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FASTTRACK CLINICAL RESEARCH
Clinical trials

Pharmacogenetics-guided dalcetrapib therapy after an acute coronary syndrome: the dal-GenE trial

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See the editorial comment for this article 'Pharmacogenetics-guided CETP inhibition: an open question?', by Anne Tybjærg-Hansen *et al.*, https://doi.org/10.1093/eurheartj/ehac398.

Abstract

Aims In a retrospective analysis of dal-Outcomes, the effect of dalcetrapib on cardiovascular events was influenced by an adenylate cyclase type 9 (*ADCY9*) gene polymorphism. The dal-GenE study was conducted to test this pharmacogenetic hypothesis.

Methods and results dal-GenE was a double-blind trial in patients with an acute coronary syndrome within 1–3 months and the AA genotype at variant rs1967309 in the *ADCY9* gene. A total of 6147 patients were randomly assigned to receive dalcetrapib 600 mg or placebo daily. The primary endpoint was the time from randomization to first occurrence of cardiovascular death, resuscitated cardiac arrest, non-fatal myocardial infarction, or non-fatal stroke. After a median follow-up of 39.9 months, the primary endpoint occurred in 292 (9.5%) of 3071 patients in the dalcetrapib group and 327 (10.6%) of 3076 patients in the placebo group [hazard ratio 0.88; 95% confidence interval (CI) 0.75–1.03; $P = 0.12$]. The hazard ratios for the components of the primary endpoint were 0.79 (95% CI 0.65–0.96) for myocardial infarction, 0.92 (95% CI 0.64–1.33) for stroke, 1.21 (95% CI 0.91–1.60) for death from cardiovascular causes, and 2.33 (95% CI 0.60–9.02) for resuscitated cardiac arrest. In a pre-specified on-treatment sensitivity analysis, the primary endpoint event rate was 7.8% (236/3015) in the dalcetrapib group and 9.3% (282/3031) in the placebo group (hazard ratio 0.83; 95% CI 0.70–0.98).

Conclusion Dalcetrapib did not significantly reduce the risk of occurrence of the primary endpoint of ischaemic cardiovascular events at end of study. A new trial would be needed to test the pharmacogenetic hypothesis that dalcetrapib improves the prognosis of patients with the AA genotype.

Clinical Trial Registration Trial registration dal-GenE ClinicalTrials.gov Identifier: NCT02525939

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Structured Graphical Abstract

Key Question

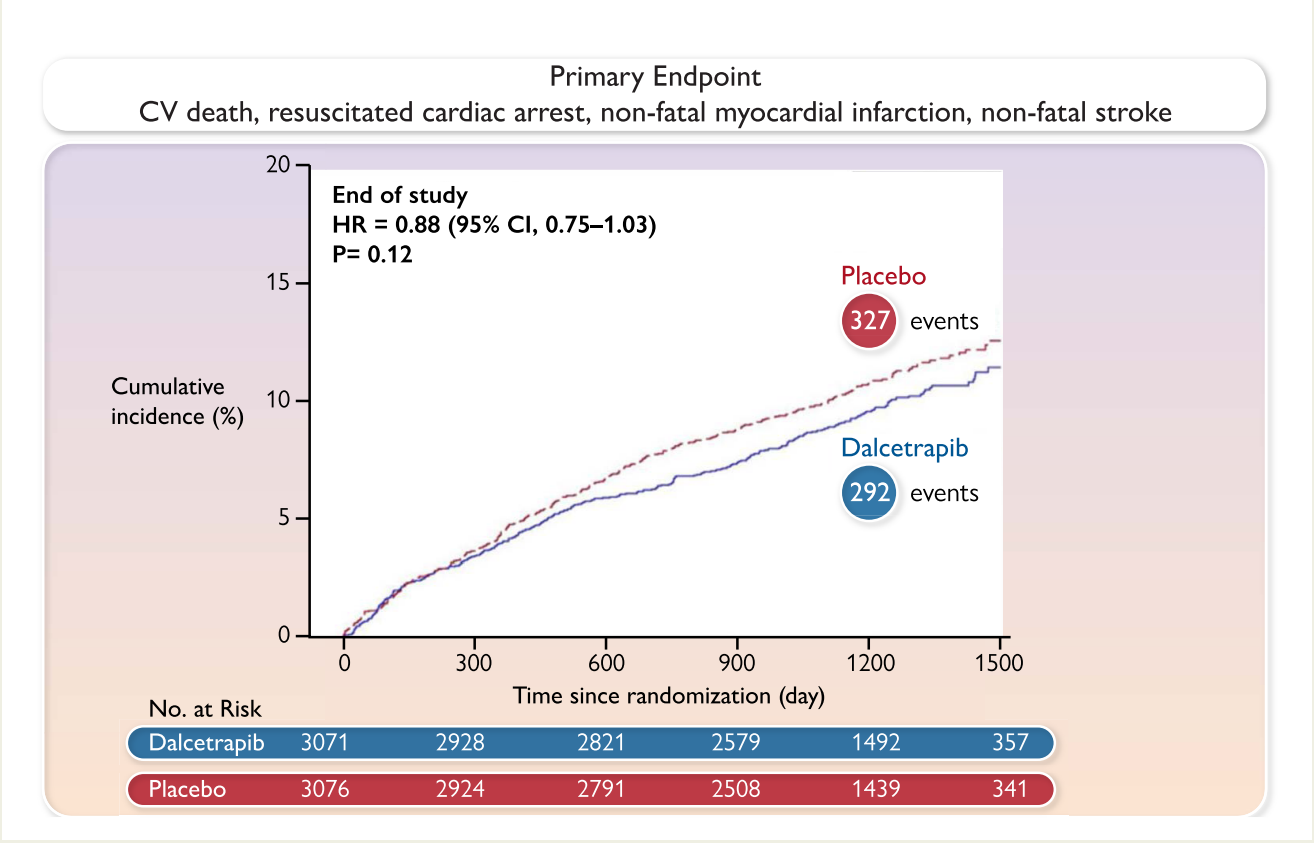
Does dalcetrapib provide cardiovascular (CV) benefits in patients with the genotype AA at variant rs1967309 in the ADCY9 gene?

Key Finding

- Primary endpoint event occurred in 9.5% of patients with dalcetrapib and 10.6% of patients with placebo (hazard ratio, 0.88; 95% CI, 0.75-1.03; P=0.12).
- On treatment analysis: Primary event occurred in 7.8% with dalcetrapib and 9.3% with placebo (hazard ratio, 0.83; 95% CI, 0.70-0.98).

Take Home Message

A new trial is needed to test the pharmacogenetic hypothesis that dalcetrapib improves the prognosis of patients with the AA genotype.



Dalcetrapib in patients with AA genotype at variant rs1967309 in the *ADCY9* gene.
CI, confidence interval; CV, cardiovascular; HR, hazard ratio.

Keywords Precision medicine • Atherosclerosis • Myocardial infarction • CETP • Adenylate cyclase type 9 (ADCY9) • Genetics

Introduction

Atherosclerotic cardiovascular disease continues to cause major morbidity despite the current standard of care including maximal doses of high-potency statins.[1] Proprotein convertase subtilisin kexin type 9 (PCSK9) inhibitors can produce very low levels of low-density lipoprotein (LDL)-cholesterol and have resulted in a reduction of 15% in composite primary cardiovascular event rates.[2–4] This creates the opportunity for further improvement of clinical outcomes with non-LDL-based approaches.[5]

In addition to their effect on reverse cholesterol transport, high-density lipoprotein (HDL) particles have anti-inflammatory, anti-oxidative, and anti-thrombotic effects.[6] Despite raising HDL-cholesterol, the cholesteryl ester transfer protein (CETP)

modulator dalcetrapib had a neutral overall effect on cardiovascular events in the dal-Outcomes study.[7] The pharmacogenomic analysis of dal-Outcomes showed that the single-nucleotide polymorphism rs1967309 in the adenylate cyclase type 9 (*ADCY9*) gene was associated with the effects of dalcetrapib on cardiovascular outcomes.[8] Patients with the AA genotype exhibited a 39% reduction in the primary composite cardiovascular endpoint when treated with dalcetrapib compared with placebo, whereas AG heterozygotes had a neutral result and those with the GG genotype showed a 27% increase in risk.[8] Supporting ultrasonography data from the dal-Plaque-2 study showed regression, stability, and progression, respectively, of carotid atherosclerosis for each of the three genotypes (AA, AG, and GG) in the dalcetrapib group.[8] Concordant findings were also obtained for the changes over time in cholesterol efflux.[9]

Inactivation of *Adcy9* in genetically modified mice protects from atherosclerosis, but only in the absence of CETP.[10] Furthermore, the demonstrated relationship between ADCY9 and CETP during recent human evolution has pointed towards a biological link between dalcetrapib's effect modifier gene *ADCY9* and its therapeutic target CETP.[11] The multiple observed interactions between ADCY9 and CETP in both animals and patients suggested that they represent a real biological phenomenon and not a spurious statistical association. Accordingly, we designed and conducted the dal-GenE study to test prospectively the pharmacogenetic hypothesis that dalcetrapib provides cardiovascular benefits when administered to patients with the favourable genotype.[12] The study design was similar to that of dal-Outcomes, with the major exception of the specific genetic inclusion criterion. The dal-GenE study represents the first large-scale cardiovascular precision medicine trial targeting atherosclerotic cardiovascular disease.[12]

Methods

Trial design

Dal-GenE was a double-blind, parallel-group, placebo-controlled, randomized trial comparing orally administered dalcetrapib 600 mg once daily with placebo in a 1:1 ratio.[12] The randomization was stratified by region and the type of acute coronary syndrome index event, to ensure that equal numbers of patients received active treatment and placebo in these strata.

The study was funded by DalCor Pharmaceuticals. The trial protocol was designed by the study executive committee and sponsor. The protocol and informed consent form were approved by the institutional review board at all centres involved in the 31 countries that participated in the trial (Supplementary material).

Study support activities of project coordination, data management, statistical oversight, and analyses were performed by the Montreal Health Innovations Coordinating Center (MHICC), and site management and monitoring were performed by MHICC, Medpace, ECLA, and GCLC. The randomization list was computer generated and uploaded in an interactive web response system (IWRS) provided by Medpace.

The database was a validated electronic case report form (eCRF) using InForm 6.0 provided by Oracle. The eCRF was developed by the MHICC and the sponsor. All eCRF users were trained as per completion guidelines and data entry was performed by the local study staff. The data cleaning activities were performed as per the MHICC data management plan. The trial was overseen by a data monitoring committee of independent experts (see Supplementary material online, *Table S1*). All efficacy endpoints were adjudicated by an independent clinical event committee. The study medication and matching placebo were provided by Recipharm (Madrid, Spain) and Calatent (Kansas City, MO, USA), respectively.

The first author (J.C.T.) and lead statistician (M.C.G.) prepared the first draft of the manuscript, had full access to the trial database, generated statistical analyses, made the decision along with co-authors to submit the manuscript for publication, and assumed responsibility for the accuracy and completeness of the data and analyses and for the fidelity to the protocol.

Trial population

Participants were enrolled across 630 investigational sites located in North America, South America, Europe, Middle East, South Africa, Australia, and New Zealand. Patients were eligible if they were at least 45 years of age, recently hospitalized for an acute coronary syndrome within the previous 1–3 months, clinically stable, treated with guidelines-based management of LDL-cholesterol at a minimum to a target level <2.6 mmol/L, and confirmed in a central laboratory to have the AA genotype at variant rs1967309 in the *ADCY9* gene in DNA derived from whole blood using the validated cobas® genotype system (Roche Molecular Systems) and a real-time polymerase chain reaction test. Genetic testing results were available to the clinical research site within 2–4 days of the blood draw.

Patients were excluded if they had New York Heart Association Class III or IV heart failure, coronary artery bypass graft surgery between the index event and the randomization, clinically apparent liver disease, a history of malignancy (except for curatively treated basal cell or squamous cell carcinoma of the skin) during the 1 year prior to screening, current or recent alcohol or drug abuse, and/or a life expectancy shorter than 3 years; serum triglyceride level above 5.65 mmol/L, glycated haemoglobin above 10%, serum creatinine above 195 μmol/L, transaminase level higher than three times the upper limit of normal, and/or haemoglobin lower than 10 g/dL; or were pregnant, breastfeeding, or of childbearing potential if not using contraception. Further details regarding eligibility criteria are provided in the Supplementary material online, *Tables S2 and S3*.

Written informed consent was obtained from all patients before enrolment. Blinded randomization was centralized and performed electronically through an automated IWRS. Allocation sequence was computer generated in a 1:1 ratio using a global randomization code and was stratified as described above. Eligible patients were randomized by research nurses through the IWRS system that provided the bottle number to give to patients. The patient randomization numbers were allocated sequentially in the order in which the participants were enrolled.

All staff involved, including study investigators and nurses, and patients were blinded to the treatment received. All randomized patients received instructions on a heart healthy diet; were counselled on appropriate lifestyle modifications such as weight control, physical activity, and smoking cessation; received contemporary evidence-based medical care; and were to complete all planned revascularization procedures prior to randomization. Clinical evaluations occurred at 1 and 6 months following randomization, and visits thereafter took place every 6 months for efficacy and safety assessments until completion of the trial.

Study endpoints

The primary efficacy endpoint was the time from randomization to the first occurrence of any component of the composite endpoint, which included death from cardiovascular causes, resuscitated cardiac arrest, non-fatal myocardial infarction, or non-fatal stroke, as positively adjudicated by the clinical endpoint committee. Each component of the primary composite endpoint was also reported. The secondary endpoints

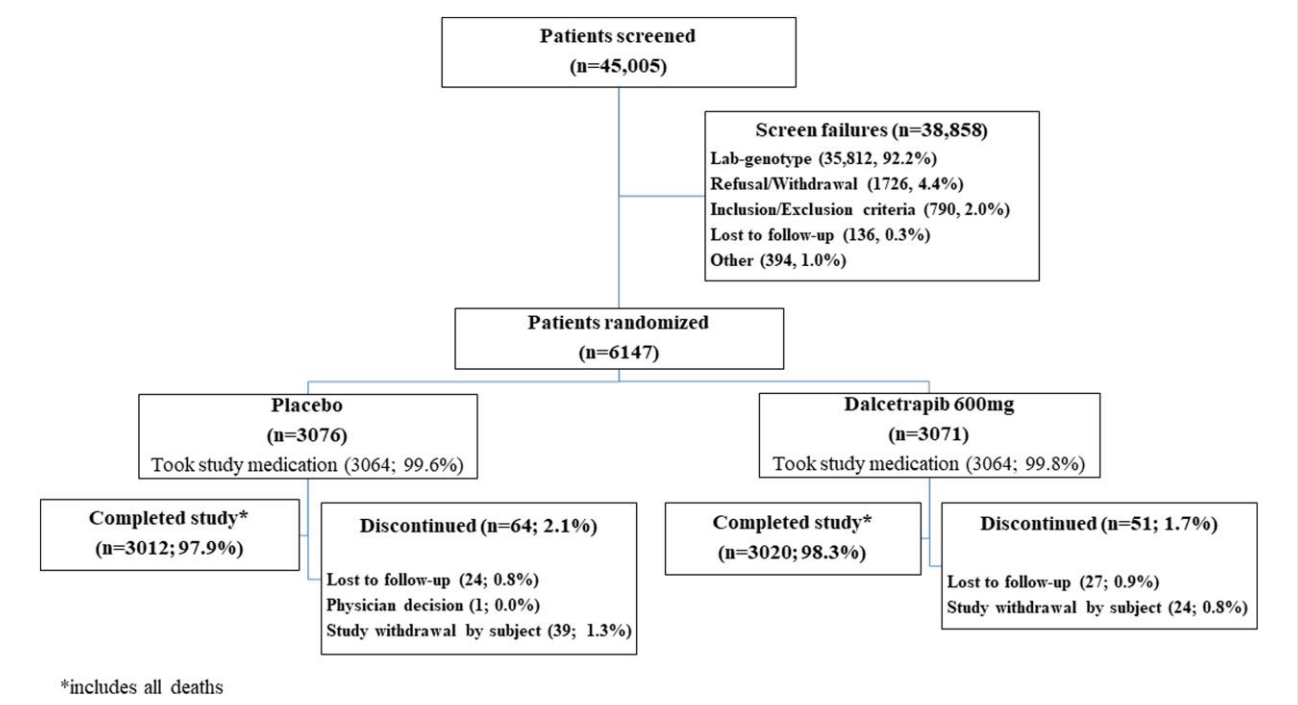


Figure 1 dal-GenE trial: patient disposition.

were the times from randomization to (i) the composite of the primary endpoint, hospital admission for acute coronary syndrome (with ECG abnormalities), or unanticipated coronary revascularization; and (ii) the composite of the primary endpoint or hospital admission for new or worsening heart failure (see Supplementary material online, *Table S4*). Exploratory endpoints included changes from baseline to 6 months in blood levels of lipid subfractions and high-sensitivity C-reactive protein. Treatment safety, including effects on diabetes, was evaluated through the assessment of adverse events and laboratory measurements.

Management of the impact of the COVID-19 pandemic on the study

The last 17 months of patient follow-up were conducted during the COVID-19 pandemic. A series of actions were taken to pro-actively manage this challenging and unprecedented situation and the potential impact of COVID-19 on trial execution. The trial protocol was amended in January 2020 to allow remote patient visits, remote monitoring, and direct drug shipments to patients. The clinical events committee included the relationship to COVID-19 in their adjudication process of study endpoints. Also, the statistical analysis plan was amended to allow for an analysis related to the direct effect of COVID-19 by excluding adjudicated events considered related or possibly related to COVID-19 and censoring patients at the date of a COVID-19-related serious adverse event.

Sample size calculation

The target sample size of 6000 randomized participants was calculated assuming an expected relative risk reduction of 22% and a statistical significance defined as a two-sided alpha of 0.05. The trial had 85% power when 582 patients had experienced a positively adjudicated primary event in the combined treatment groups assuming a 2.8-year recruitment period, a 1% yearly lost to follow-up rate, and a 7% event rate at 2 years following randomization to the placebo group. In this event-driven trial, the minimum detectable effect (at a significance level of 0.05) of dalcetrapib compared with placebo at study end was 15%.

Statistical analyses

The analyses were performed on the intention-to-treat population, which included all randomized patients with the exception of two who were randomized by error. This population was approved prior to breaking the blind. A stratified Cox proportional hazards model, accounting for the two stratification factors used for randomization, was utilized to analyse the primary endpoint. Time to event started at randomization and patients who were lost to follow-up (while event-free) were censored at the time that they were last known to be event free. The null (H_0) and alternative (H_A) hypotheses tested with the above Cox model were H_0: $\lambda = 1$ vs. H_A: $\lambda \neq 1$, where λ is the assumed constant hazard ratio for the time to occurrence of the composite events of the primary endpoint for the dalcetrapib and placebo groups. The hazard ratio, within strata, was assumed to depend on treatment alone. The analyses were conducted at the 0.05 significance level.

Secondary endpoints were expressed as time to event and analyses similar to that for the primary endpoint were conducted. Estimates of treatment effect are presented with 95% confidence intervals (CIs) and *P*-values.

In order to control the Type I error that results from the multiplicity of endpoints, the secondary endpoints were to be formally tested using the Hochberg's step-up procedure only if the primary analysis resulted in a significant treatment effect at $P < 0.05$. Otherwise, statistical tests for the secondary endpoints were to be presented for illustrative purposes.

The primary efficacy endpoint was also evaluated within subgroups of subjects (region, age, sex, and diabetes) using Cox proportional hazards models, adding to the models a term for the factor defining the subgroup and a term for the interaction between the factor and the treatment group. This interaction term was tested at the 0.1 significance level and determined whether the treatment effect was affected by the presence

Table 1 Characteristics of the trial patients

Characteristic	Dalcetrapib (*N* = 3071)	Placebo (*N* = 3076)
Age, years (mean ± SD)	62.2 ± 9.2	62.3 ± 9.3
Female sex, *n* (%)	723 (23.5)	672 (21.8)
White, *n* (%)	2855 (93.0)	2866 (93.2)
Body mass index, kg/m^2	28.6 ± 4.8	28.6 ± 4.7
Smoking, *n* (%)	681 (22.2)	682 (22.2)
Hypertension, *n* (%)	2153 (70.1)	2163 (70.3)
Diabetes, *n* (%)	797 (26.0)	782 (25.4)
LDL-cholesterol, mg/dL	71.8 ± 30.6	71.2 ± 29.8
HDL-cholesterol, mg/dL	43.5 ± 12.6	43.3 ± 12.4
Triglycerides, mg/dL	136.2 ± 82.9	135.5 ± 86.8
Hs-C-reactive protein, mg/L	3.9 ± 8.2	3.9 ± 8.8
Prior MI, *n* (%)	604 (19.7)	627 (20.4)
Prior PCI, *n* (%)	700 (22.8)	711 (23.1)
Prior CABG, *n* (%)	152 (4.9)	163 (5.3)
Prior heart failure, *n* (%)	647 (21.1)	702 (22.8)
Prior stroke/TIA, *n* (%)	183 (6.0)	152 (4.9)
Index ACS to randomization, days	53.8 ± 18.1	54.0 ± 18.3
PCI for index MI, *n* (%)	2651 (86.3)	2648 (86.1)
Aspirin use, *n* (%)	2827 (92.0)	2807 (91.3)
Other anti-platelet agent, *n* (%)	2915 (94.9)	2904 (94.4)
Statin use, *n* (%)	3021 (98.4)	3026 (98.4)
Beta-blocker, *n* (%)	2594 (84.5)	2609 (84.8)

ACS, acute coronary syndrome; CABG, coronary artery bypass graft surgery; HDL, high-density lipoprotein; Hs, high sensitivity; LDL, low-density lipoprotein; MI, myocardial infarction; PCI, percutaneous coronary intervention; TIA, transient ischaemic attack.

of the factor. In addition, under the proposed models, the treatment effect was estimated and presented with 95% CI within subgroups.

An on-treatment sensitivity analysis of the primary endpoint was prespecified, excluding participants who discontinued study treatment prematurely and who had their first primary event 14 days or more after the date of the last dose of study treatment, or participants who never took study medication and had a primary event.

A futility analysis was conducted by the independent Data Monitoring Committee, to determine if there was sufficient evidence of efficacy to justify continuation of the trial to its completion. This futility assessment, performed when approximately 70% of the target number of patients with a first positively adjudicated primary endpoint was reached, was based on the conditional power of the trial derived under various relative risk reduction assumptions.

After the trial topline results became available, the Data Monitoring Committee shared its closed reports, which suggested a favourable effect on the primary endpoint increasing up to the time of the futility analysis (reviewed on 23 January 2020, based on events adjudicated up to December 2019). Because the first occurrence of documented changes in study procedures (including delivery of medication to patients' houses) and the World Health Organization emergency committee meeting evaluating whether COVID-19 constituted a public health emergency both occurred during the week of 23 January 2020, a non-pre-specified COVID-related sensitivity analysis was conducted by censoring data on that date.

Treatments were also examined with respect to the incidence of adverse events, serious adverse events, and adverse events leading to premature study withdrawal or premature study drug withdrawal. Adverse events were grouped and summarized by body system as defined by version 19.1 of the Medical Dictionary for Regulatory Activities (MedDRA), following classification of investigator assessments into MedDRA preferred terms. Statistical analyses were performed using SAS version 9.4.

Role of the sponsor

The sponsoring organization (DalCor Pharmaceuticals) was involved in the design and management of the study; had no role in the collection, management, and analysis of the data, in the preparation of the manuscript, and in the decision to submit the manuscript for publication. The sponsor contributed to the interpretation of the data and review of the manuscript, but did not have the right to veto publication or to control the decision regarding to which journal the manuscript was submitted.

Results

Patients

Trial enrolment began in April 2016 and was completed in December 2018; the last trial visit was in June 2021. A total of 45 005 patients with recent acute coronary syndrome were screened in order to identify participants meeting the single genetic criterion and other inclusion criteria and no exclusion criteria required for randomization, particularly given the overall prevalence of the AA genotype at rs1967309 in the *ADCY9* gene of 20% in the tested population. A total of 6149 eligible patients underwent randomization, among whom two were randomized by error and excluded. The median duration of follow-up was 39.9 months. At the time of database lock and unblinding in July 2021, vital status was available for all except for 58 patients (99.1%). The rates of loss to follow-up and withdrawal of consent were 0.8 and 1.0%, respectively. Details regarding the disposition of the patients are provided in *Figure 1*.

The baseline characteristics of patients are shown in *Table 1*. Patients were enrolled a mean of 53.9 days after the index acute coronary syndrome. The mean age of participants was 62.3 years, 22.7% of the patients were women, mean body mass index was 28.6 kg/m^2, and 25.7% had diabetes. Aspirin, another anti-platelet agent, and a statin were taken by 91.6, 94.7, and 98.4% of the patients, respectively. At the end of the study, the trial medication had been prematurely discontinued in 25.7% of the patients in the dalcetrapib group and in 25.2% of the patients in the placebo group. The mean treatment duration with the trial medication was 33.5 ± 14.5 months.

Clinical efficacy endpoints at end of trial

A primary endpoint event occurred in 292 (9.5%) of the 3071 patients in the dalcetrapib group, when compared with 327 (10.6%) of the 3076 patients in the placebo group (hazard ratio 0.88; 95% CI 0.75–1.03; $P = 0.12$; *Figure 2*). *Table 2* shows the event rates and

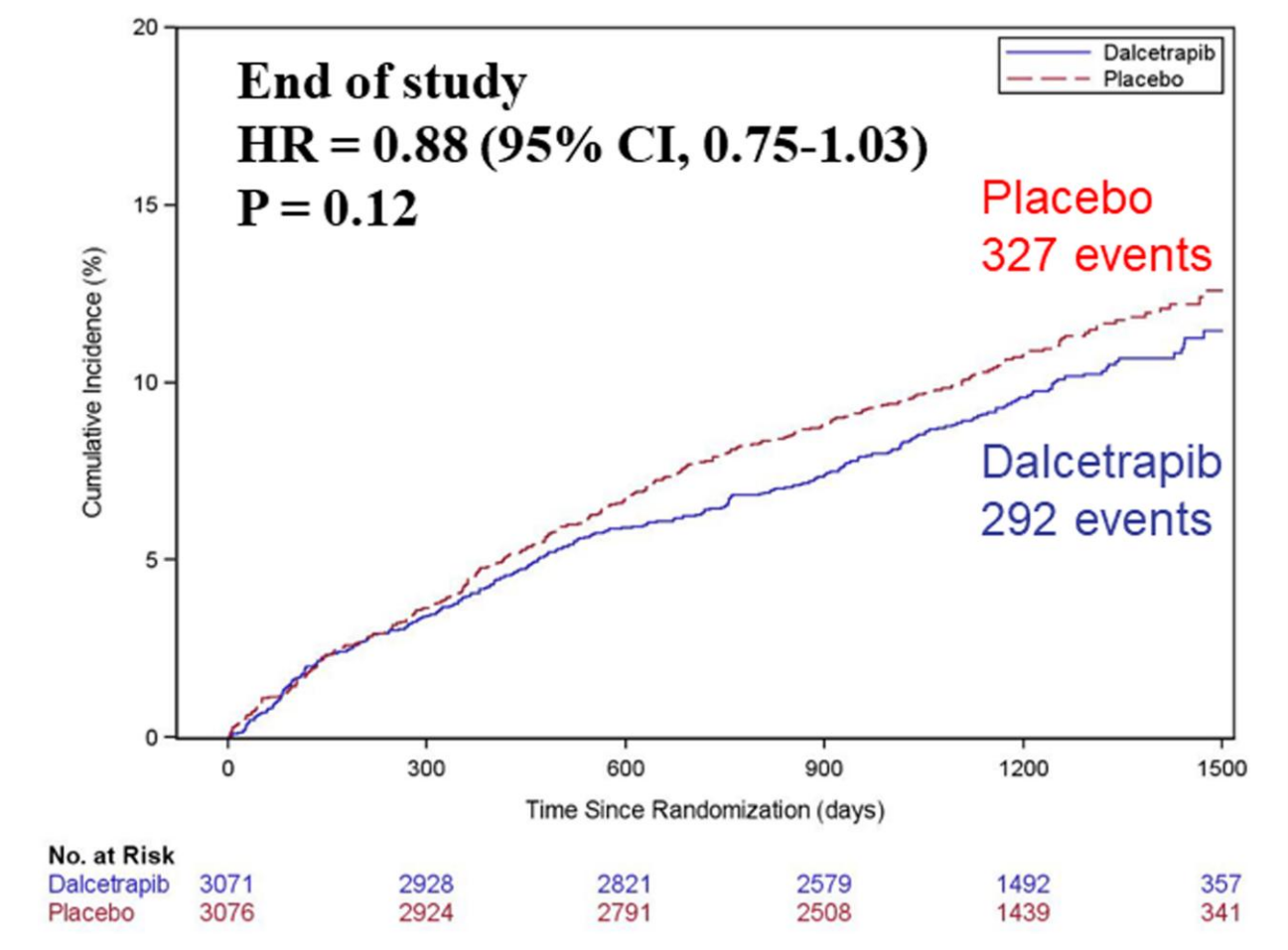


Figure 2 Kaplan–Meier curves for the primary efficacy endpoint at end of study.

hazard ratios for the components of the primary endpoint, which included death from cardiovascular causes (hazard ratio 1.21; 95% CI 0.91–1.60), resuscitated cardiac arrest (hazard ratio 2.33; 95% CI 0.60–9.02), myocardial infarction (hazard ratio 0.79; 95% CI 0.65–0.96), and stroke (hazard ratio 0.92; 95% CI 0.64–1.33). Adjustment for baseline HDL-cholesterol did not affect the primary endpoint result (hazard ratio 0.87; 95% CI 0.75–1.02; *Table 2*).

In a pre-specified on-treatment sensitivity analysis of the primary endpoint excluding participants who discontinued study treatment prematurely and who had their first primary event 14 days or more after the date of the last dose of study treatment, or participants who never took study medication and had a primary event (56 patients in the dalcetrapib group and 45 patients in the placebo group), the primary endpoint event rate was 7.8% (236/3015) in the dalcetrapib group and 9.3% (282/3031) in the placebo group (hazard ratio 0.83; 95% CI 0.70–0.98).

Exploratory analysis of clinical efficacy endpoints before the COVID-19 pandemic

The pre-specified COVID-specific analysis that excluded adjudicated events considered related or possibly related to COVID-19 yielded a result similar to that of the primary analysis. In that sensitivity analysis, a primary endpoint event occurred in 288 (9.4%) of the 3071 patients in the dalcetrapib group, when compared with 323 (10.5%) of the 3076 patients in the placebo group (hazard ratio 0.88; 95% CI 0.75–1.03). We further assessed the effects of the pandemic on the trial (see Supplementary material online, *Table S5*). There was a greater reduction in the number of non-fatal myocardial infarctions reported during the pandemic compared with pre-COVID-19 than that of deaths (see Supplementary material online, *Figure S1*), with a change of the ratio of non-fatal myocardial infarction to all-cause death from 1.74 pre-COVID-19 to 0.96 during COVID-19. The evolution of the ratio of cardiovascular to non-cardiovascular death from pre-COVID-19 to during COVID-19 is shown in Supplementary material online, *Figure S2*. In a non-pre-specified sensitivity analysis censoring on 23 January 2020, a primary endpoint event occurred in 6.9% (213/3071) and 8.4% (257/3076) of the patients in the dalcetrapib and placebo groups, respectively (hazard ratio 0.82; 95% CI 0.68–0.98; Supplementary material online, *Figure S3*). Supplementary material online, *Table S6* describes the event rates and hazard ratios for the components of the primary endpoint in that analysis and Supplementary material online, *Figure S4* depicts the evolution of the hazard ratio for the primary endpoint by calendar date.

Table 2 Rates and hazard ratios for major clinical outcomes at end of study

Clinical outcome	Dalcetrapib (*N* = 3071)	Placebo (*N* = 3076)	Hazard ratio (95% CI)	*P*-value
Primary composite endpoint, *n* (%)	292 (9.5)	327 (10.6)	0.88 (0.75–1.03)	0.12
Components of primary endpoint CV death, *n*	64	55		
Resuscitated cardiac arrest, *n*	5	2		
Non-fatal MI, *n*	171	221		
Non-fatal stroke, *n*	52	49		
All CV death, *n* (%)	104 (3.4)	86 (2.8)	1.21 (0.91–1.60)	
All cardiac arrest, *n* (%)	7 (0.2)	3 (0.1)	2.33 (0.60–9.02)	
All myocardial infarction, *n* (%)	180 (5.9)	226 (7.3)	0.79 (0.65–0.96)	
All stroke, *n* (%)	55 (1.8)	59 (1.9)	0.92 (0.64–1.33)	
Primary, hospital admission for ACS, or unanticipated coronary revascularization,[a] *n* (%)	471 (15.3)	471 (15.3)	1.00 (0.88–1.13)	
All unanticipated coronary revascularization,[b] *n* (%)	318 (10.4)	307 (10.0)	1.04 (0.89–1.21)	
All urgent unanticipated coronary revascularization,[b] *n* (%)	112 (3.6)	139 (4.5)	0.80 (0.62–1.03)	
All elective unanticipated coronary revascularization,[b] *n* (%)	226 (7.4)	194 (6.3)	1.17 (0.97–1.42)	
Primary or hospital admission for heart failure,[a] *n* (%)	321 (10.5)	346 (11.2)	0.92 (0.79–1.07)	
All-cause death, *n* (%)	180 (5.9)	179 (5.8)	1.00 (0.82–1.24)	
Primary or all-cause death,[b] *n* (%)	360 (11.7)	407 (13.2)	0.87 (0.76–1.01)	
Primary or non-COVID-19 death,[b] *n* (%)	345 (11.2)	393 (12.8)	0.87 (0.75–1.00)	

Adjustment for baseline HDL-cholesterol did not affect the primary endpoint result (hazard ratio 0.87; 95% CI 0.75–1.02).
ACS, acute coronary syndrome; CI, confidence interval; CV, cardiovascular; MI, myocardial infarction.
[a]Pre-specified secondary endpoint.
[b]Not pre-specified.

Subgroups and biomarkers

Efficacy results in pre-specified subgroups at end of study and censored on 23 January 2020 are shown in *Figure 3* and Supplementary material online, *Figure S5*. Results for the changes in lipid subfractions, high-sensitivity C-reactive protein, and blood pressure as well as new-onset diabetes are shown in Supplementary material online, *Table S7*. There was a higher increase in HDL-cholesterol and a smaller decrease in high-sensitivity C-reactive protein with dalcetrapib compared with placebo ($P < 0.0001$ for both).

Safety and adverse events

The incidence of serious adverse events was 29.3% in the dalcetrapib group and 30.0% in the placebo group ($P = 0.55$), and the incidence of adverse events was 77.5 and 76.8% of the patients in the two groups ($P = 0.53$, *Table 3*). At least one treatment-emergent gastrointestinal adverse event occurred in 27.0% of the patients in the dalcetrapib group, when compared with 24.0% of the patients in the placebo group ($P = 0.006$). Diarrhoea was reported in 9.8 and 6.1% of patients in the two trial groups ($P < 0.0001$). Nausea occurred in 3.3% of patients in the dalcetrapib group and 2.1% of those in the placebo group ($P = 0.007$). Headache and cough were also reported as adverse events more frequently in the dalcetrapib group (>2.0%) than in the placebo group.

Discussion

Dal-GenE did not demonstrate a statistically significant result on the primary composite endpoint of cardiovascular death, resuscitated cardiac arrest, non-fatal myocardial infarction, or non-fatal stroke at end of trial. There were fewer non-fatal myocardial infarctions with dalcetrapib compared with placebo and a numerical excess of cardiovascular deaths and resuscitated cardiac arrests at end of study (*Structured Graphical Abstract*).

The study endpoints appear to have been impacted by the last 17 months of follow-up that occurred during COVID-19 pandemic, when the reporting of non-fatal events (including myocardial infarction) was reduced but deaths (especially non-cardiovascular deaths) were increased. However, the pre-specified COVID-specific analysis that excluded adjudicated events considered related or possibly related to COVID-19 yielded a result similar to that of the primary analysis. Study results censored on 23 January 2020 showed a nominally significant reduction of 18% in the occurrence of the primary endpoint with dalcetrapib compared with placebo. Thus, dalcetrapib appeared to be associated with this benefit up to the emergence of COVID-19, which was attenuated during the pandemic. COVID-19 occurred as early as January 2020 around the world and probably even earlier in some countries and had a global impact

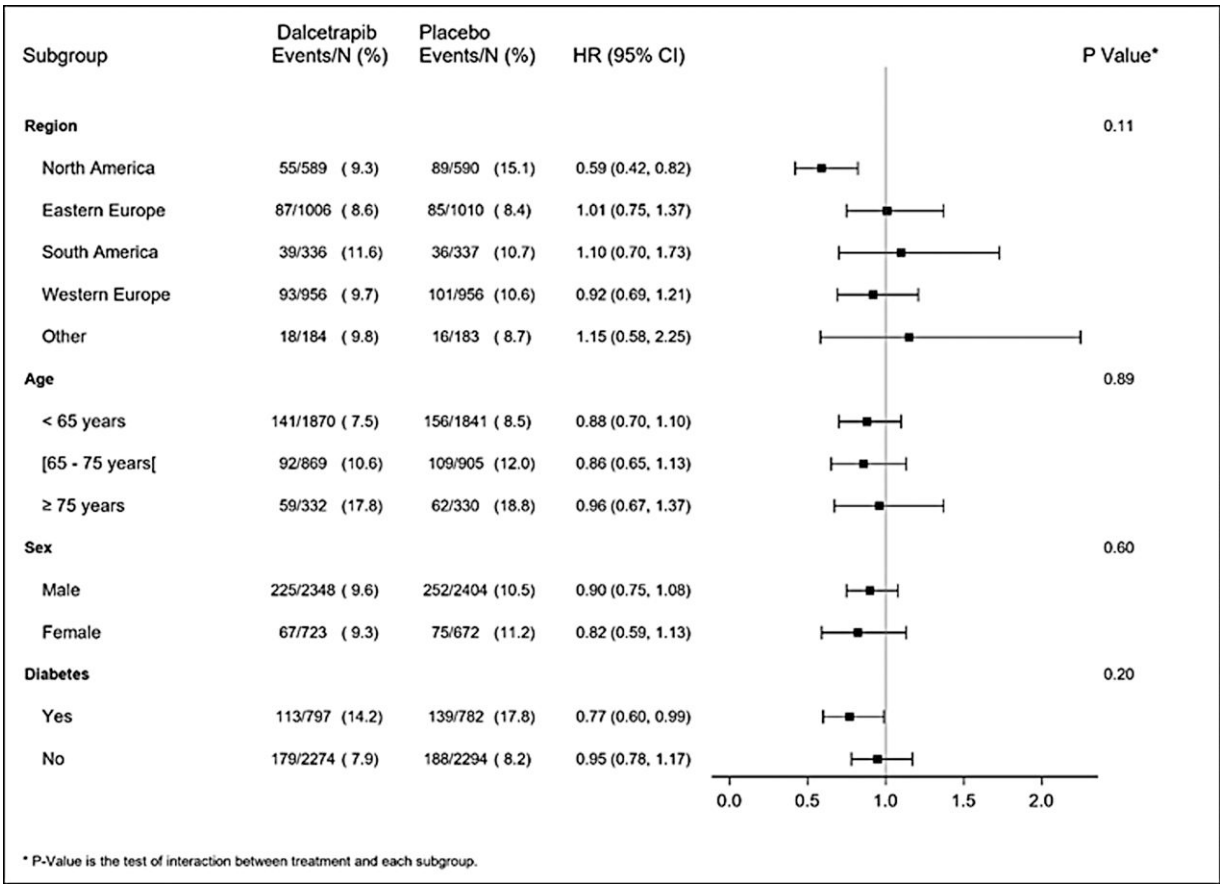


Figure 3 Efficacy results in pre-specified subgroups at end of study.

on cardiovascular care outside of dal-GenE.[13–15] In particular, the numbers of patients diagnosed and hospitalized with myocardial infarction decreased precipitously, while mortality increased.[13–15] During that period, hospitals were overloaded with severe COVID-19 cases, and patients with cardiovascular disease often did not seek medical care because they were more concerned with the fear of contracting the virus than by the actual complications of their cardiac condition. Occasionally, patients could not even reach hospitals because of various restrictions and difficulties. In dal-GenE, the much greater reduction in non-fatal events during the pandemic compared with pre-COVID than that of deaths is consistent with the published impact of the pandemic on cardiovascular care.[13–15] The reduction in the number of reported myocardial infarctions during the pandemic in dal-GenE reduced the relative weight of this key component on the composite primary endpoint. This phenomenon was compounded by the increase in all-cause and non-cardiovascular deaths during the same period.

The reduced numbers of myocardial infarctions at end of study and of the composite primary endpoint before the pandemic with dalcetrapib are concordant with the results in the population of patients with the AA genotype at position rs1967309 in the *ADCY9* gene in dal-Outcomes.[8] These results are also supported by the regression of carotid artery disease as well as the increase in cholesterol efflux with dalcetrapib in patients with the AA genotype.[8,9] In addition, the *CETP* target gene for dalcetrapib and the *ADCY9* effect modifier gene, both residing on chromosome 16, have shown evidence of co-evolution through human history, possibly through a selection pressure.[11] Further evidence of an interplay between these two gene products was obtained from genetically modified mice, with ADCY9 inactivation clearly protecting from atherosclerosis and improving endothelial function only in the absence of CETP.[10] In contrast to the effect on cholesterol efflux, the increase of HDL-cholesterol with dalcetrapib in patients is not dependent on the genotype at position rs1967309 in the *ADCY9* gene.[9] This discrepancy underscores that plasma HDL-cholesterol does not provide a reliable assessment of reverse cholesterol transport. The decrease over time in high-sensitivity C-reactive protein was smaller with dalcetrapib than that with placebo in dal-GenE, but the mechanism for this effect of medications targeting CETP is not known. This result appears to be discordant from prior observations with dalcetrapib.[5]

This interaction between the drug target and second gene determining therapeutic response underscores well the potential of precision medicine. One major objective of precision medicine is to better match personal patient characteristics with the therapeutic intervention to optimize the likelihood of beneficial actions while reducing the exposure to unneeded adverse drug experiences. Implementation of each precision medicine strategy, however,

Table 3 Proportions of patients with adverse events

Adverse event	Dalcetrapib (*N* = 3071)	Placebo (*N* = 3076)	*P*-value
Any AE, *n* (%)	2380 (77.5)	2363 (76.8)	0.53
Any SAE, *n* (%)	900 (29.3)	923 (30.0)	0.55
Gastrointestinal AE, *n* (%)	829 (27.0)	737 (24.0)	0.006
Gastrointestinal SAE, *n* (%)	96 (3.1)	99 (3.2)	0.84
Diarrhoea AE, *n* (%)	301 (9.8)	188 (6.1)	<0.0001
Nausea AE, *n* (%)	100 (3.3)	66 (2.1)	0.007
Neoplasm SAE, *n* (%)	107 (3.5)	139 (4.5)	0.04
Neoplasm AE, *n* (%)	206 (6.7)	215 (7.0)	0.66
Headache AE, *n* (%)	146 (4.8)	96 (3.1)	0.001
Cough AE, *n* (%)	140 (4.6)	95 (3.1)	0.003
Infection SAE, *n* (%)	204 (6.6)	211 (6.9)	0.73
Hypothyroidism AE - n (%)	32 (1.0)	14 (0.5)	0.008
Macular degeneration AE, *n* (%)	6 (0.2)	3 (0.1)	0.32

AE, adverse event; SAE, serious adverse event.

requires adequately powered clinical trials testing such personalized medicine approaches.

A case–control analysis has indicated that the point estimates for the effect of evacetrapib compared with placebo in patients with the AA, AG, and GG genotypes were 0.88, 1.04, and 1.18, respectively ($P = 0.06$).[16] One of the potential concerns associated with that secondary analysis was that it did not involve random selection of control subjects.[17] Nevertheless, the point estimate of the hazard ratio in that analysis of patients with the AA genotype was identical to that at end of study in dal-GenE. A meta-analysis of the dalcetrapib and evacetrapib pharmacogenomic data yielded a relative risk reduction of 20% in cardiovascular event rates when patients with the AA genotype at rs1967309 received the CETP inhibitor compared with placebo.[18] In contrast, the effects of anacetrapib were not affected by *ADCY9* genotype in REVEAL.[19,20] The differences in chemical structures, binding sites, and functional effects of dalcetrapib and anacetrapib may at least in part explain the different pharmacogenomic results.[21] The strong CETP inhibition caused by anacetrapib reduces the fractional clearance rate of HDL's ApoA-I[22] and physiologically resembles complete CETP deficiency with its very large HDL particles.[23] In contrast, dalcetrapib causes partial CETP modulation of heterotypic transfer of cholesteryl esters between HDL- and Apo-B-containing lipoproteins, without affecting homotypic transfers between HDL particles.[24]

Dal-GenE represents the first combined clinical development of a new anti-atherosclerosis therapy and its companion diagnostic test and has demonstrated the feasibility of performing a large pharmacogenetic-guided clinical trial and providing genotype result within 2–days of blood draw for the approximately 45 005 patients screened in 31 countries. The diagnostic test had been carefully evaluated prior to utilization in the clinical trial. The widespread adoption of genotyping by local research teams and participants was better than anticipated, which supports the fact that the cardiovascular field is ready for pharmacogenetic strategies.

The safety and tolerability profile of dalcetrapib has been demonstrated with the dosage (600 mg daily) beyond which no additional pharmacodynamic benefit is obtained.[25] Accordingly, the same dosage of dalcetrapib was used in dal-GenE. As expected, no safety concern was raised by the use of dalcetrapib in patients with the AA genotype in dal-GenE. Diarrhoea was the only adverse event of interest that was more frequently observed with dalcetrapib compared with placebo.

The main limitation of this study is the occurrence of the COVID-19 pandemic during study conduct. The effect on coronary revascularization was neutral and represented the summation of trends for reduced urgent and increased elective interventions. Urgent coronary revascularization did not contribute to the time to first event analysis of the first secondary composite endpoint, because it occurred after a myocardial infarction or an acute coronary syndrome. Finally, the pre-specified on-treatment analysis is not formally randomized.

Conclusion

In conclusion, dalcetrapib did not significantly reduce the risk of occurrence of the primary composite endpoint at end of study, with fewer non-fatal myocardial infarctions, and a numerical excess of cardiovascular deaths and resuscitated cardiac arrests. A new trial would be needed to test the pharmacogenetic hypothesis that dalcetrapib improves the prognosis of patients with the AA genotype at rs1967309 in the *ADCY9* gene after an acute coronary syndrome.

Supplementary material

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

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Sanofi; research grant and honoraria from Servier; a patent on pharmacogenomics-guided CETP inhibition was obtained, and J.C.T. and M.P.D. are mentioned as authors. M.P.D. has a minor equity interest in DalCor and has received honoraria from DalCor. M.A.P. receives research support from Novartis; he serves as a consultant for AstraZeneca, Corvidia, DalCor, GlaxoSmithKline, Jazz, MyoKardia, Novartis, Novo Nordisk, Roche, Sanofi, Servier, and Takeda; and has equity in DalCor. D.D.W. has received remuneration for participation in clinical trial committees from CSL Ltd, Daiichi Sankyo, DalCor, the Medicines Company, Novo Nordisk, Regeneron, Resverlogix, and Sanofi, and honoraria for lectures from DalCor and Pfizer. W.K. reports personal fees (consulting) from DalCor and consulting fees from AstraZeneca, Novartis, Pfizer, The Medicines Company, Kowa, a Corvidia, Esperion, Genentech, Omeicos, Novo Nordisk, Daiichi Sankyo, and Amgen; personal fees (honorarium for lectures) from AstraZeneca, Novartis, Amgen, Sanofi, Bristol-Myers-Squibb, and Berlin-Chemie; grants and non-financial support (provision of reagents for biomarker measurements free of charge) from Roche Diagnostics, Beckmann, Singulex, and Abbott, outside the submitted work. A.P.M. has received personal fees for participation in study committee from DalCor, and personal fees for participation in study committees from AstraZeneca, Bayer, Novartis, and Fresenius. J.J.V.M. reports grants paid to his institution and fees as steering committee member from DalCor Pharma UK for the Dal-GenE study, during the conduct of the study; grants paid to his institution and fees as steering committee member from Oxford University and Bayer; grants paid to his institution from Theracos; grants paid to his institution and fees as steering committee from Abbvie; grants paid to his institution from Pfizer, Merck AstraZeneca, GlaxoSmithKline, and Novartis. J.W.J. and his department have received research grants from and/or was speaker (with or without lecture fees) in CME-accredited meetings sponsored by Amgen, Athera, Biotronik, Boston Scientific, Dalcor, Daiichi Sankyo, Lilly, Medtronic, Merck-Schering-Plough, Novartis, Novo Nordisk, Pfizer, Roche, Sanofi-Aventis, The Medicine Company, The Netherlands Heart Foundation, CardioVascular Research The Netherlands (CVON), The Netherlands Heart Institute and the European Community Framework KP7 Programme. H.D.W. reports grants paid to his institution and fees as steering committee member from DalCor Pharma UK for the Dal-GenE study, during the conduct of the study; grants paid to his institution and fees as steering committee member from Sanofi-Aventis, Regeneron Pharmaceutics, National Institutes of Health (USA), CSL Behring, American Regent, Sanofi-Aventis Australia Pty Ltd, and Esperion Therapeutics Inc, outside the submitted work. F.L.T., T.H., and D.M.B. are employed by and have minor equity interest in DalCor. Other authors report no conflicts of interest.

Data availability

Data underlying this article is owned by DalCor Pharmaceuticals. Data will be shared on request to the corresponding author following permission of DalCor Pharmaceuticals.

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