

# A single infusion of MDCO-216 (ApoA-1 Milano/POPC) increases ABCA1-mediated cholesterol efflux and pre-beta 1 HDL in healthy volunteers and patients with stable coronary artery disease

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Received 17 September 2015; revised 21 September 2015; accepted 23 September 2015; online publish-ahead-of-print 11 December 2015

## Aims

Apolipoprotein A-1 (ApoA-1), based on epidemiology, is inversely associated with cardiovascular (CV) events. Human carriers of the ApoA-1 Milano variant have a reduced incidence of CV disease. Regression of atherosclerotic plaque burden was previously observed on intravascular ultrasound (IVUS) with ETC-216, a predecessor of MDCO-216. MDCO-216, a complex of dimeric ApoA-1 Milano and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, is being developed to reduce atherosclerotic plaque burden and CV events. We investigated the efficacy and safety of a single infusion of MDCO-216 in healthy volunteers and in patients with coronary artery disease (CAD).

## Methods and results

Twenty-four healthy volunteers and 24 patients with documented CAD received a 2-h infusion of MDCO-216 in a randomized, placebo controlled, single ascending dose study. Five cohorts of healthy volunteers and four cohorts of CAD patients received ApoA-1 Milano doses ranging from 5 to 40 mg/kg. Subjects were followed for 30 days. Dose-dependent increases in ApoA-1, phospholipid, and pre-beta 1 HDL and decreases in ApoE were observed. Prominent and sustained increases in triglyceride, and decreases in HDL-C, endogenous ApoA-1 and ApoA-II occurred at doses >20 mg/kg and profound increases in ABCA1-mediated cholesterol efflux were observed. Other lipid and lipoprotein parameters were generally unchanged. MDCO-216 was well tolerated.

## Conclusions

MDCO-216-modulated lipid parameters profoundly increased ABCA1-mediated cholesterol efflux and was well tolerated. These single-dose data support further development of this agent for reducing atherosclerotic disease and subsequent CV events.

## Keywords

Atherosclerosis • Coronary disease • Lipids • Lipoproteins • Cholesterol efflux

## Introduction

For several decades, statins have had a significant impact on reducing cardiovascular (CV) events through lowering of LDL-C.<sup>1</sup> However, considerable residual CV risk remains.<sup>2</sup> Epidemiological and experimental data support the targeting of HDL-C apolipoprotein A-1 (ApoA-1)<sup>3</sup> to further reduce atherosclerosis burden and CV events.<sup>4</sup>

Based on epidemiology, several therapies that elevate HDL-C and ApoA-1 have been evaluated in large clinical trials. Torcetrapib and dalcetrapib, which inhibit cholesteryl ester transfer protein (CETP), a protein that promotes transfer of cholesteryl esters from HDL to LDL particles, failed to improve CV outcomes, despite marked improvements in the atherogenic lipid profile.<sup>5,6</sup> Furthermore, torcetrapib was associated with increased mortality and morbidity and increased blood

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pressure related to activation of the renin–angiotensin–aldosterone system.<sup>7</sup> Niacin effects multiple biomarkers including HDL-C and ApoA-1 but also failed to reduce CV events in combination with statin in two recent studies.<sup>8,9</sup> Interestingly, these drugs have limited effect on parameters of HDL function such as cholesterol efflux capacity.<sup>10,11</sup> Potentially, it is the function of the HDL, rather than the HDL-C concentration that is important in atherosclerosis and CV disease.<sup>12</sup>

Despite these set-backs, several HDL- or ApoA-1-related therapies are still being developed. One of these, MDCO-216 which consists of a complex of highly purified ApoA-1 Milano (13 mg/mL) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) (14 mg/mL), has previously been studied as a predecessor compound ETC-216. Regression of atherosclerotic plaque burden was observed in a Phase II intravascular ultrasound (IVUS) study.<sup>13</sup> MDCO-216 is based on apolipoprotein A-1 Milano (ApoA-1M)<sup>14</sup> which has been identified in individuals in a village in northern Italy. The amino acid sequence of wild-type ApoA-1 contains arginine at position 173 which is replaced by cysteine in carriers of ApoA-1M.<sup>15</sup> These carriers are heterozygous and apparently protected from the development of atherosclerosis despite HDL-C concentrations in the lowest 5th percentile (10–30 mg/dL), low ApoA-1 concentrations,<sup>16</sup> and elevated triglyceride. In contrast to patients with low HDL-C, there are no signs of premature atherosclerosis in the carriers of ApoA-1M. These subjects also have increased cholesterol efflux potential compared with individuals with wild-type ApoA-1<sup>17</sup> and this formed the basis for the consideration of developing this apolipoprotein as a treatment for atherosclerotic disease.<sup>18</sup>

Following improvements to the manufacturing process,<sup>19</sup> MDCO-216 has been re-introduced into humans in a single ascending dose (SAD) Phase I study, the first results of which are described here.

## Methods

### Study design

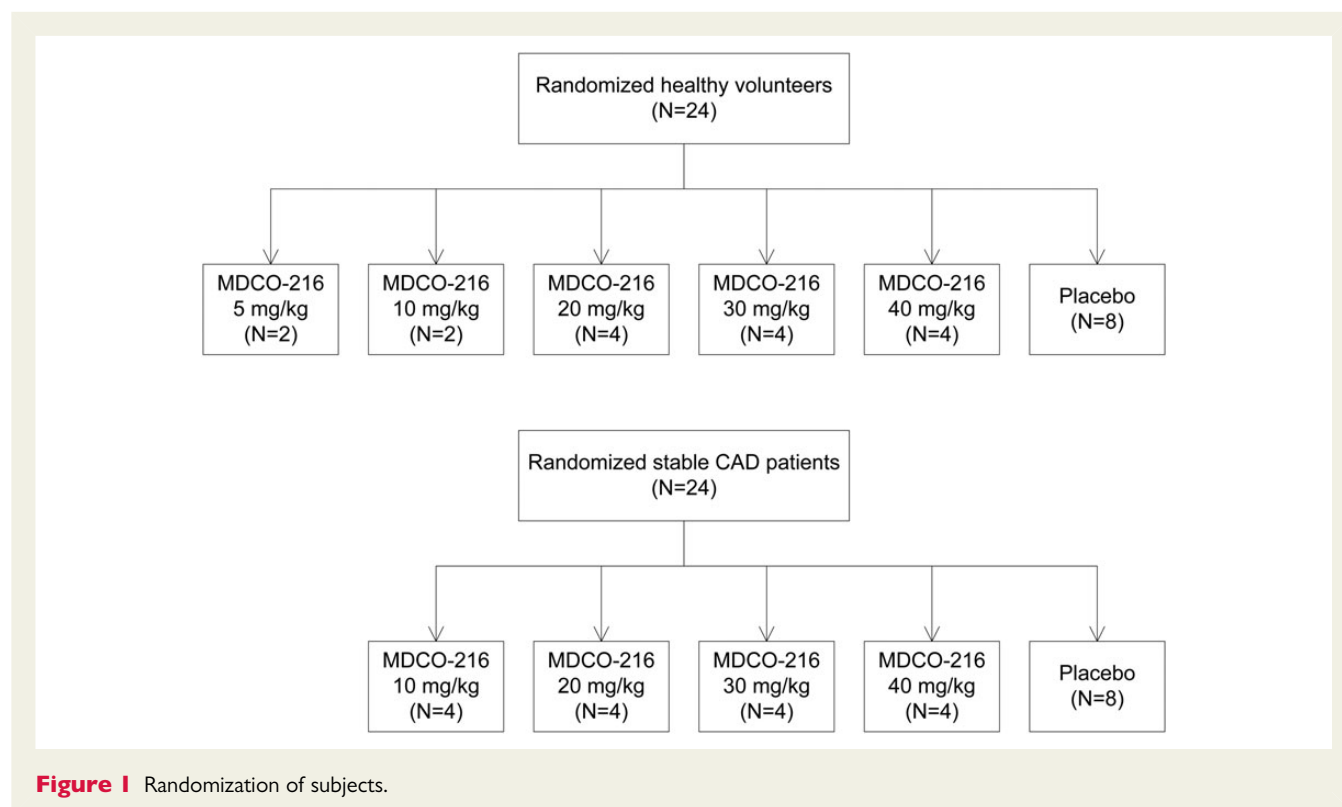
The study protocol was developed by the Sponsor, The Medicines Company, in collaboration with the Centre for Human Drug Research (CHDR) in Leiden, the Netherlands, and complied with the Declaration of Helsinki. The protocol was approved by accredited local (BEBO, Assen, the Netherlands) and national (CCMO, the Hague, the Netherlands) independent medical ethics committees. After signing informed consent and meeting all inclusion and exclusion criteria, 24 healthy volunteers and 24 patients with stable CAD were randomized between February and October 2013 and received a 2 h infusion of MDCO-216 or placebo (Figure 1). A detailed description is provided in Supplementary material online.

### Randomization and blinding

Subjects were assigned in a 2:1 ratio to MDCO-216 or matching placebo. Five cohorts of Healthy volunteers received single doses of 5–40 mg/kg ApoA-1M. Subsequently, four cohorts of stable CAD patients received doses of 10–40 mg/kg ApoA-1M. Dose escalation was only permitted after review of all safety data to Day 7 and approval from a Safety Review Committee consisting of specialists in Cardiology, Vascular Medicine, the Principal Investigator, and Medical Director from the sponsor. In the first two cohorts of healthy volunteers (5 and 10 mg/kg), two subjects received MDCO-216 and one subject received placebo. In all remaining cohorts, four subjects received MDCO-216 and two received placebo.

### Concomitant treatment

Subjects were excluded from the trial if there was a recent or current history of investigational drug products. Concurrent use of HDL raising



therapies was not permitted, as these may have an impact on the pharmacodynamic endpoints of the trial. Stable CAD patients were allowed to remain on all current medications for CV risk factors.

## Pre-defined endpoints

This trial investigated the safety, tolerability, pharmacodynamics, and pharmacokinetics of single escalating doses of MDCO-216 in healthy volunteers and in stable CAD patients. Endpoint evaluation was based on an assessment of safety (including adverse events, laboratory parameters, inflammatory cytokine responses, and anti-MDCO-216 antibodies), pharmacokinetics ( $C_{max}$ ,  $t_{1/2}$ ,  $V_d$ , Cl,  $AUC_{last}$ , and  $AUC_{inf}$ ), and pharmacodynamics (including lipids and *ex vivo* cholesterol efflux capacity).

## Core laboratories

Lipid assays were conducted at the lipid laboratory of the Academic Medical Center in Amsterdam, the Netherlands. Cholesterol efflux assessments were conducted at Vascular Strategies, Plymouth Meeting PA, USA. Other pharmacodynamics parameters were measured at Pacific Biomarkers Inc. in Seattle, WA, USA and at Liposciences Inc. in Raleigh, NC, USA.

Blood chemistry, haematology, and virology were analysed by the Central Clinical Chemistry, Haematology, and Virology Laboratory Leiden University Medical Center, the Netherlands. RND ELISA and whole blood *ex vivo* assays for cytokine IL-6 and TNF- $\alpha$  testing were performed at Good Biomarker Sciences in Leiden, the Netherlands and anti-MDCO-216 assessments were conducted at PPD Inc. in Richmond, USA.

## Statistical plan and analysis

A sample size of 24 healthy volunteers and 24 patients with stable CAD was considered adequate to meet the objectives of this study.

All data were analysed descriptively and/or displayed graphically according to dose level. Placebo-treated patients from all dose groups were pooled. No confirmatory hypothesis was planned to be established in this study; therefore, no formal statistical hypothesis testing was performed. However, when appropriate, *P*-values and two-sided 90 or 95% confidence intervals were derived to demonstrate the strength of the findings.

## Role of the sponsor

The sponsor participated in discussions with CHDR regarding the design and conduct of the study and provided logistical support. Data analyses were assessed jointly. All authors were involved in reviewing and revising the manuscript prior to submission and had full access to the data and final responsibility for the decision to submit for publication.

## Results

### Patient characteristics and patient flow

Thirty-seven healthy volunteers were screened. Three were excluded based on abnormal laboratory results and two because of medical history. Twenty-four were randomized with eight potential replacement subjects.

Thirty-two CAD patients were screened. Two were excluded because of a prolonged QTc interval, and one because of body weight. Twenty-four were randomized with five potential replacement subjects.

Table 1 displays the baseline characteristics of the enrolled subjects. All completed the study per protocol.

**Table 1** Baseline subject characteristics and lipid values

	Healthy volunteers	Stable CAD patients
Mean age (range)	25.6 years (18–54)	62.2 years (46–75)
Gender	10 male, 14 female	23 male 1 female
Race	23 Caucasian, 1 Black	22 Caucasian, 1 Asian, 1 mixed
Mean weight (range)	70.1 kg (54–92)	84.7 kg (62–105)
Mean height (range)	176 cm (164–197)	177 cm (164–193)
Mean BMI (range)	22.5 (19–25)	27 (21–32)
ApoA-1 (mg/dL) (SD)	157.3 (35.3)	121.5 (23.1)
Total cholesterol (mmol/L) (SD)	4.56 (0.77)	4.28 (0.80)
HDL-C (mmol/L) (SD)	1.43 (0.33)	1.21 (0.35)
LDL-C (mmol/L) (SD)	2.18 (0.44)	2.22 (0.54)
Phospholipid (mmol/L) (SD)	2.55 (0.47)	2.38 (0.26)
Pre-beta 1 HDL (mmol/L) (SD)	40.8 (14.6)	65.5 (24.9)
Triglyceride (mmol/L) (SD)	0.95 (0.28)	1.46 (0.61)
Endogenous ApoA-1 (mg/dL) (SD)	157.2 (35.3)	121.4 (23.1)
ApoA-II (mg/dL) (SD)	30.2 (4.9)	27.9 (4.12)
Apo-B (mg/dL) (SD)	77.5 (15.9)	78.4 (20.6)
Apo-E (mg/dL) (SD)	1.93 (0.55)	1.90 (0.42)
ABCA1% (efflux/4 h) (SD)	6.23 (2.03)	6.29 (2.52)
ABCG1% (efflux/4 h) (SD)	6.24 (1.07)	6.97 (0.96)
SRB1% (efflux/4 h) (SD)	5.82 (1.17)	4.71 (0.77)

## Lipids, lipoproteins, and particle profile

Following infusion of MDCO-216, ApoA-1 increased rapidly in a dose-dependent manner in the healthy volunteers with an  $\sim 100$  mg/dL maximal increase from baseline at the end of the infusion (2 h) in the 40 mg/kg cohort. In the stable CAD patients, similar increases were observed for ApoA-1 at 30 and at 40 mg/kg (Figure 2). In the CAD patients, at the two highest MDCO-216 doses, ApoA-1 levels dropped below baseline levels after 24 h and returned to baseline levels between Day 6 and Day 28 (Figure 2). Pre-beta 1 HDL, measured using ELISA, increased in a dose-dependent manner, with maximal levels reached at the end of infusion with a return to baseline levels at 24 h (Figure 3). Despite the higher baseline levels observed in CAD patients, similar increases were observed in both populations.

In both populations, phospholipids increased rapidly during infusion in a dose-dependent manner. The concentration either remained stable (40 mg/kg) or continued to increase (5, 10, and 20 mg/kg) up to 8 h reaching baseline levels on Day 7 (see Supplementary material online, Figure S1). Total cholesterol followed a similar time profile, although the absolute changes were small, especially in the healthy volunteers (see Supplementary material online, Figure S2).

HDL-cholesterol, endogenous ApoA-1 and ApoA-II concentrations decreased, maximally at 48 h post-administration, reaching baseline levels by Day 30 (see Supplementary material online, Figures

S3–S5). This was most profound at the two highest doses and more pronounced in stable CAD patients compared with healthy volunteers.

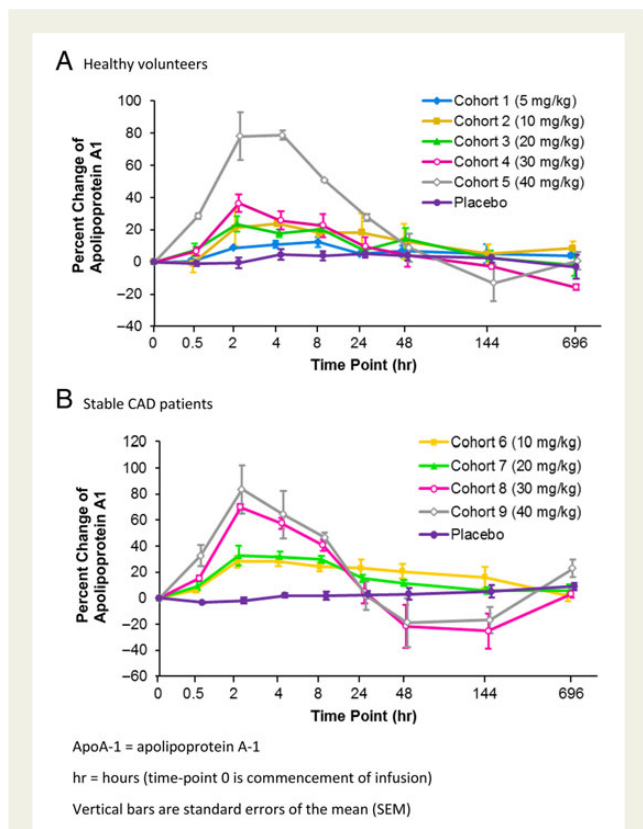
LDL-cholesterol decreased in stable CAD patients in the 30 and 40 mg/kg cohorts from 4 to 8 h and returned to baseline between 8 h and 7 days post-administration. This effect was not observed with lower doses or in the healthy volunteers (see Supplementary material online, Figure S6). Apo-B levels increased at  $\sim 48$  h in both study populations, with the greatest increases and most prolonged effects at the highest doses, returning to baseline levels by Day 30 except for the 40 mg/kg cohorts (see Supplementary material online, Figure S7).

Triglycerides dose-dependently increased in both study populations with a maximum  $\sim$ three-fold increase from baseline observed at 8 h post-administration. Triglycerides remained elevated until study Day 7 in the 30 and 40 mg/kg cohorts (see Supplementary material online, Figure S8).

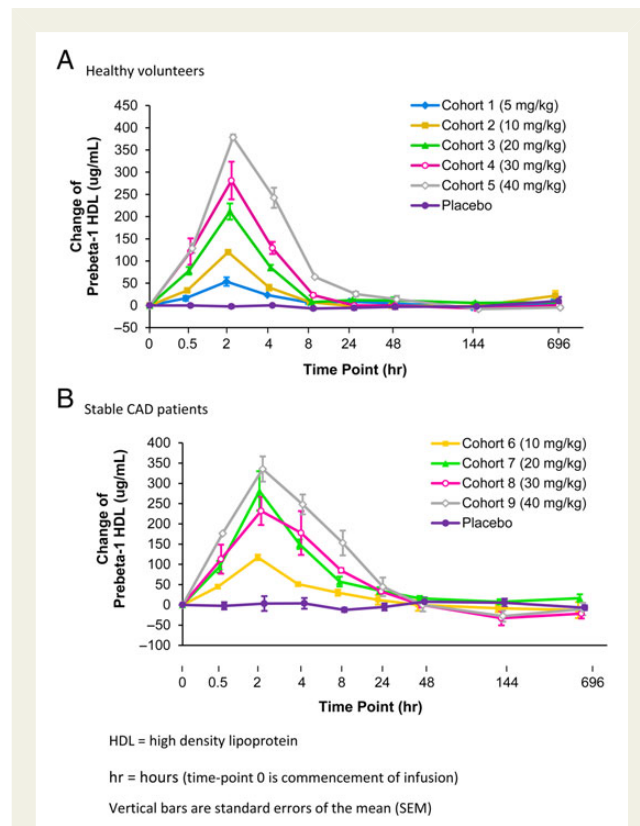
ApoE levels decreased in both study populations for up to 8 h in stable CAD patients compared with 48 h in healthy volunteers (see Supplementary material online, Figure S9).

## Cholesterol efflux capacity

MDCO-216 treatment increased ABCA1-mediated cholesterol efflux up to four-fold from baseline at the highest doses in both cohorts



**Figure 2** Apolipoprotein A-1: change from baseline (%) (A) healthy volunteers, (B) stable coronary artery disease patients. ApoA-1, apolipoprotein A-1, hr, hours (time-point 0 is commencement of infusion). Vertical bars are standard errors of the mean.



**Figure 3** Pre-beta 1 HDL: change from baseline ( $\mu$ g/dL). (A) Healthy volunteers. (B) Stable coronary artery disease patients. HDL, high density lipoprotein; hr, hours (time-point 0 is commencement of infusion). Vertical bars are standard errors of the mean.

(Figure 4). Efflux was maximal at the end of the infusion, and decreased over time to reach baseline levels at Day 7. In the healthy volunteers, efflux was significantly increased at 20 mg/kg, whereas in CAD patients a dose of 10 mg/kg already substantially increased efflux. In healthy volunteers, maximal efflux was observed at 30 mg/kg, in CAD patients 20 mg/kg, indicating a steep dose–response curve. The individual magnitude of responses and time course of the response was generally similar (see Supplementary material online, Figure S10) within each cohort and this was independent of baseline values.

A dose-dependent increase of SR-BI-mediated cholesterol efflux was observed, with maximal effects observed at the end of infusion, returning to baseline levels after 24 h in both populations (see Supplementary material online, Figure S11).

ABCG1-mediated cholesterol efflux was modestly increased between end of infusion and Days 2–7, with no clear dose dependency in any cohort (see Supplementary material online, Figure S12).

Pharmacokinetic results and other pharmacodynamic parameters not presented in this manuscript will be discussed in separate manuscripts.

## Adverse clinical events

MDCO-216 (5–40 mg/kg) as a single dose was safe and generally well tolerated with no SAEs or deaths reported in both healthy volunteers and stable CAD patients.

The most common adverse events reported in the study in MDCO-216 and placebo subjects were headache, catheter site pain and fatigue. These were generally mild and were not observed in an increased frequency at higher doses or compared with placebo.

The only severe non-serious AE that was observed was a case of DVT in a healthy volunteer following 30 mg/kg MDCO-216. Given this participant's previous history of DVT, a direct causative relation to study treatment was considered unlikely by the investigator, although causality to study drug cannot be excluded. No other adverse events or laboratory results suggestive of coagulopathy were observed in this study.

## Laboratory findings

No clinically relevant changes in safety laboratory parameters were observed after infusion of MDCO-216. Glucose–insulin homeostasis was not assessed in this trial, although plasma glucose was unchanged over the course of the study in all dose groups. Transient elevations were seen in serum amylase and pancreatic lipase in two patients following 10 and 20 mg/kg MDCO-216, respectively, and in two placebo subjects. The absence of clinical signs and symptoms did not suggest pancreatic damage caused by MDCO-216.

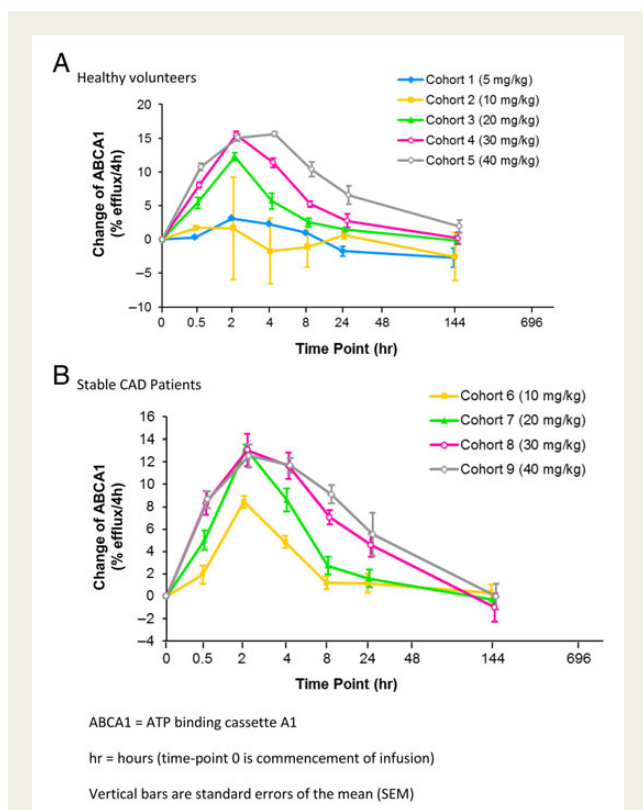
No pro-inflammatory effect, as assessed by plasma levels of IL-6 and TNF- $\alpha$ , was observed after infusion of MDCO-216 *in vivo* or after incubation of whole blood with MDCO-216 *ex vivo*. No induction of ADA was observed after infusion of MDCO-216.

## Discussion

This clinical trial assessed the safety, tolerability, pharmacodynamics, and pharmacokinetics of single doses of MDCO-216 (a complex of ApoA-1M and POPC manufactured by a new process) administered as a 2-h infusion. MDCO-216 was administered initially to healthy volunteers in a SAD design followed by patients with stable CAD. Dose escalation only occurred following review by a Safety Review Committee of safety data collected to Day 7. MDCO-216 was generally well tolerated with no evidence of infusion or hypersensitivity reactions previously observed with ETC-216.

Given the high incidence of CV events, even in patients on statins, additional therapies are required for dyslipidaemia.<sup>20,21</sup> Thus far, in contrast to epidemiology, pharmacological increases of HDL-C have not led to improved CV outcomes, suggesting increases of HDL-C per se may not be protective, at least on top of statin. The function of HDL is possibly more important than the level of HDL-C achieved on drug therapy,<sup>22</sup> although it is not well understood what specifically defines HDL function. Cholesterol efflux capacity is a potential surrogate marker for HDL function,<sup>23</sup> although not all studies have concluded that efflux may be cardioprotective.<sup>24</sup> Potential anti-inflammatory effects should also be considered.

Recent work<sup>25</sup> has shown that ApoA-1 and HDL-C increases are not well correlated with improvements in cholesterol efflux. In the recent dal-ACUTE study with the CETP inhibitor dalcetrapib HDL-C was increased by ~30% but efflux was only improved by an absolute 1% or relative 10% from baseline in acute coronary syndrome patients, further strengthening the argument for a discordance between the quantity of HDL-C and the function of the



**Figure 4** ABCA1-mediated efflux: change from baseline (% efflux/4 h). (A) Healthy volunteers, (B) stable coronary artery disease patients. ABCA1: ATP binding cassette A1; hr, hours (time-point 0 is commencement of infusion). Vertical bars are standard errors of the mean.

HDL particles. This finding was supported by the dal-OUTCOMES study where similar biomarker changes were observed, without any benefit on longer term CV outcomes. Therapies such as niacin and fenofibrate, which also raise HDL-C, have also failed to provide benefit when patients are treated with statins<sup>26,27</sup> and this may be due to the inability of these therapies to normalize parameters of HDL function such as cholesterol efflux. Interestingly, the initial four-fold increase of cholesterol efflux with MDCO-216 at the highest doses is associated with later decreased HDL-C levels.

Cholesterol efflux capacity is markedly impaired in patients with CHD compared with healthy individuals, especially in ACS patients. In this study, baseline efflux levels were similar between volunteers and patients which may reflect the stable clinical status of the CAD patients. As cholesterol efflux is generally independent of HDL-C and ApoA-1 levels,<sup>28</sup> this further raises the potential importance of the need to test therapies such as MDCO-216 that can increase measures of HDL function such as cholesterol efflux to levels comparable with healthy individuals. Also of interest in the current study was the general consistency of response noted in subjects in both cohorts especially at doses of 20 mg/kg and above.

Analyses from two recently reported studies<sup>29,30</sup> have provided insights into the relevance of cholesterol efflux and CV outcomes. In the Dallas Heart Study, a 67% lower CV risk was observed in the lowest versus the highest quartile of cholesterol efflux capacity. Baseline HDL-C had no impact on this relationship. Adding cholesterol efflux capacity to traditional CV risk factors was also associated with improvement in indices of classification and discrimination. In a nested case–control study from the Epic-Norfolk study, the authors concluded that HDL-C efflux capacity was significantly and inversely associated with CV events independent of traditional CV risk factors, even after adjusting for levels of HDL-C and ApoA-1.

Currently, a prospective clinical trial is needed with new therapeutic agents to assess biomarkers of HDL function and specifically cholesterol efflux in terms of the clinical relevance to outcomes such as coronary death, myocardial infarction, and stroke. This could provide a surrogate biomarker guiding early phase trials for new lipid modifying therapies for CV disease. Imaging modalities such as intra-vascular ultrasound and the correlation of changes in imaging parameters such as percent atheroma volume (PAV) with changes in biomarkers such as cholesterol efflux may also help to validate these surrogates given the link that has been inferred between changes in PAV and clinical outcomes.<sup>31</sup>

Given the multiple potential benefits of improvements in HDL function, new therapies to improve HDL function may be of particular relevance in patients with acute coronary syndromes where clinically meaningful improvements in parameters such as high-sensitivity C-reactive protein, vascular function, and efflux are not observed despite marked reduction in LDL-C and increases in HDL-C with statins.<sup>32,33</sup>

ETC-216, the predecessor to MDCO-216, was previously shown to produce regression of atherosclerosis as measured by IVUS. Therefore, MDCO-216 is considered a promising new therapy for atherosclerosis and CV events, although the optimal timing of initiation of therapy relative to an acute coronary event is yet to be determined. ABCA1 efflux is markedly increased by up to four-fold with MDCO-216, despite little change or even a decrease in

HDL-C. In studies with CSL-112, a novel formulation of ApoA-1, a similar magnitude and time course of increases of ABCA1 efflux has also been observed with doses up to 135 mg/kg.<sup>34</sup> These therapies will need to be evaluated in future clinical trials which assess their effects on atherosclerotic disease and CV outcomes.

In conclusion, MDCO-216 was well tolerated with no significant safety findings. The marked changes on lipid-related biomarkers support the future assessment of MDCO-216 in larger and longer term clinical trials with the hope of finding a new therapy for patients with CHD and gaining a better understanding of HDL, ApoA-1, and HDL function. Whether this and other therapies will lead to improvements in CV outcomes remains unproven, but given the remaining incidence of CV events new therapies are required in addition to those currently used in clinical practice.<sup>35</sup>

## Supplementary material

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

## Acknowledgements

We are grateful to Uwe Otto for monitoring the trial, the support staff at CHDR in Leiden, the Netherlands for supervising subject conduct and Dr Tiepu Liu of The Medicines Company, Parsippany, NJ, USA for statistical and programming support.

## Funding

This study was sponsored by the Medicines Company, Parsippany, NJ, USA who also performed project management. Funding to pay the Open Access publication charges for this article was provided by The Medicines Company, Parsippany, New Jersey.

**Conflict of interest:** D.G.K., A.B., S.E.B., H.K., and P.L.J.W. are employees of The Medicines Company and receive share options. J.B., J.A.A.R., and M.M. are employees of the Centre for Human Drug Research, Leiden, the Netherlands.

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