The effect of cholesteryl ester transfer protein inhibition on lipids, lipoproteins, and markers of HDL function after an acute coronary syndrome: the dal-ACUTE randomized trial

Kausik K. Ray1*, Marc Dittmarsch2, David Kallend2, Eric J. Niesor2, Gabriela Suchankova2, Ruchi Upmanyu3, Judith Anzures-Cabrera3, Valerie Lehnert2, Meike Pauly-Evers2, Ingar Holme4, Josef Štásek5, Maarten W. J. van Hessen6, and Peter Jones7, on behalf of the dal-ACUTE Investigators

1Cardiovascular Sciences Research Centre, St George’s University of London, Cranmer Terrace, London, SW17 ORE UK; 2F. Hoffmann-La Roche Ltd, Basel, Switzerland; 3Roche Products Ltd, Welwyn Garden City, UK; 4Department of Endocrinology, Obesity and Preventive Medicine, Oslo University Hospital, Ulleval, Oslo, Norway; 5Charles University Faculty Hospital, Hradec Králové, Czech Republic; 6Groene Hart Ziekenhuis, Gouda, The Netherlands; and 7Baylor College of Medicine, Houston, TX, USA

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Aims
The effects of cholesteryl ester transfer protein (CETP) inhibition on lipids, inflammation, and markers of high-density lipoprotein (HDL) function, following an acute coronary syndrome (ACS), are unknown.

Methods and results
The dal-ACUTE study randomized 300 patients (1:1) to dalcetrapib 600 mg/day or placebo within 1 week of an ACS. The primary endpoint was percent change in HDL-cholesterol (HDL-C) after 4 weeks. Secondary endpoints included apolipoprotein levels, markers of HDL function, and inflammation. Dalcetrapib treatment increased HDL-C and apolipoprotein A1 by 33.7 and 11.8%, respectively (both $P<0.001$) and total cholesterol efflux by 9.5% ($P=0.003$) after 4 weeks, principally via an increase in non-ABCA1-mediated efflux, without statistically significant changes in pre-$\beta$1-HDL levels. The increase in total efflux with dalcetrapib correlated most strongly with increases in apolipoprotein A1 and HDL-C ($r=0.46$ and $0.43$, respectively) rather than the increase in pre-$\beta$1-HDL ($r=0.32$). Baseline and on-treatment ABCA1-mediated efflux correlated most strongly with pre-$\beta$1-HDL levels; in contrast, non-ABCA1-mediated efflux correlated better with apolipoprotein A1 and HDL-C levels.

Conclusions
High-density lipoprotein raised through CETP inhibition with dalcetrapib improves cholesterol efflux, principally via a non-ABCA1-mediated pathway. While HDL-C was increased by one-third, apolipoprotein A1 and total efflux were increased only by one-tenth, supporting the concept of dissociation between improvements in HDL function and HDL-C levels, which may be of relevance to ongoing trials and the development of therapeutic interventions targeting HDL.

Keywords
Coronary disease • Lipids • Lipoproteins

Introduction
High-density lipoprotein (HDL) particles potentially possess several anti-atherosclerotic properties, including the ability to accept cholesterol from the blood vessel wall for elimination from the circulation via the reverse cholesterol transport pathway.1 High-density lipoprotein cholesterol (HDL-C), however, is a measure of the total cholesterol content of several HDL particle subtypes which vary in shape, size and, importantly, function.2 Observational data supporting a protective association between HDL-C and cardiovascular disease3 have stimulated the development of treatments that raise HDL-C. The simplistic paradigm that achieving a higher

* Corresponding author. Tel: +44 02082666879, fax: +44 02082753416, Email: kray@sgul.ac.uk

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HDL-C is protective has now been challenged by several lines of evidence, supporting the possibility that the projected clinical utility of HDL might be more related to specific HDL subclasses and function as assessed by cholesterol efflux capacity rather than HDL-C levels per se. Large outcome studies for two different cholesteryl ester transfer protein (CETP) inhibitors were recently prematurely terminated due to (i) an increase in all-cause mortality for torcetrapib and (ii) clinical futility for dalcetrapib. Both agents raised HDL-C, by 72 and 30%, respectively. In the dal-OUTCOMES trial, dalcetrapib raised HDL-C in patients with a recent history of an acute coronary syndrome (ACS) but failed to reduce cardiovascular events for reasons that remain unclear. Exploratory analyses from the dal-OUTCOMES study suggest that neither HDL-C levels at baseline nor on-treatment in both the placebo and dalcetrapib groups were associated with clinical events, raising several possibilities including the concept that HDL particles, produced in a pro-inflammatory milieu, were dysfunctional. The dal-ACUTE study was designed prior to the termination of the dalcetrapib clinical programme to provide information on short-to-medium-term effects of CETP inhibition with dalcetrapib on HDL-C, apolipoprotein A1, HDL function, and inflammatory biomarkers immediately after an ACS, and is to date the largest study of the effects of HDL-raising interventions on cholesterol efflux, as a measure of HDL function.

Methods

Study design

The dal-ACUTE study was a phase 3, double-blind, randomized, placebo-controlled trial performed at 31 centres in the Czech Republic, Netherlands, UK, and USA from 24 March 2011 to 26 March 2012 (NCT01323153). The protocol was reviewed and approved by the Institutional Review Board or Ethics Committee at each participating centre and the study was conducted in accordance with the Declaration of Helsinki/local regulations. All participants provided written informed consent.

Study participants

Between March 2011 and October 2011, men and women aged 45 years or over, hospitalized for an ACS, defined as spontaneous myocardial infarction (MI) or unstable angina, were assessed for study eligibility during a 1-week-screening period. Once considered clinically stable, but no later than 1 week after the index ACS event, eligible patients (see Supplementary material online, Appendix) were randomized to study treatment on Day 1 (baseline) using a computer-generated global randomization code. Patients were allocated sequentially in a 1:1 ratio stratified by country to receive dalcetrapib 600 mg/day or placebo for 20 weeks, with blinding maintained by matching placebo and dalcetrapib tablets, and by withholding HDL-C, apolipoprotein A1, and biomarker concentrations from the investigator and their personnel. Study treatment was performed on a background of guideline-based medical care.

Study procedures

Patients returned to clinic at weeks 4, 8, 12, and 20 for assessment and at week 24 (4 weeks after the last dose) for safety follow-up. Blood samples were obtained for the measurement of fasting standard lipid parameters, apolipoproteins (A1, B, and E), measures of HDL function (specifically pre-β1-HDL levels and cholesterol efflux capacity), high-sensitivity C-reactive protein, and HDL-associated serum amyloid A (HDL-SAA). Cholesterol efflux capacity was assessed in apolipoprotein B-depleted plasma and used as an acceptor of cholesterol from cholesterol-loaded J774 macrophages. Pre-β1-HDL was quantitated in EDTA plasma using the sandwich ELISA from Sekisui (Japan), which detects a specific epitope of apolipoprotein A1 present in pre-β1-HDL.

Study endpoints

The pre-specified primary efficacy endpoint in this study was per cent change in HDL-C from baseline after 4 weeks between dalcetrapib and placebo. Secondary endpoints included change between treatment groups in other lipids and apolipoprotein levels measured at 4, 8, 12, and 20 weeks, and measures of change in HDL function (pre-β1-HDL levels and cholesterol efflux capacity), HDL-SAA, and high-sensitivity C-reactive protein levels measured at 4 and 20 weeks.

Safety was evaluated by assessing adverse events, laboratory tests, vital signs, and pre-defined positively adjudicated project-specific cardiovascular events, i.e. coronary heart disease death, non-fatal MI, hospitalization for unstable angina (electrocardiogram abnormalities without biomarker elevations), resuscitated cardiac arrest, stroke, hospitalization for congestive heart failure, and any revascularization procedure.

Statistical analysis

Efficacy analyses were performed on the intention-to-treat population (all patients receiving at least one dose of study medication). For the primary endpoint, a linear model was constructed with the percentage change in HDL-C from baseline at week 4 as the dependent variable, with centred baseline HDL-C as a covariate, and treatment group and country as fixed effects. Point estimates and corresponding 95% confidence intervals were calculated for both treatment arms, and a difference between the two groups, tested with a two-sided alpha of 0.05, was considered significant. For secondary endpoints, a mixed-model repeated-measures analysis was used to model the per cent change from baseline over time. The model included country, week of treatment, treatment group, and the interaction of week of treatment and treatment group as fixed effects, and centred baseline of the variable to be analysed as a covariate. Rather than using the patient as a random effect, a covariance structure for the random error terms was assumed as previous data indicated no major differences in variance over time.

Study overview

The trial was designed by the executive committee which included an independent statistician (Ingmar Holme) and was conducted by the sponsor. The analyses reported in this article were performed by the sponsor (Ruchi Umpayu). All authors had full access to all the results of the study and had final responsibility for the decision to submit for publication.

Results

Patients

Of 320 screened patients, 300 were randomized (1:1) to placebo or dalcetrapib 600 mg/day on average 3.9 days after the index ACS event. Two patients randomized to dalcetrapib did not receive the study drug and were excluded from all analyses. Baseline demographics were well-matched between groups (Table 1 and Supplementary material online, Appendix) and reflected current standards of care. Baseline HDL-C (39.0 and 40.8 mg/dL, respectively, in the placebo and dalcetrapib groups) was similar to levels observed in the dal-OUTCOMES study (corresponding levels 42.2 and 42.5 mg/dL).
respectively), but median high-sensitivity C-reactive protein was higher in dal-ACUTE (9.2 and 8.3 mg/L, respectively, for placebo and dalcetrapib) compared with dal-OUTCOMES (median 1.5 mg/L for both).

Lipids and lipoproteins

The placebo-corrected increases in HDL-C and apolipoprotein A1 observed with dalcetrapib at Week 4 of 33.7 and 11.8%, respectively (Figure 1), were maintained throughout the study and were non-inferior to those observed at Week 4 in dal-OUTCOMES (≏30 and 10%, respectively). These effects were not significantly different between subgroups except among those in the lowest apolipoprotein B/A1 quartile where they were significantly higher (Supplementary material online, Appendix). Low-density lipoprotein cholesterol (LDL-C) and apolipoprotein B levels declined post-ACS, but did not differ between placebo and dalcetrapib at any time point (Figure 1). In the weeks following ACS, apolipoprotein E levels fell in the placebo-treated group compared with baseline but were maintained in the dalcetrapib group such that apolipoprotein levels were higher by 11.4% in the dalcetrapib group at 4 weeks, and at 20 weeks the difference was 16.1%.

Markers of high-density lipoprotein function

Pre-β1-HDL, the rate-limiting acceptor of cholesterol effluxed by the ATP-binding cassette transporter (ABC) A1 pathway, did not change significantly over time, nor between dalcetrapib and placebo (Figure 2), although, interestingly, it was significantly reduced among those in the highest quartile of high-sensitivity C-reactive protein at baseline (Supplementary material online, Appendix). In contrast, both non-ABCA1-mediated efflux and total efflux were significantly increased by dalcetrapib at 4, 12, and 20 weeks (Figure 2) with evidence of an attenuation among those with high body mass.
index, current smokers, and individuals with higher apolipoprotein B/A1 ratios at baseline (Supplementary material online, Appendix).

Baseline and Week 4 correlations between different measures of efflux and various lipid parameters are shown in Table 2. At baseline, pre-β1-HDL levels correlated more strongly with ABCA1-mediated-efflux compared with non-ABCA1-mediated efflux ($r = 0.50$ vs. $0.17$) but, in contrast, both HDL-C and apolipoprotein A1 levels were more strongly correlated with non-ABCA1-mediated vs. ABCA1-mediated efflux ($r = 0.69$ vs. $0.07$ and $r = 0.68$ vs. $0.26$, respectively). At baseline, apolipoprotein E correlated with total cholesterol efflux, ABCA1-mediated and non-ABCA1-mediated efflux ($r = 0.25$, $r = 0.21$, and $r = 0.16$, respectively), albeit modestly. For HDL-SAA at baseline, there was an inverse correlation with ABCA1-mediated efflux and total efflux, but both were modest ($r = -0.27$ and $r = -0.19$, respectively).

After 4 weeks of treatment, pre-β1-HDL levels remained more strongly correlated with ABCA1 compared with non-ABCA1-mediated efflux and did not appear substantially different between dalcetrapib and placebo or from baseline correlations in general. As seen at baseline, HDL-C and apolipoprotein A1 levels at 4 weeks remained more strongly correlated with non-ABCA1-mediated efflux than ABCA1-mediated efflux and this correlation with non-ABCA1 efflux did not differ substantially between treatments or from baseline correlations. At 4 weeks, the correlation

Figure 1 Effect of treatment on mean high-density lipoprotein cholesterol, apolipoprotein A1, low-density lipoprotein cholesterol, apolipoprotein B, and apolipoprotein E levels. Error bars represent 95% confidence intervals. ***P-value < 0.001, **P-value < 0.01 and *P-value < 0.05 (two-sided) for the difference between groups. To convert the values for cholesterol to millimoles per litre, multiply by 0.2586. Numbers for placebo/dalcetrapib at: baseline $n = 148/148$, Week 4 $n = 146/141$, Week 8 $n = 144/136$, Week 12 cholesterol $n = 143/135$, Week 12 apolipoproteins $n = 143/134$, and Week 20 $n = 140/130$. HDL, high-density lipoprotein cholesterol and LDL, low-density lipoprotein cholesterol.
Figure 2  Effect of treatment on per cent change in parameters of high-density lipoprotein functionality. Error bars represent 95% confidence intervals; ***P-value < 0.001 and **P-value < 0.01 for the difference between groups. Numbers for pre-β1-HDL placebo/dalcetrapib at: Week 4 n = 142/133 and Week 20 n = 134/123. Numbers for efflux parameters placebo/dalcetrapib at: Week 4 n = 110/102, Week 12 n = 102/95, and Week 20 n = 104/96. ABC, ATP-binding cassette transporter and HDL, high-density lipoprotein.

Table 2  Pearson correlations between cholesterol efflux and lipid parameters at baseline and Week 4

<table>
<thead>
<tr>
<th></th>
<th>ABCA1-specific efflux</th>
<th>Non-ABCA1-specific efflux</th>
<th>Total efflux</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>r²</td>
<td>P-value</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-β1-HDL</td>
<td>Combined</td>
<td>0.50</td>
<td>0.25</td>
</tr>
<tr>
<td>HDL-C</td>
<td>Combined</td>
<td>0.07</td>
<td>0.00</td>
</tr>
<tr>
<td>Apo A1</td>
<td>Combined</td>
<td>0.26</td>
<td>0.07</td>
</tr>
<tr>
<td>Apo E</td>
<td>Combined</td>
<td>0.21</td>
<td>0.04</td>
</tr>
<tr>
<td>HDL-SAA</td>
<td>Combined</td>
<td>−0.27</td>
<td>0.07</td>
</tr>
<tr>
<td>Week 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-β1-HDL</td>
<td>Dalcetrapib</td>
<td>0.47</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.54</td>
<td>0.29</td>
</tr>
<tr>
<td>HDL-C</td>
<td>Dalcetrapib</td>
<td>0.26</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.03</td>
<td>0.00</td>
</tr>
<tr>
<td>Apo A1</td>
<td>Dalcetrapib</td>
<td>0.31</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.12</td>
<td>0.01</td>
</tr>
<tr>
<td>Apo E</td>
<td>Dalcetrapib</td>
<td>0.35</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.41</td>
<td>0.17</td>
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<tr>
<td>HDL-SAA</td>
<td>Dalcetrapib</td>
<td>−0.02</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.09</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Apo, apolipoprotein; HDL, high-density lipoprotein; HDL-C, HDL-cholesterol; HDL-SAA, HDL-associated serum amyloid A.
between levels of apolipoprotein E and ABCA1-mediated efflux were stronger, both for dalcetrapib and placebo arms, such that the variation in ABCA1-efflux explained by apolipoprotein E levels were approximately four- to five-fold higher in both groups compared with baseline, although the correlations were not dissimilar at Week 4 between randomized treatment groups. In contrast, the correlation between apolipoprotein E levels and both total and non-ABCA1 efflux were markedly different in the dalcetrapib-treated group at Week 4 compared with baseline and vs. placebo. Whereas the correlation between apolipoprotein E and non-ABCA1-mediated efflux was virtually identical to baseline in the placebo arm, in the dalcetrapib-treated group the correlation between apolipoprotein E and non-ABCA1 mediated efflux was more marked, such that there was an ≏13-fold increase in the variation in non-ABCA1-mediated efflux explained by apolipoprotein E levels at 4 weeks vs. at baseline ($r^2 0.38$ vs. 0.03) and a corresponding approximately three-fold increase in the variation in total efflux explained by apolipoprotein E ($r^2 0.38$ vs. 0.13). Hence, at 4 weeks, there were marked differences between dalcetrapib vs. placebo in the correlations in total and non-ABCA1-mediated efflux ($r^2 0.38$ vs. 0.20 and 0.38 vs. 0.05, respectively). High-density lipoprotein-associated serum amyloid A levels at Week 4 (which was significantly lower than at baseline) were now not longer correlated with efflux after 4 weeks treatment with dalcetrapib or placebo.

Correlations between change from baseline in total efflux and change from baseline in various lipid parameters at Week 4 are shown in Table 3. When the change in total cholesterol efflux at 4 weeks from baseline was correlated against the change in HDL-C, apolipoprotein A1, pre-$\beta$-HDL, apolipoprotein E, and HDL-SAA, a higher proportion of the variation in total efflux could be explained by the change in apolipoprotein A1 and HDL-C than change in pre-$\beta$1-HDL. Change in total cholesterol efflux at 4 weeks weakly correlated positively with change in apolipoprotein E levels but only for the placebo group. In contrast, the change in total cholesterol efflux at 4 weeks was inversely correlated with change in HDL-SAA levels for both placebo and dalcetrapib.

**Inflammation**

High-sensitivity C-reactive protein and HDL-SAA levels declined over time but did not differ between placebo and dalcetrapib after 4 and 20 weeks (Figure 3). Across baseline quartiles of high-sensitivity C-reactive protein, HDL-SAA was progressively higher and pre-$\beta$1-HDL progressively lower in the intention-to-treat population, but these differences were less apparent over time. For details see Supplementary material online, Appendix.

**Safety**

Dalcetrapib was generally well tolerated, with no significant differences in number of adverse events between dalcetrapib and placebo, although more positively adjudicated cardiovascular events were observed in the dalcetrapib arm after 20 weeks. For details see Supplementary material online, Appendix.

**Discussion**

The dal-ACUTE study demonstrated that, in the pro-inflammatory environment following an ACS, CETP inhibition with dalcetrapib...

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**Table 3** Pearson correlations between change in total efflux and change in lipid parameters from baseline to Week 4

<table>
<thead>
<tr>
<th>Change in lipid parameter</th>
<th>Change in total efflux</th>
<th>$r$</th>
<th>$r^2$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-$\beta$-HDL</td>
<td>Dalcetrapib</td>
<td>0.33</td>
<td>0.11</td>
<td>0.0010</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.34</td>
<td>0.11</td>
<td>0.0003</td>
</tr>
<tr>
<td>HDL-C</td>
<td>Dalcetrapib</td>
<td>0.43</td>
<td>0.19</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.38</td>
<td>0.15</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Apo A1</td>
<td>Dalcetrapib</td>
<td>0.46</td>
<td>0.21</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.52</td>
<td>0.27</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Apo E</td>
<td>Dalcetrapib</td>
<td>0.07</td>
<td>0.00</td>
<td>0.5074</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.26</td>
<td>0.07</td>
<td>0.0071</td>
</tr>
<tr>
<td>HDL-SAA</td>
<td>Dalcetrapib</td>
<td>-0.21</td>
<td>0.04</td>
<td>0.0397</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>-0.32</td>
<td>0.10</td>
<td>0.0008</td>
</tr>
</tbody>
</table>

Apo, apolipoprotein; HDL, high-density lipoprotein; HDL-C, HDL-cholesterol; HDL-SAA, HDL-associated serum amyloid A.

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**Figure 3** Time courses of mean high-sensitivity C-reactive protein and high-density lipoprotein-associated serum amyloid A levels in the dal-ACUTE study. Error bars represent 95% confidence intervals. HDL, high-density lipoprotein; HDL-SAA, HDL-associated serum amyloid A; hsCRP, high-sensitivity C-reactive protein; SAA, serum amyloid A.
significantly increased HDL-C concentrations by 32%, apolipoprotein A1 by 11% and, importantly, total cholesterol efflux by 9.5% at 4 weeks; which were sustained over 20 weeks. However, there were no significant effects on pre-β1-HDL during 20 weeks of treatment and consequently no significant increase in ABCA1-mediated cholesterol efflux. Taken together, these findings suggest that the increase in the HDL pool following CETP inhibition results in the generation of functional HDL, capable of cholesterol efflux, working principally in vitro through non-ABCA1-mediated pathways. In addition, raising HDL-C and apolipoprotein A1 with dalcetrapib following an ACS did not alter the natural time course of inflammatory markers, as measured by high-sensitivity C-reactive protein and HDL-SAA, nor the time course of atherogenic lipids or lipoproteins, as measured by LDL-C and apolipoprotein B levels. In contrast, apolipoprotein E levels remained similar to baseline in the dalcetrapib group but fell in the placebo group, such that the levels in the dalcetrapib group were 11.4% higher after 4 weeks.

Among the subgroups assessed, the effect of CETP inhibition on HDL-C and apolipoprotein A1 concentration was most marked among those with apolipoprotein B/A1 ratios in the lowest quartiles. Importantly, and in contrast to prior suggestions, this inhibition of CETP raised both HDL-C and apolipoprotein A1 among a population with a baseline high-sensitivity C-reactive protein of 8–9 mg/L, with no attenuation of effect across high-sensitivity C-reactive protein quartiles. Notably, ABCA1-mediated cholesterol efflux at 4 weeks was reduced among those within the highest quartiles of baseline high-sensitivity C-reactive protein, whereas smoking and higher apolipoprotein B/A1 levels reduced non-ABCA1-mediated efflux. Taken together, the presented data suggest that CETP inhibition with dalce- trapib predominantly affects non-ABCA1-mediated efflux, while specific patient characteristics influence the extent of the changes observed in lipids, apolipoproteins, and HDL-mediated efflux.

Although high levels of CETP inhibition with dalcetrapib were shown in vitro and in animal models to consistently increase HDL remodelling and production of pre-β1-HDL, in the dal-ACUTE trial pre-β1-HDL levels were not statistically higher with dalcetrapib. Likewise, there was no significant increase in ABCA1-dependent cholesterol efflux which is, in part, driven by pre-β1-HDL as the preferred acceptor. As non-ABCA1-dependent efflux was increased by dalcetrapib, these results suggest that the HDL particles (mostly HDL2) produced via this CETP inhibition preferentially accept cholesterol from macrophages via a synergistic ‘remodelling and shuttling mechanism’ (see below) or via other routes, such as ABCG1. Whereas apolipoprotein E levels fell in the placebo group in the weeks after an ACS, the levels remained similar to baseline and were statistically higher among the dalcetrapib-treated patients. Higher apolipoprotein E levels have previously been linked to ABCG1-dependent cholesterol efflux among CETP-deficient subjects, supporting the concept that the higher apolipoprotein E levels are related to CETP inhibition and under these circumstances possibly more likely bound to HDL, although not specifically measured in this study.

We explored the determinants of HDL-related efflux (as a proxy for function) by assessing the correlation between cholesterol efflux and various HDL-related parameters. However, in light of recent findings, it should be reflected that HDL efflux is not definitively considered protective and remains a controversial topic. The correlations between HDL-C, apolipoprotein A1, pre-β1-HDL, and different efflux parameters at baseline were generally similar to on-treatment correlation coefficients among both placebo- and dalcetrapib-treated subjects at 4 weeks, suggesting that functionality was maintained rather than changed in the dalcetrapib group and correlates with increases in HDL particles and HDL-C. Observed increases in efflux are likely explained by an increase in the quantity of HDL rather than the quality. Non-ABCA1-mediated efflux was more strongly correlated with apolipoprotein A1 levels than pre-β1-HDL, whereas the converse was true for ABCA1-mediated efflux. In addition, the correlation (r) in total cholesterol efflux change from baseline to 4 weeks was stronger with change in apoli- poprotein A1 and HDL-C levels rather than with change in pre-β1-HDL. Given that CETP inhibition with dalcetrapib did not alter pre-β1-HDL, one plausible hypothesis is that this CETP inhibition raises apolipoprotein A1 by (i) inhibiting catabolism of mature large HDL particles rather than increasing apolipoprotein A1 synthesis, and (ii) inhibiting heterotopic transfer of cholesterol esters from apolipoprotein A1 to apolipoprotein B-containing lipoproteins, which results in an increase in apolipoprotein A1 and HDL-C concentrations (albeit differentially). The present data should be interpreted in light of the fact that the efflux assay is based on efflux of 3H-cholesterol, and especially for non-ABCA1-specific efflux a major part of the efflux represents isotope exchange and not net efflux. The implication is that the increase in net cholesterol efflux is likely even more discrepant from the change in HDL-C levels than were noted in this trial. Similarly, it is also plausible that net cholesterol efflux is such a small percentage of the isotopic efflux that this assay is not useful, except perhaps for interventions that significantly increase pre-β-HDL.

The relevance of apolipoprotein E levels and HDL-SAA to choles- terol efflux merits further consideration. First, dalcetrapib did not alter apolipoprotein E levels but rather attenuated the decline in levels observed in the placebo group. Consequently, among dalcetrapib-treated patients there was no significant correlation between the change in apolipoprotein E and change in total choles- terol efflux. In contrast, there was a weak but significant positive correlation among the placebo group. Among the dalcetrapib-treated patients, the correlation between absolute apolipoprotein E levels and non-ABCA1-mediated and total efflux were several-fold stronger than at baseline and when compared with placebo. Taken together, these data suggest that higher apolipoprotein E levels (most likely incorporated into larger HDL particles associated with CETP inhibition) strongly favour non-ABCA1-mediated efflux. In contrast, immediately after an ACS, HDL-SAA levels were weakly inversely correlated with ABCA1-dependent and total efflux. As HDL-SAA levels declined equally in both the placebo and dalcetrapib groups over time, the HDL-SAA levels at Week 4 were no longer correlated with any form of efflux. However, in both dalcetrapib- and placebo-treated groups, change in HDL-SAA from baseline was weakly inversely correlated with ABCA1-mediated and total efflux, albeit with a slightly stronger association in the placebo group. Taken together, these data may suggest that HDL-SAA is indeed a determinant of ABCA1-mediated efflux, but a weak one with perhaps an absolute level above which it impacts on HDL function.

The potential clinical relevance of the present data must be consid- ered in light of the dal-OUTCOMES study which, despite ~30%
increase in HDL-C and 10% increase in apolipoprotein A1 with dalcetrapib, failed to demonstrate any benefit on cardiovascular outcomes. One hypothesis prior to the present data was that the HDL generated by CETP inhibition in the ACS milieu may be dysfunctional, and that inflammatory conditions deplete pre-β1-HDL and reduce ABCA1-mediated efflux largely independent of changes in HDL-C and apolipoprotein A1 levels. 20–22 In our study, dalcetrapib did not influence the inflammatory markers high-sensitivity C-reactive protein and HDL-SAA. There were no differences in cholesterol efflux across the patient high-sensitivity C-reactive protein subgroups by quartile, except that ABCA1-mediated efflux was lower in the high high-sensitivity C-reactive protein subgroup; however, the HDL raised by CETP inhibition did appear to be functional. Importance, as the inverse correlations between HDL-SAA and ABCA1-specific efflux within the first week post-ACS do not persist beyond 4 weeks, the hypothesis that the neutral result in dal-OUTCOMES was due to the fact the dalcetrapib raised dysfunctional HDL can be totally refuted by the nature of the study design. The dal-OUTCOMES trial excluded patients who presented within 4 months of the index ACS event. Hence, the large majority of clinical events in the dal-OUTCOMES study occurred at much later time points, beyond the index presentation when HDL-SAA does not impact on efflux. Furthermore, in dal-ACUTE, the change in total efflux was more strongly correlated with changes in apolipoprotein A1 and HDL-C, so it remains to be seen whether newer CETP inhibitor agents, which increase apolipoprotein A1 and HDL-C by a greater extent, will increase total efflux further and/or reduce clinical events. However, these agents also affect LDL-C, e.g. anacetrapib lowers extent, will increase total efflux further and/or reduce clinical events. Furthermore, in dal-ACUTE, the change in total efflux was more strongly correlated with changes in apolipoprotein A1 and HDL-C, so it remains to be seen whether newer CETP inhibitor agents, which increase apolipoprotein A1 and HDL-C by a greater extent, will increase total efflux further and/or reduce clinical events. However, these agents also affect LDL-C, e.g. anacetrapib lowers LDL-C by up to 40%, 23 making it difficult to separate HDL-mediated benefits from those mediated by LDL-C decrease. Additionally, while efflux improved with dalcetrapib, we remain uncertain about the level of ‘normal’ efflux among healthy people. Indeed, among healthy volunteers, Khera et al. 5 observed that the levels of apolipoprotein A1 and HDL-C accounted for <40% of the variation in cholesterol efflux capacity, suggesting that one should not expect a doubling of efflux for a doubling of HDL-C or apolipoprotein A1. A relevant consideration is that a 9.5% improvement in efflux may not have been enough to normalize the effects of deranged efflux among individuals with established cardiovascular disease. This hypothesis is supported by the observation that ACS patients fail to show improvements in vascular function, high-sensitivity C-reactive protein, and oxidized LDL following apolipoprotein A1 infusions, despite raising HDL-C, and lowering LDL-C. 24 Whether this is due to the underlying disease or concomitant therapies, e.g. with statins, is unclear, but the latter induces micro RNA33 levels and impairs cholesterol efflux from the HDL increased by dalcetrapib, possibly via a decrease in ABCA1 and ABCG1 expression. 25 It should also be recognized that, as HDL is a pleiotropic lipoprotein with multiple favourable effects on atherosclerotic pathways, 1 we cannot exclude a lack of benefit on other potentially more important effects mediated via HDL. Finally, while CETP inhibition in our study appears to modulate efflux principally via non-ABCA1-mediated pathways, we cannot exclude the possibility that agents which markedly increase or mimic pre-β1-HDL, e.g. apolipoprotein A1 infusion, or which principally mediate efflux via the ABCA1 transporter, would produce a different clinical outcome. 26,27 While the dalcetrapib programme may have terminated, this study of the natural history of lipids, lipoproteins, and HDL function among those receiving and naive to CETP inhibition provide novel observations which may benefit ongoing studies in the field.

In conclusion, dal-ACUTE demonstrated that dalcetrapib increased apolipoprotein A1 and HDL-C in plasma and total cholesterol efflux from macrophages in vitro. While HDL-C was increased by one-third, efflux was only increased by one-tenth. The data provide further strength to the hypothesis that there is a disconnect between HDL particle function, HDL-C concentration, and cardiovascular outcomes. In particular, the correlation between change in cholesterol efflux and cardiovascular events warrants further exploration of the dal-OUTCOMES study.

Supplementary material
Supplementary material is available at European Heart Journal online.

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