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Original article

Rationale and design of dal-VESSEL: a study to assess the safety and efficacy of dalcetrapib on endothelial function using brachial artery flow-mediated vasodilatation

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Abstract

Objective:
Dalcetrapib increases high-density lipoprotein cholesterol (HDL-C) levels through effects on cholesteryl ester transfer protein (CETP). As part of the dalcetrapib dal-HEART clinical trial programme, the efficacy and safety of dalcetrapib is assessed in coronary heart disease (CHD) patients in the dal-VESSEL study (ClinicalTrials.gov identifier: NCT00655538), the design and methods of which are presented here.

Research design and study method:
Men and women with CHD or CHD risk equivalent, with HDL-C levels <50 mg/dL were recruited for a 36-week, double-blinded, placebo-controlled trial. After a pre-randomisation phase of up to 8 weeks, patients received dalcetrapib 600 mg/day or placebo in addition to their existing treatments. Brachial flow-mediated dilatation (FMD) measured by B-mode ultrasound represents endothelial function and is a validated marker for early atherosclerosis and cardiovascular disease risk.

Main outcome measures:
The primary efficacy outcome is change from baseline in brachial FMD after 12 weeks. The primary safety endpoint is 24-hour ambulatory blood pressure monitoring (ABPM) assessed at week 4. Secondary endpoints include brachial FMD at 36 weeks, ABPM at 12 and 36 weeks, lipid profile, CETP mass and activity, and markers of inflammation, oxidation, and cardiovascular risk. Clinical endpoints are assessed as a composite endpoint for the dal-HEART Program.

Current status:
In 19 European clinical centres, 476 subjects met inclusion criteria and have entered the study. In conclusion, the dal-VESSEL study is the largest multicentre trial with brachial FMD ever performed. The study assesses efficacy and safety of dalcetrapib on endothelial function, blood pressure, lipids, and clinical outcomes in CHD patients with below average HDL-C and will therefore provide vital information regarding its potential role in the preventative treatment of CHD risk.

Introduction

Endothelial dysfunction, lipid accumulation, and migration of inflammatory cells into the artery wall lead to the development of atherosclerotic plaques in the vessel wall[1]. The cardiovascular consequences of this progressive disease account for 30% of all deaths worldwide[2]. Guidelines on cardiovascular disease prevention focus on risk factor management, including the reduction of low-density lipoprotein cholesterol (LDL-C) with hydroxymethylglutaryl coenzyme A reductase inhibitors (statins)[3,4]. Despite the unambiguous efficacy of statins,

the majority of cardiovascular events are not prevented by the administration of these agents[5], leaving room for improved preventative strategies.

High-density lipoprotein cholesterol (HDL-C) is a promising target for pharmacologic intervention, as both case-controlled and population-based epidemiologic data indicate that higher HDL-C levels are associated with lower risk of cardiovascular disease[6]. A review of four large, prospective epidemiologic studies showed that an increase of 1 mg/dL (0.03 mmol/L) in HDL-C was associated with a 2–3% reduction in cardiovascular disease risk[7]. HDL has many properties which are believed to contribute to its protective role, including reverse cholesterol transport, antioxidant, anti-inflammatory, and antithrombotic effects. Dalcetrapib increases HDL-C by reducing cholesteryl ester transfer protein (CETP) activity. CETP mediates the transfer of cholesteryl ester from HDL-C to apolipoprotein B containing lipoproteins and the simultaneous transfer of triglycerides in the opposite direction (Figure 1). In genotype association studies, lower levels of CETP activity have been shown to be associated with a lower incidence of cardiovascular events[8,9]. There is a potential opportunity therefore for additional pharmacotherapies to further reduce cardiovascular disease morbidity and mortality if used in conjunction with standard of care.

Dalcetrapib has been shown to induce dose-related decreases in CETP activity and increases in HDL-C levels and is well-tolerated[10–12]. Specifically, no signs of blood pressure increase, electrolyte changes, or mineralocorticoid excess have been observed at therapeutic doses[10–12]. Evaluation of the latter are of particular importance, as these were the signs of the toxicity exhibited by torcetrapib, the first CETP inhibitor to enter large-scale clinical trials[13]. The toxicity displayed by torcetrapib was most likely related to an off-target toxicity of the torcetrapib molecule, rather than to CETP inhibition itself[14,15].

The dal-VESSEL study is being conducted to assess the safety of dalcetrapib, particularly in relation to blood pressure and vascular endothelial function, as part of the overall dal-HEART (dalcetrapib HDL Evaluation, Atherosclerosis & Reverse cholesterol Transport) Program (Figure 2). Reported here is the study design of this phase IIB, randomised, double-blind, placebo-controlled clinical trial.

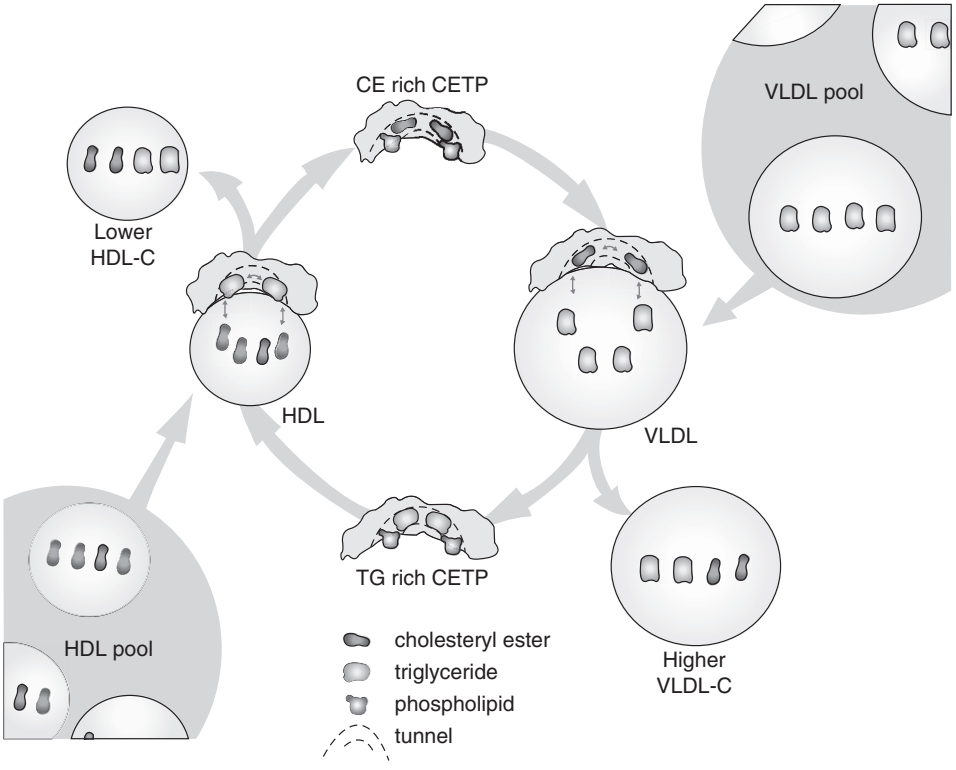

Figure 1. Schematic of the mechanism of CETP mediated lipid transfer. Cholesteryl ester transfer protein (CETP) mediates transfer of cholesteryl ester (CE) from high-density lipoprotein (HDL) to apolipoprotein (Apo) B-containing lipoproteins, e.g. low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL), and the simultaneous transfer of triglycerides (TG) in the opposite direction. Neutral lipids (CE or TG) occupy a continuous 'tunnel' traversing the core of CETP, plugged at either end by phospholipids. Upon binding of CETP, to the lipoprotein, these phospholipids are released allowing neutral lipid exchange to occur. Dalcetrapib binding to CETP prevents CETP from binding to lipoproteins, thus preventing net transfer of CE from HDL and thereby increasing HDL-C levels. Figure adapted with permission from Qiu X *et al. Nat Struct Mol Biol* 2007;14:106.

Patients and methods

Study design

The dal-VESSEL study (ClinicalTrials.gov identifier: NCT00655538; accessed September 1, 2010) is a prospective, randomised, double-blind, placebo-controlled multicentre study that compares 600 mg dalcetrapib once daily with matching placebo, in addition to usual care, in patients with coronary heart disease (CHD) or CHD risk equivalent. The dal-VESSEL protocol was investigator-initiated and the final trial design was performed in collaboration with the study sponsor. The protocol was reviewed and approved by the institutional review board at each of the participating centres and all participants provided written informed consent. The study is being conducted at 19 clinics in France, Germany, Italy, Switzerland, and The Netherlands and is conducted in compliance with the principles of the Declaration of Helsinki and performed according to Good Clinical Practice guidelines.

Patients meeting the entry criteria enter a pre-randomisation phase of up to 8 weeks, to allow adjustment of lipid-lowering therapy. Eligible study participants are randomised according to a computer-generated global randomisation code and assigned in a 1:1 double-blinded fashion, stratified by centre, to receive either 600 mg dalcetrapib or matching placebo tablets for a 36-week period, followed by a 2-week safety follow-up. B-mode ultrasound flow-mediated dilatation (FMD) measurements are performed at baseline, 12 and 36 weeks (Figure 3). Ambulatory blood pressure monitoring (ABPM) is to be performed on a normal working day, at baseline, 4, 12 and 36 weeks. Per the protocol, no change in antihypertensive treatments is allowed from randomisation to week 4.

Inclusion and exclusion criteria

Main inclusion criteria at screening are males and females aged 18–75 years; a diagnosis of CHD or CHD risk equivalent based on National Cholesterol Education Program

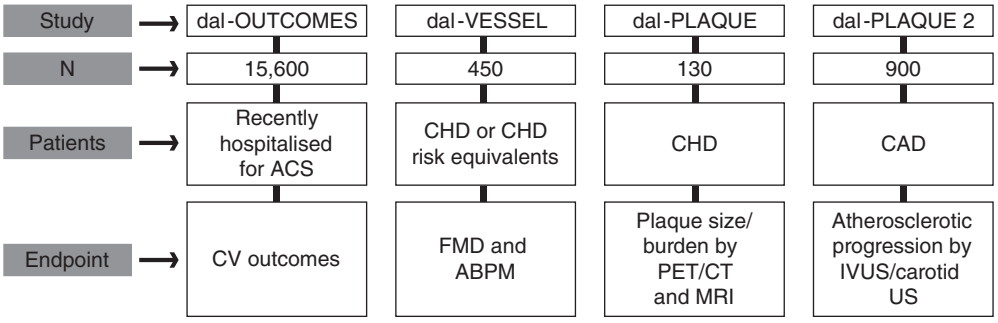

Figure 2. The dal-HEART (dalcetrapib HDL Evaluation, Atherosclerosis & Reverse cholesterol Transport) Program. The dal-HEART Program tests a novel hypothesis, using double-blind, randomised, placebo-controlled studies, that raising HDL-C through CETP inhibition will attenuate cardiovascular risk. ABPM, ambulatory blood pressure monitoring; ACS, acute coronary syndrome; CAD, coronary artery disease; CHD, coronary heart disease; CT, computed tomography; CV, cardiovascular; FMD, flow-mediated dilatation; IV, intravascular; MRI, magnetic resonance imaging; PET, positron emission tomography; US, ultrasound.

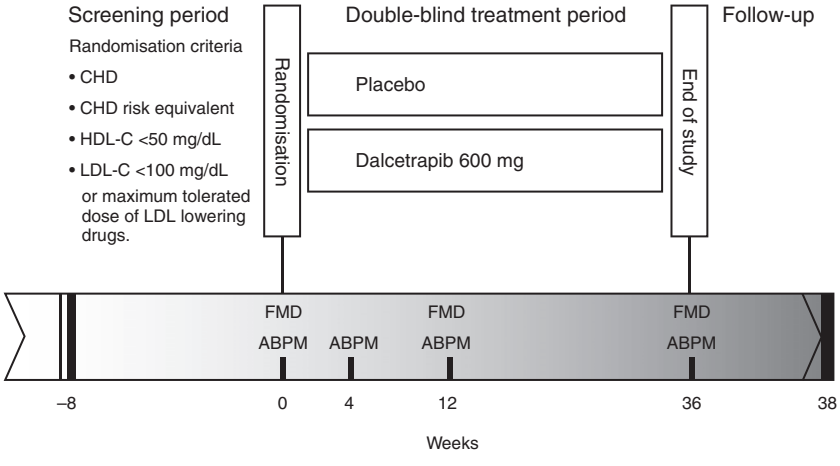

Figure 3. dal-VESSEL study design. CHD, coronary heart disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FMD, flow-mediated dilatation; ABPM, ambulatory blood pressure monitoring.

Adult Treatment Panel III, e.g. clinical atherosclerotic disease, diabetes or >20% 10-year risk of CHD events; HDL-C <50 mg/dL (<1.3 mmol/L) and triglyceride level ≤400 mg/dL (≤4.5 mmol/L). Additional inclusion criteria at randomisation are an evaluable baseline FMD scan, an evaluable 24-hour ABPM assessment and treatment with statin and/or other LDL-C-lowering drugs to a stable LDL-C level <100 mg/dL (<2.6 mmol/L), unless taking maximum tolerated doses of therapy based on their medical condition or intolerance to statin.

Females are excluded if they are pregnant, breast feeding, or if they are of childbearing potential without effective contraception. Other exclusion criteria are concomitant HDL-C raising therapy (niacin, fibrates, bile acid sequestrants, rimonabant, CETP therapy, or other), uncontrolled blood pressure, patients likely to require alterations of antihypertensive treatment during the first 4 weeks of treatment, uncontrolled diabetes, recent clinically significant coronary or cerebral vascular event, familial hypercholesterolaemia, any clinically significant medical condition that could interfere with the conduct of the study, presence of any abnormality on a laboratory evaluation performed prior to randomisation that is considered by the investigator to be clinically important, history of receiving dalcetrapib in a clinical trial in the previous 12 months, or subjects previously exposed to torcetrapib.

Study endpoints

The primary efficacy endpoint is to assess the effect of dalcetrapib added to usual medical care on endothelial function as measured by brachial artery FMD. The primary statistical analysis evaluates %FMD change from baseline to 12 weeks between treatment arms. A secondary statistical analysis includes FMD at 36 weeks. Furthermore, the effect of dalcetrapib on lipids (total cholesterol and triglycerides), lipoproteins (HDL and LDL), apolipoprotein A1 and apolipoprotein B, lipoprotein subfractions, CETP mass and activity, biomarkers of inflammation (high sensitivity C-reactive protein, interleukin-6, soluble platelet-selectin, sE-selectin, soluble intercellular adhesion molecule, soluble vascular cell adhesion molecule, phospholipase A2, matrix metalloproteinase-3 and -9, adiponectin, myeloperoxidase, tissue plasminogen activator, plasminogen activator inhibitor) and insulin sensitivity will be investigated. In addition, a composite cardiovascular endpoint including CHD death, non-fatal myocardial infarction, hospitalisation for documented acute coronary syndrome, resuscitated cardiac arrest, stroke, hospitalisation for congestive heart failure, and any revascularisation procedures will be analysed as part of the overall phase II and III dal-HEART Program.

The primary safety objective of this study is to evaluate the effect of dalcetrapib on blood pressure as measured by 24-hour ABPM after 4 weeks. Secondary endpoints include 24-hour ABPM at weeks 12 and 36, diurnal rhythm of blood pressure at 4, 12, and 36 weeks, daytime and night time mean blood pressure at 4, 12 and 36 weeks, adverse events, laboratory tests (haematology, biochemistry, urinalysis), electrocardiogram, and vital signs (blood pressure measurement using the auscultatory method and pulse).

Statistical analyses plan

The primary efficacy analysis will be intent-to-treat and will include all patients randomised with a post-baseline assessment at 12 weeks. Patients will be assigned to treatment groups as randomised for analysis purposes. The primary efficacy variable is the change in brachial artery %FMD determined by ultrasound between baseline and 12 weeks. The primary hypothesis to be tested is that of a non-inferior effect of dalcetrapib on endothelial function compared with placebo treatment. Based on two assumptions, (1) that a >15% relative decrease in FMD in the dalcetrapib arm after 12 weeks of treatment indicates a disadvantageous effect on endothelial function, and hence on cardiovascular health, and (2) that average %FMD at the start of the study would be approximately 4–4.5%, it was calculated that an absolute decrease in %FMD from baseline of 0.65% or less in the dalcetrapib 600 mg/day arm would indicate non-inferiority (i.e., a ≤15% relative decrease in FMD). In addition, the hypothesis that %FMD will differ between treatment groups, such that dalcetrapib 600 mg/day will be significantly more effective than placebo in increasing %FMD after 3 months of treatment, is also to be tested. Non-inferiority will be tested first and if non-inferiority is concluded, the superiority test will be performed. A two-sided significance level will be used for this hierarchical testing of the hypotheses. Based on previous assessments of reproducibility (Eric de Groot, MD, PhD, personal communication) the pooled data standard deviation of %FMD change from baseline is assumed to be 2.0%. Assuming a two-sided α of 0.05 and a β of 0.1 (power of 90%), 200 subjects per treatment arm will allow for observation of a between group difference in %FMD of 0.65%. To account for a 10% drop-out in follow up, 450 patients are planned to be randomised per protocol.

With regard to the safety analysis, the population will include all patients who received at least one dose of study medication and had a safety assessment at baseline. Patients will be assigned to treatment groups as randomised for analysis purposes. If there are patients who switch from their initally randomised treatment, then if warranted, selected summaries will be reproduced based

on patients being assigned to treatment groups as treated for analysis purposes. The primary safety hypothesis to be tested is that of a non-harmful effect of dalcetrapib on blood pressure compared with placebo treatment after 4 weeks' treatment. No harm is concluded if the upper 95% confidence limit for the difference in ABPM change from baseline is less than 2 mmHg. Under the assumptions that there is no difference between treatments in the expected mean change from baseline in ABPM, the pooled data standard deviation of 24-hour ABPM change from baseline is 6 mmHg, and the null hypothesis is rejected if the upper 95% confidence limit for the difference in means (dalcetrapib minus placebo) is less than a no-harm threshold of 2 mmHg, then with 90% power and a dropout rate of 10%, a total sample size of 450 randomised patients is sufficient.

For the secondary efficacy endpoint, the hypothesis will be tested that %FMD will differ between treatment groups, such that dalcetrapib 600 mg/day will be significantly more effective than placebo in increasing %FMD after 36 weeks of treatment. This hypothesis will also be tested for the effect of dalcetrapib on 24-hour ABPM, blood lipids, CETP mass and activity, biomarkers of inflammation, insulin sensitivity, and a composite cardiovascular endpoint as part of the overall dal-HEART Program.

For the primary and secondary efficacy, and the safety analyses, a linear model will be assumed including treatment and centre (small centres will be grouped together) as fixed effects along with a covariate term for the baseline value of %FMD or 24-hour ABPM. Additional models will also be used in those cases where warranted to improve the model fit, include terms for baseline LDL-C, baseline HDL-C and a treatment by centre interaction. *P*-values, point estimates, and 95% CIs will be determined for each of the summary statistics.

Brachial B-mode ultrasound imaging

Prior to FMD assessment, patients are to adhere to the preparation requirements for the scan. All patients are requested to fast, starting at least 12 hours prior to the scan (water is allowed), as well as refrain from strenuous exercise during that period. Caffeine, smoking, and intake of vitamin C are not allowed from 6 hours prior to the scan. Regular medication is to be taken, but no other medication is allowed prior to the FMD scan. Measurements take place in a quiet, temperature-controlled (20–24°C) room. From 15 minutes prior to start of the scan, subjects remain at rest in the supine position.

B-mode ultrasound scans of the right brachial artery are obtained using a Sonix SP ultrasound machine (Ultrasonix, Vancouver, Canada) equipped with a 7.5 MHz linear array transducer (or ultrasound probe). All Sonix SPs are equipped with a validated and compliant machine application protocol. Patients are scanned in the supine position. The brachial artery measures approximately 3–5 mm in diameter and may shift slightly in position during the FMD procedure. Measuring 0.0–0.4 mm brachial lumen changes, in particular in follow-up studies, therefore poses considerable ultrasound and mechanical challenges. To cope with these issues the right arm is stabilised in an integrated arm rest and ultrasound probe holder positioning device (AMC Vascular Imaging, Academic Medical Center, Amsterdam, The Netherlands). The device consists of three microscope stages at right angles to each other and a hydraulically damped flexible arm with a specifically designed probe holder clamp in which the ultrasound transducer is attached. The patient's arm is stabilised in two foam-embedded half cylinders which are placed on the same board as the probe holder. The arm rest enables stable and standardised positioning of the arm, using the medial epicondyle of the humerus as an anatomical landmark. This stable and per protocol arm positioning allows defined positioning of the blood pressure cuff around the lower arm.

The distance of the ultrasound transducer with respect to the medial epicondyle is measured to ensure that the same segment of the brachial artery is studied in the initial and follow-up scans. The ultrasound transducer is then attached to the probe holder. The arm of the probe holder is flexible to position the probe and can be fixed once the probe is positioned. The microscope stage to which the probe holder is attached enables sub-millimetre adjustments by the sonographer watching the ultrasound monitor. In this way the transducer position can be moved in lateral, longitudinal, and rotational directions to obtain the optimal transducer position in respect to the arterial walls and to correct for the small arterial movements when the blood pressure cuff is released.

The right brachial artery is visualised in the longitudinal direction. Depth and gain settings are set to optimise the lumen/arterial wall contrast. Magnification of the ultrasound image is set at 140% high-definition zoom.

With the patient's arm and the transducer correctly positioned, the sonographer starts the ultrasound instrument application protocol. The protocol starts with a 1-minute baseline ultrasound recording, followed by 5 minutes of forearm ischaemia (not recorded), induced by inflating a vascular pressure cuff, placed downwards from the medial epicondyle, to 250 mmHg. Hyperaemic blood flow is induced by deflating the cuff, after which 3 minutes of ultrasound recording follows. Image acquisition is ECG gated on the R-wave and the ultrasound images saved in a Digital Imaging and Communications in Medicine (DICOM) clip. During cuff occlusion no images are acquired to allow for a smaller DICOM clip file size. In order to ensure the secure and regulatory compliant transmission of data from the image acquisition sites to the

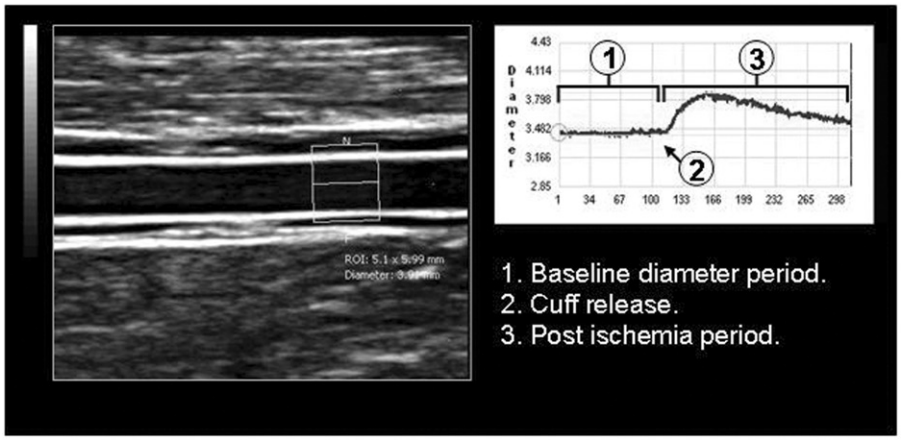

Figure 4. B-mode ultrasound of brachial artery flow-mediated dilatation (FMD). This figure shows a longitudinal image of the brachial artery. A diastolic image is recorded in each heartbeat. For off-line image analyses, a region of interest (white box) is selected for continuous automated measurement of brachial artery diameter. On the right, the brachial diameters (*y*-axis) are shown for each recorded image (*x*-axis). The average baseline diameter can be calculated from the recorded frames prior to cuff inflation. The maximum diameter is measured after cuff release.

centralised server, a standard device data transfer box is utilised (CERTU Medical, Amsterdam, The Netherlands). Together with the mirrored servers, image data is made available in real time to The London Core Lab (London, UK). Image administration and processing systems comply with the necessary international regulations and guidelines. Scan analysis is performed by FMD experts at The London Core Lab and the coordinating quality control, information technology, and data management activities by AMC Vascular Imaging (Amsterdam, The Netherlands).

Repeat scans and ultrasound image analysis

The use of brachial FMD to follow endothelial function in a multicentre clinical trial is challenging due to numerous factors which contribute to measurement variability. In part, this variability can be counterbalanced by standardising environmental and measurement means and methods. In addition to creating favourable conditions of standardisation, and application of the good working practices of sonographers and readers for a controlled clinical trial, the extent and sources of variability need to be quantified, understood, and described. To meet these quality control requirements in the dal-VESSEL study, extensive repeat scans and scan analyses are being performed to understand the extent and sources of variation in FMD. To assess intra-sonographer interscan FMD repeatability, for each study timepoint a subset of up to 35% of trial participants is scanned twice by all sonographers, with at least one night in between scans. All scans are assessed qualitatively within 6 hours by the FMD core laboratory to provide feedback to sonographers and are consecutively assessed in full (e.g., qualitatively and quantitatively) for quality and efficacy purposes. Scans for a given patient, including the repeat scans, are all analysed by one reader. Some of the scans are analysed in full more than once, to assess inter- and intra-reader variability. For quality control purposes, a subset of scans analysed in the core lab were also assessed by the quality control lab in Amsterdam (cross-validation).

For image analysis, the reader selects a region of interest in the longitudinal image of the brachial artery. Upon definition of the correct interfaces of the scan, the Brachial Analyser software performs an automated tracing of the lumen–wall boundaries of the near and far wall of the images of the clip of the scan (Figure 4).

The Brachial Analyzer software quantifies a brachial artery lumen diameter for each image of the scan, before and after cuff occlusion. Average baseline diameter (D_{base}) and the maximum post-cuff deflation diameter (D_{max}) are used to calculate the absolute flow-mediated vasodilatation (FMD_{abs}) and %FMD. FMD_{abs} is defined as: $FMD_{abs} = D_{max} - D_{base}$ and %FMD, the primary endpoint of the dal-VESSEL trial, is defined as: $\%FMD = 100 (D_{max} - D_{base})/D_{base}$.

ClinicalTrials.gov identifier: NCT00655538.

Current results

Patient enrolment

Enrolment into dal-VESSEL was initiated in June 2008. A total of 476 patients have been randomised, with the last patient randomised in August 2009. Baseline characteristics of patients who received at least one dose of study treatment (n=472) are shown in Table 1. The proportion

Table 1. Baseline characteristics of patients who received at least one dose of study treatment.

Characteristic*	Patients ($n = 472$)
Age, years	62.1 ± 7.55
Male sex, *n* (%)	427 (90)
Body mass index	29.10 ± 4.66
Medical history of, *n* (%)	
Coronary heart disease	306 (65)
Symptomatic carotid artery disease	35 (7)
Peripheral arterial disease	42 (9)
Abdominal aortic aneurysm	14 (3)
Type II diabetes	211 (45)
Hypertension	349 (74)
Smoking, *n* (%)	
Never	96 (20)
Former	253 (54)
Current	123 (26)
Statin use†, *n* (%)	450 (95)
Blood pressure, mmHg	
Systolic	135.0 ± 14.32
Diastolic	79.4 ± 8.82
Cholesterol, mg/dL (mmol/L)	
Total	149.26 ± 23.98 (3.86 ± 0.62)
LDL-C	80.05 ± 19.64 (2.07 ± 0.51)
HDL-C	38.67 ± 7.27 (1.00 ± 0.19)
Triglycerides, mg/dL (mmol/L)	153.23 ± 73.42 (1.73 ± 0.83)

*All reported as mean ± standard deviation unless otherwise stated.
†Patients with at least one treatment.

of male patients is slightly higher than that predicted for a CHD study population, but is unlikely to impact the results of the study. Results will be reported in 2011.

Discussion

Epidemiologic data have shown that both lower LDL-C levels and increased HDL-C levels are associated with reduced cardiovascular disease risk. However, while there are many effective treatments for lowering LDL-C, currently available therapies that increase HDL-C have either limited efficacy or are restricted by tolerability issues. Dalcetrapib increases HDL-C levels through a novel mechanism by reducing CETP activity. The dal-VESSEL study is a randomised, double-blind, placebo-controlled, multicentre trial, designed to assess the effects of dalcetrapib complementary to statins or other LDL-C-lowering therapy, on endothelial function and blood pressure in patients with CHD or CHD risk equivalent and HDL-C levels below 50 mg/dL.

There is a strong rationale for assessing endothelial function and blood pressure to investigate the efficacy and safety of dalcetrapib. In the Investigation of Lipid Level Management Using Coronary Ultrasound to Assess Reduction of Atherosclerosis by CETP Inhibition and HDL Elevation (ILLUSTRATE) trial, and the Rating Atherosclerotic Disease change by Imaging with A New CETP Inhibitor (RADIANCE) 1 and 2 trials[13,16–18], the CETP inhibitor torcetrapib was shown to have no beneficial effect on coronary and carotid atherosclerosis progression. Furthermore, the morbidity and mortality trial, Investigation of Lipid Level Management to Understand Its Impact in Atherosclerotic Events (ILLUMINATE), showed an increased risk of cardiovascular events and death from any cause after torcetrapib treatment[14]. It has been hypothesised that the negative clinical effects of torcetrapib are due to the off-target toxicity observed with this agent, specifically, substantial blood pressure increases, electrolyte changes, and increases in circulating aldosterone levels[14,15]. These off-target effects are thought to be at least partly due to activation of the renin-angiotensin-aldosterone system (RAAS) and could have mitigated the potential impact of HDL-C elevation. Recent data from a genetic association study suggest these side-effects are likely to be specific to torcetrapib and not related to CETP inhibition[19]. Activation of the RAAS, increases in blood pressure, and inflammation all cause an increase in the production of reactive oxygen species and therefore contribute to endothelial dysfunction[20]. Alteration in endothelial function affects the accumulation of lipids and inflammatory cells in the artery wall, thereby accelerating atherogenesis and the occurrence of clinical complications. Preclinical studies of dalcetrapib, which has a different mechanism of action to torcetrapib, have excluded off-target effects on blood pressure and the RAAS[21,22]. To date, there have been no reports of a greater incidence of adverse events relating to blood pressure, electrolyte changes or aldosterone production compared with placebo in clinical studies of dalcetrapib[10,11]. Investigating whether CETP modulation[23] by dalcetrapib does not negatively affect endothelial function, without increases in blood pressure and inflammatory markers, is imperative for understanding if dalcetrapib is likely to be of assistance in the prevention of cardiovascular disease and its complications.

The inclusion of 24-hour ABPM after 4, 12 and 36 weeks, as a safety endpoint in this study, was intended to provide the most rigorous assessment to date of the effect of acute and chronic treatment with dalcetrapib on blood pressure. In ILLUMINATE, almost 50% of patients receiving torcetrapib experienced an increase in systolic blood pressure >2.5 mmHg within 1 month[13]. In dal-VESSEL, ABPM after 4 weeks was chosen as the primary safety endpoint, with no change to anti-hypertensive therapy allowed within the first 4 weeks, to evaluate potential acute effects of dalcetrapib on blood pressure. In addition, ABPM measurements at 12 and 36 weeks will evaluate potential chronic effects on blood pressure. In the dal-PLAQUE and dal-PLAQUE 2 studies, part of the dal-HEART Program, the effect of dalcetrapib on atherosclerotic plaque size and burden and atherosclerotic disease progression will be assessed. The dal-VESSEL and dal-PLAQUE studies will increase our understanding of the safety and tolerability profile of dalcetrapib, while

dal-OUTCOMES will serve more fully to define the clinical utility of dalcetrapib for reduction of cardiovascular morbidity and mortality[24].

In the dal-VESSEL study, endothelial function is assessed by brachial artery FMD, which is largely nitric oxide-dependent[25,26]. Previous studies have shown that brachial artery FMD is closely related to the coronary endothelium-dependent vasomotor response to acetylcholine[27]. This indicates endothelial dysfunction is a generalised process and is not necessarily confined to vascular beds with clinically overt atherosclerosis. The predictive value of brachial artery FMD for cardiovascular disease is supported by data from a number of studies, two of which were conducted at multiple centres[28–31]. In the multicentre population-based Cardiovascular Health Study (CHS), which followed 2792 adults, aged 72–98 years for 5 years, FMD proved to be a significant predictor of cardiovascular events with a hazard ratio of 0.91 (95% CI, 0.83–0.99), $p = 0.02$ per unit SD of FMD[28]. Although the CHS participants are older (mean age 78 years) than those enrolled in the dal-VESSEL study (mean age 62 years), in other trials that assessed statins, blood pressure-lowering medication, and lifestyle interventions in relatively younger adult patients and children[32–35], brachial FMD response (and coronary dilatation in response to acetylcholine in one study)[33] has been shown to be concordant with clinical endpoints. For example, in multicentre studies inverse correlations were observed between FMD and achieved systolic blood pressure after antihypertensive treatment ($r = -0.25$; $p < 0.05$)[32] and between the arterial response to acetylcholine and LDL cholesterol level at follow-up after statin therapy ($r = -0.46$, $p < 0.05$)[33], and in a large single-centre study between FMD and average serum cholesterol level in young children after dietary intervention started in infancy ($r = -0.83$, $p = 0.004$)[35]. Conversely, in a single-centre study in middle-aged men and women with multiple coronary risk factors, short-term life-style intervention (diet and exercise) resulting in modest improvements in exercise capacity and LDL cholesterol (−11% reduction) but no change in blood pressure, did not improve vascular endothelial function as measured by brachial artery FMD[34]. The advantage of FMD is that short term exposure to drugs can be assessed. Furthermore, due to the noninvasive nature of this modality, patients can be repeatedly assessed in a short period of time.

The dal-VESSEL study is the first large multicentre drug efficacy and safety study to use FMD as the primary efficacy endpoint. Consistency in FMD measurement between centres is maintained by using one scan protocol and standardised equipment. Most importantly, as described above, the integrated arm rest/ultrasound probe holder, used at all sites, was designed to allow for optimum imaging of the small brachial arteries in all stages of the FMD imaging protocol. The Ultrasonix Sonix SP ultrasound machine is programmable and has a trial-specific validated application protocol to guide the sonographer while carrying out FMD scans. The near-field linear array transducer permits detailed imaging of the small superficially located brachial artery. Furthermore, the application protocol permits cardiac gating and recording of a diastolic B-mode ultrasound image in each heartbeat, continuously during the first and last 3 minutes of the protocol. Ultrasound scans can be sent automatically via a secure transfer system, the data transfer box, to the core laboratory for data storage and analysis.

The protocol has been designed for optimum patient preparation and scanning, aimed at minimising the environmental influences on FMD derived from diet, medication, smoking, physical exercise, and temperature. For maximal adherence to the protocol, all sonographers underwent training and certification for the dal-VESSEL project, under the supervision of experienced FMD trainers, prior to being allowed to scan patients.

Conclusion

dal-VESSEL is an important component in the dal-HEART Program assessing dalcetrapib. The study is part of the de-risking strategy surrounding dalcetrapib and will help to determine the safety profile of dalcetrapib by assessing its effects on endothelial function, blood pressure, and vascular inflammation complementary to regular lipid-altering treatment in patients with CHD or CHD risk equivalent. dal-VESSEL is the largest multicentre study so far to employ FMD as a primary endpoint and its design includes extensive repeat scan and image analysis procedures as well as rigorous in-trial quality assurance and control measures, to ensure data quality and standardisation. The dal-VESSEL trial will therefore provide important information on the efficacy and safety of dalcetrapib. Further study is needed, however, to determine whether interventions aimed at increasing HDL-C through actions on CETP that are devoid of off-target toxicity will ultimately improve CHD outcomes. The dal-OUTCOMES study is being conducted to address this question[24].

Transparency

Declaration of funding
This study was funded by F. Hoffmann-La Roche Ltd.

Declaration of financial/other relationships
T.B., D.K. and V.L. have disclosed that they are employees of F. Hoffmann-La Roche. J.J.P.K. has disclosed that he has received research funding from AstraZeneca, Roche, Eli Lilly, Novartis, Merck, Merck Schering Plough, Isis Pharmaceuticals and Genzyme. He also has served as an advisor to AstraZeneca, Pfizer, Isis Pharmaceuticals, Genzyme, Roche, Novartis, Merck, Merck Schering Plough, Boehringer Ingelheim, Karo Bio,

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