counterpart). Atorva = atorvastatin; Rosuva = rosuvastatin; Simva = simvastatin.

efflux and changes in lipid variables at week 12 are shown in [Table 2](#). Changes in CETP mass, HDL-C, and apoA-I were directly correlated, whereas the change in CETP activity and apoB were inversely correlated with changes in total and non-ABCA1-specific efflux in the 2 pooled evacetrapib treatment groups. Change in CETP mass was also directly correlated with changes in ABCA1-specific efflux in the 2 groups. Changes in pre-beta-1 HDL were directly correlated

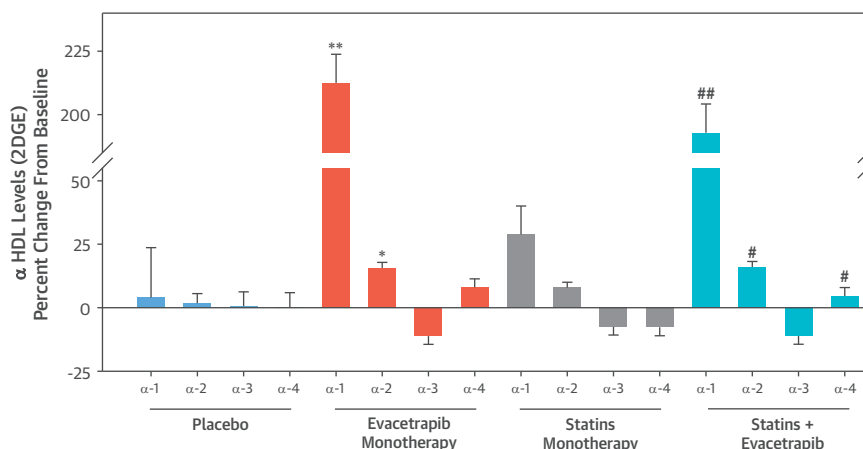
with total, non-ABCA1-, and ABCA1-specific efflux in the evacetrapib plus statin group, but were not correlated with the evacetrapib monotherapy group.

## DISCUSSION

This study represents the largest and most systematic evaluation of the effects of a CETP inhibitor on HDL CEC and on the related measure of pre-beta-1



**FIGURE 2** Percent Change in Alpha-1, -2, -3, and -4 HDL Subclasses by 2DGE



Evacetrapib monotherapy or in combination with statin increased alpha-1 and alpha-2 HDL levels. Results are presented as least-square mean  $\pm$  SE. \* $p < 0.05$ ; \*\* $p < 0.001$  (vs. placebo); # $p < 0.05$ ; ## $p < 0.001$  (vs. pooled statins). Sample sizes: placebo,  $n = 20$ ; pooled evacetrapib monotherapy,  $n = 60$ ; pooled statins,  $n = 60$ ; pooled statins+evacetrapib,  $n = 59$ . Abbreviations as in Figure 1.

HDL as assessed by 2 independent methods. We investigated the potent CETP inhibitor evacetrapib as monotherapy or in combination with 3 different statins on these HDL-related parameters, and found that evacetrapib, both as monotherapy and combined with statins, significantly increased total CEC, non-ABCA1-specific CEC, ABCA1-specific CEC, and pre-beta-1 HDL (Central Illustration). These robust findings demonstrate that evacetrapib has effects on HDL function beyond its effect in raising plasma HDL-C levels.

The efflux of macrophage cholesterol to HDL-related acceptors may be a mechanism that is atheroprotective (16). ABCA1 transports cholesterol to lipid-poor apoA-I particles, which are often described as pre-beta-1 HDL, whereas ABCG1 and scavenger receptor class B, type I (SR-BI) (which account for most of non-ABCA1-specific efflux) transport cholesterol to larger cholesteryl ester-containing HDL particles. Together, these pathways are responsible for the bulk of macrophage total cholesterol efflux to HDL (23,24), especially when macrophages are loaded with cholesterol. Assays that measure the CEC of HDL (using serum after depletion of apoB-containing lipoproteins) in individuals have been evaluated with regard to their relationship to ASCVD. Cross-sectionally, CEC is strongly and inversely associated with prevalent carotid intimal-medial thickness (11), angiographic coronary artery disease (11), and prevalent clinical coronary artery disease (12) after adjusting for HDL-C levels. Prospectively, CEC is strongly and inversely associated with incident cardiovascular events after adjusting for HDL-C levels (13,25).

These results have raised the question of whether interventions that increase CEC might favorably affect reduction of cardiovascular events. One novel finding of this study was the relationship between CETP activity (or mass) and efflux measures at baseline and after evacetrapib treatment. CETP activity was inversely correlated with all efflux measures at baseline, whereas on-treatment CETP activity was inversely correlated with non-ABCA1-specific (and total) efflux, but not with ABCA1-specific efflux. Conversely, CETP mass had no relationship with efflux measures at baseline, whereas on-treatment CETP mass was directly correlated with almost all efflux measures (especially non-ABCA1-specific efflux). These findings indicate that CETP activity, in a given individual, may influence CEC and generation of pre-beta-1 HDL. These data support the findings from Mendelian randomization studies that linked common CETP variants associated with low CETP activity to lower cardiovascular risk (7). Association of some variants and CETP activity with CEC and pre-beta-1 HDL concentrations have been described (26).

**STUDY LIMITATIONS.** One potential limitation in the current assessment of CEC is that macrophages used in ex vivo assays are exposed to different conditions than patients' macrophages in vivo and are not pre-exposed to the study drug. However, the results obtained with statin monotherapy in this study suggest that the test macrophages behaved as expected, because decreased intracellular cholesterol and oxysterol concentrations induced by statins are known to lead to increased transcription of micro-RNA33

**TABLE 1** Pearson's Correlation Coefficients (*r*) for Correlations Between Baseline Total, Non-ABCA1-Specific, or ABCA1-Specific Cholesterol Efflux Capacity and Lipid Variables in All Participants (*n* = 377)

	Total	Non-ABCA1-Specific	ABCA1-Specific
CETP mass	-0.03	0.09	-0.11
CETP activity	-0.29*	-0.19*	-0.27*
hsCRP	-0.18*	-0.14†	-0.15†
HDL-C	0.45*	0.61*	0.19*
ApoA-I	0.53*	0.61*	0.31*
ApoB	0.24*	0.03	0.34*
ApoE	0.31*	0.17*	0.33*
Pre-beta-1 (IF)	0.46*	0.22*	0.52*
Pre-beta-1 (2DGE)‡	0.46*	0.22†	0.52*
Alpha-1‡	0.38*	0.57*	0.11

\**p* < .001. †*p* < 0.01. ‡For lipid variables measured by 2DGE, *n* = 199.  
2DGE = 2-dimensional gel electrophoresis; ABCA1 = adenosine triphosphate-binding cassette transporter A1; ApoA-I = apolipoprotein A1; ApoB = apolipoprotein B; ApoE = apolipoprotein E; CETP = cholesteryl ester transfer protein; IF = immunofixation; HDL-C = high-density lipoprotein cholesterol; hsCRP = high-sensitivity C-reactive protein.

**TABLE 2** Pearson's Correlation Coefficients (*r*) for Correlations Between on Treatment or Percent Change in Total, Non-ABCA1-Specific, or ABCA1-Specific Cholesterol Efflux Capacity and on Treatment or Percent Change in Lipid Variables in the Pooled Evacetrapib Monotherapy (*n* = 111) and Pooled Statins Plus Evacetrapib (*n* = 114) Groups

	On Treatment		Percent Change	
	Pooled Evacetrapib Monotherapy	Pooled Statins + Evacetrapib	Pooled Evacetrapib Monotherapy	Pooled Statins + Evacetrapib
<b>Total</b>				
CETP mass	0.12	0.26*	0.35†	0.54†
CETP activity	-0.34†	-0.11	-0.34†	-0.27*
ApoB	0.01	-0.05	-0.19	-0.25*
HDL-C	0.50†	0.61†	0.39†	0.56†
ApoA-I	0.49†	0.69†	0.41†	0.57†
Pre-beta-1‡	0.46†	0.51†	0.05	0.42†
Alpha-1§	0.38*	0.51†	0.28	0.17
<b>Non-ABCA1-specific</b>				
CETP mass	0.28*	0.43†	0.36†	0.42†
CETP activity	-0.34†	-0.35†	-0.32†	-0.41†
ApoB	-0.05	-0.27*	-0.13	-0.33†
HDL-C	0.71†	0.87†	0.29*	0.41†
ApoA-I	0.69†	0.88†	0.33†	0.41†
Pre-beta-1‡	0.26*	0.39†	-0.07	0.31*
Alpha-1§	0.63†	0.80†	0.34*	-0.01
<b>ABCA1-specific</b>				
CETP mass	-0.05	0.07	0.28*	0.25*
CETP activity	-0.22	0.10	-0.15	-0.00
ApoB	0.06	0.13	-0.05	0.11
HDL-C	0.14	0.23	0.18	0.22
ApoA-I	0.15	0.34†	0.26†	0.22
Pre-beta-1‡	0.46†	0.47†	0.14	0.34†
Alpha-1§	0.07	0.12	0.08	-0.04

\**p* < 0.01. †*p* < 0.001. ‡Pre-beta-1 correlations are for the immunofixation measurements. §Number for lipid variables measured by 2DGE: pooled evacetrapib monotherapy (*n* = 60) and pooled statin plus evacetrapib (*n* = 59).

Abbreviations as in Table 1.

and decreased transcription of liver X receptor, respectively. This results in down-regulation of the expression of ABCA1 and ABCG1 (27). Whether evacetrapib affects the expression of ABCA1 and ABCG1 in human macrophages *in vivo* is unknown.

CETP inhibition has been well-established to increase plasma HDL-C levels, but its effect on HDL function, as assessed by CEC, has been uncertain. Torcetrapib monotherapy resulted in increased total CEC at the 60 mg/day dose used in the cardiovascular outcomes study ILLUMINATE (Investigation of Lipid Level management to Understand its iMPact IN ATtherosclerotic Events) (9) and at the 120 mg/day dose (28) in proportion to the magnitude of HDL-C increase with these 2 doses. The better cholesterol efflux with the higher dose of torcetrapib is consistent with the observation that subjects with greater HDL-C and apoA-I increases on torcetrapib showed more pronounced regression of coronary atherosclerosis and appeared to have better cardiovascular outcomes (29). Anacetrapib monotherapy, at a dose 3-fold higher than that being studied in the cardiovascular outcomes study REVEAL (Randomized Evaluation of the Effects of Anacetrapib Through Lipid Modification), also increased total CEC (30). However, differences in assay methodology make it difficult to compare data, because the torcetrapib and anacetrapib studies used isolated HDL, whereas a supernatant of apoB-depleted serum was used in the present study. Finally, dalcetrapib 600 mg/day in combination with a statin also minimally increased total CEC (31), in proportion with a relatively small HDL-C increase, which was consistent with the no effects on cardiovascular outcomes in the dal-OUTCOMES (Effects of the Cholesterol Ester Transfer Protein Inhibitor Dalcetrapib in Patients with Recent Acute Coronary Syndrome) study (6). In that study, Ray *et al.* (31) used the same methodology to evaluate CEC that was used in the present study, but they were not able to show any effect of dalcetrapib treatment on ABCA1-specific CEC. Thus, the modest increase in total CEC with torcetrapib, anacetrapib, and dalcetrapib was largely due to non-ABCA1-specific efflux, with ABCG1 playing a major role in mediating the efflux potential. No previous studies have rigorously assessed the impact of CETP inhibition on ABCA1-specific efflux or on pre-beta-1 HDL, which is the major acceptor of ABCA1-mediated efflux.

In this comprehensive study of evacetrapib, the most unique finding was a substantial, consistent increase in ABCA1-specific efflux, both in monotherapy and in combination with a statin. This was accompanied by significant increases in pre-beta-1 HDL levels as measured by 2 independent assays. The correlations between pre-beta-1 HDL concentrations and



ABCA1-specific efflux at baseline and on treatment suggest a potential role for pre-beta-1 HDL concentrations as a surrogate marker of ABCA1-specific efflux. These data also imply that ABCA1-specific efflux capacity was maintained on a per particle basis under evacetrapib treatment, because ABCA1-specific efflux and pre-beta-1 HDL concentrations increased proportionately. CETP inhibition is known to increase the average size and cholesteryl ester content of HDL particles and would not necessarily be expected to increase the concentrations of ABCA1-driven, lipid-poor pre-beta-1 HDL particles. However, our results clearly establish that evacetrapib did have this effect. The mechanisms of this effect remain unclear, but may be related to the poorly understood processes that generate lipid-poor, apoA-I-containing pre-beta-1 HDL particles. It is generally accepted that HDL remodeling processes can result in desorption of apoA-I from the surface of HDL particles, which generates lipid-poor apoA-I. It is possible that potent CETP inhibition makes HDL particles better substrates for certain types of remodeling activities, such as those carried out by phospholipid transfer protein, endothelial lipase, hepatic lipase, or SR-BI, which generate lipid-poor pre-beta-1 HDL particles. The mechanisms by which potent CETP inhibition increase ABCA1-mediated CEC and pre-beta-1 HDL require further study.

Because our study involved statin monotherapy arms for comparison with evacetrapib in combination with statins, we had the opportunity to perform the largest systematic analysis of the effects of different statins on CEC to date. Previous studies (27,32) were small, were generally limited to 1 statin, and used methods to assess CEC that were less well-established. The recent report by Niesor *et al.* (27) showed that statins increased micro-R33 expression and decreased ABCA1 expression and cholesterol efflux from peripheral tissues; there was a differential effect of relatively hydrophobic statins compared with more hydrophilic statins on the degree of cholesterol efflux reduction. The present study adds incremental information with regard to the effect of different statins on ABCA1 cholesterol efflux, because both rosuvastatin and simvastatin significantly reduced total CEC, non-ABCA1-specific efflux, and ABCA1-specific efflux, whereas atorvastatin had no significant effect on CEC. This is interesting in light of the previous observations that atorvastatin had less effect in raising HDL-C levels compared with rosuvastatin and simvastatin (33). The mechanisms by which statins reduce CEC are not understood. Even more interesting, the effect of evacetrapib, when given in combination with a statin, on CEC and pre-beta-1 HDL appears to be slightly reduced with respect to evacetrapib monotherapy,

although the correlation between changes in total and ABCA1-specific CEC and pre-beta-1 HDL levels remained robust. The mechanisms underlying the effects of potent CETP inhibition combined with statin therapy on CEC and pre-beta-1 HDL will need to be elucidated in future studies.

## CONCLUSIONS

Potent CETP inhibition with evacetrapib unexpectedly increased not only total CEC but also ABCA1-specific CEC and pre-beta-1 HDL in a dose-dependent fashion. Furthermore, this effect was slightly reduced by co-administration with statins, which proved to decrease CEC as monotherapy. These results reveal the complex effects of potent CETP inhibition on HDL remodeling and function that require more investigation. They also raise the possibility, in light of recent prospective data that inversely linked CEC to incident cardiovascular events, that this increase in CEC might have the potential to reduce cardiovascular risk.

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**REPRINT REQUESTS AND CORRESPONDENCE:** Dr. Daniel J. Rader, Perelman School of Medicine at the University of Pennsylvania, Smilow Center for Translational Research, 3400 Civic Center Boulevard, Building 421, 11-125, Philadelphia, Pennsylvania 19104-5158. E-mail: [rader@mail.med.upenn.edu](mailto:rader@mail.med.upenn.edu).

## PERSPECTIVES

**COMPETENCY IN MEDICAL KNOWLEDGE:** HDL particles promote cholesterol efflux from macrophages, and CEC is inversely associated with prevalent cardiovascular disease and incident cardiovascular events, independently of blood levels of HDL-C. ABCA1 plays a crucial role in cholesterol efflux from macrophages, and lipid-poor pre-beta-1 HDL particles are the rate-limiting acceptors of cholesterol via this pathway. A 12-week course of treatment with evacetrapib, either alone or in combination with statin therapy, increases ABCA1-specific cholesterol efflux and pre-beta-1 HDL levels.

**TRANSLATIONAL OUTLOOK:** Further study is needed to determine whether the increase in CEC brought about by treatment with evacetrapib can reduce cardiovascular risk.

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**KEY WORDS** apolipoproteins, cholesterol, lipoproteins

**APPENDIX** For supplemental tables, please see the online version of this article.