PCSK9 inhibition with evolocumab (AMG 145) in heterozygous familial hypercholesterolaemia (RUTHERFORD-2): a randomised, double-blind, placebo-controlled trial

*Frederick J Raal, Evan A Stein, Robert Dufour, Traci Turner, Fernando Civeira, Lesley Burgess, Gisle Langslet, Russell Scott, Anders G Olsson, David Sullivan, G Kees Hovingh, Bertrand Cariou, Ioanna Gouni-Berthold, Ransi Somaratne, Ian Bridges, Rob Scott, Scott M Wasserman, Daniel Gaudet, for the RUTHERFORD-2 Investigators**

Summary

Background Heterozygous familial hypercholesterolaemia is characterised by low cellular uptake of LDL cholesterol, increased plasma LDL cholesterol concentrations, and premature cardiovascular disease. Despite intensive statin therapy, with or without ezetimibe, many patients are unable to achieve recommended target levels of LDL cholesterol. We investigated the effect of PCSK9 inhibition with evolocumab (AMG 145) on LDL cholesterol in patients with this disorder.

Methods This multicentre, randomised, double-blind, placebo-controlled trial was undertaken at 39 sites (most of which were specialised lipid clinics, mainly attached to academic institutions) in Australia, Asia, Europe, New Zealand, North America, and South Africa between Feb 7 and Dec 19, 2013. 331 eligible patients (18–80 years of age), who met clinical criteria for heterozygous familial hypercholesterolaemia and were on stable lipid-lowering therapy for at least 4 weeks, with a fasting LDL cholesterol concentration of 2·6 mmol/L or higher, were randomly allocated in a 2:2:1:1 ratio to receive subcutaneous evolocumab 140 mg every 2 weeks, evolocumab 420 mg monthly, or subcutaneous placebo every 2 weeks or monthly for 12 weeks. Randomisation was computer generated by the study sponsor, implemented by a computerised voice interactive system, and stratified by LDL cholesterol concentration at screening (higher or lower than 4·1 mmol/L) and by baseline ezetimibe use (yes/no). Patients, study personnel, investigators, and Amgen study staff were masked to treatment assignments within dosing frequency groups. The coprimary endpoints were percentage change from baseline in LDL cholesterol at week 12 and at the mean of weeks 10 and 12, analysed by intention-to-treat. This trial is registered with ClinicalTrials.gov, number NCT01763918.

Findings Of 415 screened patients, 331 were eligible and were randomly assigned to the four treatment groups: evolocumab 140 mg every 2 weeks (n=111), evolocumab 420 mg monthly (n=110), placebo every 2 weeks (n=55), or placebo monthly (n=55). 329 patients received at least one dose of study drug. Compared with placebo, evolocumab at both dosing schedules led to a significant reduction in mean LDL cholesterol at week 12 (every-2-weeks dose: 59·2% reduction [95% CI 53·4–65·1], monthly dose: 61·3% reduction [53·6–69·0]; both p<0·0001) and at the mean of weeks 10 and 12 (60·2% reduction [95% CI 54·5–65·8] and 65·6% reduction [59·8–71·3]; both p<0·0001). Evolocumab was well tolerated, with rates of adverse events similar to placebo. The most common adverse events occurring more frequently in the evolocumab-treated patients than in the placebo groups were nasopharyngitis (in 19 patients [9%] *vs* five [5%] in the placebo group) and muscle-related adverse events (ten patients [5%] *vs* 1 [1%]).

Interpretation In patients with heterozygous familial hypercholesterolaemia, evolocumab administered either 140 mg every 2 weeks or 420 mg monthly was well tolerated and yielded similar and rapid 60% reductions in LDL cholesterol compared with placebo.

Funding Amgen Inc.

Lancet 2015; 385: 331–40

Published **Online** October 2, 2014 http://dx.doi.org/10.1016/S0140-6736(14)61399-4

See **Comment** page 307

*The RUTHERFORD-2 study investigators are listed in the appendix

Carbohydrate and Lipid Metabolism Research Unit, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa (Prof F J Raal PhD); **Metabolic and Atherosclerosis Research Center, Cincinnati, OH, USA** (Prof E A Stein PhD, T Turner MD); **Institut de Recherches Cliniques de Montreal** (R Dufour MD) **and ECOGENE-21, Dyslipidemia, Diabetes and Atherosclerosis Research Group, Department of Medicine** (Prof D Gaudet MD), **University of Montreal, Montreal, QC, Canada; University Hospital Miguel Servet, Zaragoza, Spain** (F Civeira MD); **TREAD Research, Department of Internal Medicine, Tygerberg Hospital, Cape Town, South Africa** (Prof L Burgess MB); **Lipid Clinic, Oslo University Hospital, Oslo, Norway** (G Langslet MD); **Lipid and Diabetes Research Group, University of Otago, Christchurch, New Zealand** (Prof Ru Scott MD); **Linkoping University and Stockholm Heart Centre, Stockholm, Sweden** (Prof A G Olsson MD); **Department of Clinical Biochemistry, Royal Prince Alfred Hospital, Camperdown, NSW, Australia** (D Sullivan MD); **Vascular Medicine, Academic Medical Centre, Amsterdam, Netherlands** (G Kees Hovingh MD); **Institut du Thorax, Nantes University**

Introduction

Heterozygous familial hypercholesterolaemia is the most common dominantly inherited disorder in human beings worldwide,[1] and recent extensive screening in several countries suggests that it affects between one in 250 and one in 300 people worldwide.[2,3] Thus, more than 3 million people probably have the disorder in the USA and Europe alone. Familial hypercholesterolaemia is caused by mutations in genes encoding key proteins involved in LDL cholesterol metabolism, which leads to reduced cellular uptake of LDL cholesterol, increased plasma LDL cholesterol concentrations, and premature development of cardiovascular disease.[2] More than 90% of affected patients have mutations in the LDL receptor gene, and more than 1700 such mutations have been described.[4] On fibroblast culture, most of these mutations are shown to be associated with residual receptor function (receptor defective) or absence of

Hospital, Nantes, France (B Cariou MD); **Center for Endocrinology, Diabetes and Preventive Medicine, University of Cologne, Cologne, Germany** (I Gouni-Berthold MD); **Amgen Inc, Thousand Oaks, CA, USA** (R Somaratne MD, Ro Scott MD, S M Wasserman MD); **and Amgen Ltd, Cambridge Science Park, Milton, Cambridge, UK** (I Bridges MSc)

Correspondence to: Prof Frederick J Raal, Department of Medicine, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown, 2193 Johannesburg, South Africa
frederick.raal@wits.ac.za

See Online for appendix

function (receptor negative).[5,6] LDL receptor-negative alleles tend to be associated with higher LDL cholesterol concentrations and earlier onset of coronary artery disease than receptor-defective alleles.[7] Mutations in the apolipoprotein B gene account for a further 5% of familial hypercholesterolaemia cases,[8] and proprotein convertase subtilisin/kexin type 9 (PCSK9) gain-of-function mutations are present in less than 1% of cases.[9] However, in most studies, despite next-generation sequencing, no mutation can be identified in up to 20% of patients with a clinical diagnosis of definite heterozygous familial hypercholesterolaemia.[10]

Statin therapy has significantly improved the treatment of familial hypercholesterolaemia, with apparent reductions in cardiovascular morbidity and mortality reported in registry and longitudinal cohort studies.[11,12] However, many patients do not achieve desirable LDL cholesterol concentrations despite intensive statin therapy, even if treatment is combined with other lipid-modifying drugs such as ezetimibe.[13,14] Phase 1 and 2 trials have shown that further LDL cholesterol reductions of 55–60% can be achieved when PCSK9 inhibitors are added to existing lipid-lowering treatments. These reductions in LDL cholesterol are similar to those reported in patients without familial hypercholesterolaemia.[15–18]

The aims of this phase 3 trial were to assess the safety and efficacy of evolocumab (AMG 145) administered subcutaneously every 2 weeks or every month in a large and diverse cohort of patients with heterozygous familial hypercholesterolaemia with LDL cholesterol concentrations of 2·6 mmol/L or higher despite intense lipid-lowering therapy.

Methods

Study design and participants

RUTHERFORD-2 was a multicentre, randomised, double-blind, placebo-controlled study undertaken at 39 sites (mostly specialised lipid clinics, mainly attached to academic centres) in Australia, Asia, Europe, New Zealand, North America, and South Africa between Feb 7 and Dec 19, 2013. Patients 18–80 years of age with a clinical diagnosis of heterozygous familial hypercholesterolaemia according to Simon Broome criteria[19] at screening and on a stable dose of a statin with or without other approved lipid-modifying therapy (eg, ezetimibe, resins, stanols, or niacin, but excluding fibrates) for at least 4 weeks before screening were eligible for inclusion. Patients were excluded at screening if they had a diagnosis consistent with homozygous familial hypercholesterolaemia or if they had undergone lipoprotein apheresis within the previous 4 months. The full exclusion criteria are listed in the appendix.

The protocol and informed consent form were approved by each site's institutional review board or independent ethics committee, and all patients provided written informed consent before enrolment.

Randomisation and masking

Eligible patients were randomly assigned in a 2:2:1:1 ratio to receive subcutaneous evolocumab 140 mg every 2 weeks, evolocumab 420 mg monthly, placebo every 2 weeks, or placebo monthly for 12 weeks. Randomisation was computer generated by the sponsor (Amgen), stratified by LDL cholesterol concentration at screening (higher or lower than 4·1 mmol/L) and baseline ezetimibe use (yes/no), and implemented by a computerised voice interactive system. Patients, study personnel, investigators, and Amgen study staff were masked to treatment assignments within dosing frequency groups. Masking was achieved by the fact that the autoinjector pens containing either the study drug (evolocumab) or placebo looked identical so neither the patients nor the investigators could tell whether they contained study drug or placebo.

Procedures

At screening, patients were assessed for eligibility and underwent a physical examination; a 12-lead ECG; and a fasting lipid, haematological, and blood chemistry analysis. Optional consent (on a separate consent form) was obtained for pharmacogenetic subanalysis, since some of ethics committees at the various sites did not approve collection of DNA for shipment outside of the country. Patients were also given a placebo injection to assess their tolerance of the autoinjector pen. Study visits were at screening, and at weeks 0 (day 1), 2, 8, 10, and 12. Study drug or placebo was administered in the clinic at weeks 0, 2, 8, and 10 for the every-2-weeks dosing groups, and at weeks 0 and 8 for the monthly dosing groups; all other injections (ie, those at week 4 for both groups and at week 6 for those receiving 2-weekly injections) were self-administered by the patients at home. We recorded information about adverse events, concomitant medications, vital signs, and fasting lipids at each visit. We assessed apolipoproteins at weeks 0, 10, and 12; anti-evolocumab antibodies at weeks 0 and 12; serum levels of unbound PCSK9 at week 0, 2, 10, and 12; and blood chemistry and haematology at weeks 0, 8, and 12. For the patients who received the study drug every 2 weeks, the final adverse event data were collected at week 14 by telephone.

We calculated LDL cholesterol concentration using the Friedewald formula, with reflexive testing through preparative ultracentrifugation when the calculated LDL cholesterol was 1·0 mmol/L or lower or triglyceride concentrations were 4·5 mmol/L or higher.[15,20] We measured lipoprotein(a) by immunoturbidimetry using an isoform independent assay as previously described.[15] Safety was monitored by an independent data monitoring committee, and deaths and major cardiovascular events were adjudicated by an independent clinical events committee.

Outcomes

The coprimary endpoints were percentage change in plasma LDL cholesterol from baseline to week 12 and at the mean of weeks 10 and 12. Secondary endpoints were

the absolute change from baseline in LDL cholesterol and the percentage of patients achieving a target of LDL cholesterol lower than 1·8 mmol/L at the same time-points; and the mean percentage change from baseline in other lipids, apolipoproteins, high-sensitivity C-reactive protein, and unbound PCSK9. Key safety outcomes included treatment-emergent and serious adverse events, increases in creatine kinase and hepatic enzymes, the development of anti-evolocumab antibodies, and adjudicated cardiovascular adverse events. Adverse events were classified according to the Medical Dictionary of Regulatory Activities, version 16.1.

Samples from patients who agreed to participate in the genetic subanalysis were sequenced by Progenika Inc (Medford, MA, USA) for mutations in the whole LDL receptor gene, including large deletions or rearrangements, and apolipoprotein B exon 26. Those patients who had an LDL receptor mutation were grouped by LDL receptor functional class (defective or negative).[5,6] Patients with LDL receptor mutations that have been described as causative of familial hypercholesterolaemia but whose function has not yet been established or described were grouped as unclassified; if no mutation was identified in the LDL receptor or apolipoprotein B genes, patients were grouped as no mutation identified.

Statistical analysis

Our planned enrolment of 300 patients (100 in each evolocumab group and 50 in each placebo group) had at least 96% power to detect a 20% or greater reduction in the LDL cholesterol concentration in both evolocumab groups compared with placebo, with a common SD of 20%, after accounting for treatment attenuation and assuming that 2% of the participants would not receive at least one dose of a study drug. All our analyses included data from any patient who received at least one dose of the study drug, and we analysed patients within the dosing frequency group to which they were randomly assigned (ie, every 2 weeks *vs* monthly).

We analysed the coprimary and cosecondary continuous efficacy endpoints within each dosing frequency group using a repeated measures linear model, with

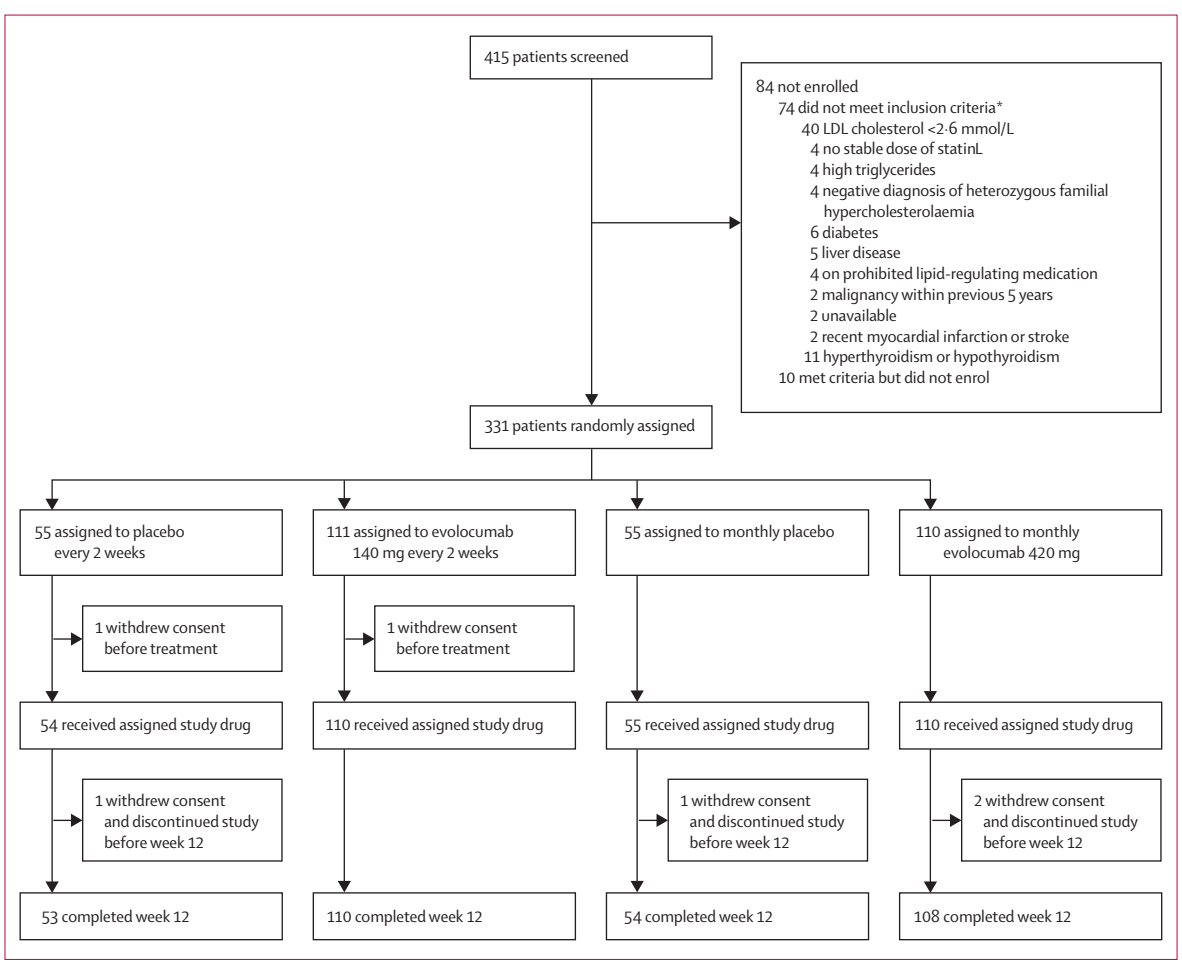

Figure 1: **Trial profile**
*Participants could be excluded at screening for several reasons.

terms for treatment group, stratification factor, scheduled visit, and the interaction of treatment and scheduled visit. We did not impute any missing data, except for the secondary endpoint of achievement of LDL cholesterol lower than 1·8 mmol/L, for which, for statistical testing purposes, non-achievement was imputed for patients with a missing value. We used the Cochran-Mantel-Haenszel test to analyse binary efficacy endpoints. Multiplicity adjustment was based on a combination of sequential testing, the Hochberg procedure,[21] and fallback procedure to control the overall significance level for all coprimary and cosecondary endpoints. A sensitivity analysis of the coprimary endpoints was done by application of a repeated measures linear effects model and Quade test[22] to patients who adhered to the scheduled study drug administration and did not have any missing data for the coprimary endpoints. We ran the covariate analysis using a similar repeated measures model but included the covariates of interest, one at a time, as a fixed effect.

We did a post-hoc exploratory subgroup analysis of the percentage change from baseline in LDL cholesterol and other lipid parameters by genotype status (confirmed or unconfirmed) and by LDL receptor class (defective, negative, or unclassified) or apolipoprotein B mutation status. We analysed the data using a repeated measures model with terms for treatment group, scheduled visit, and the interaction of treatment and scheduled visit. We also estimated the mean treatment effect differences and 95% CIs between subgroups. We used descriptive statistics to assess baseline demographics and baseline lipid parameters for all patients and by genotype status, and the incidence of adverse events and raised laboratory values.

This trial is registered with ClinicalTrials.gov, number NCT01763918.

	Placebo every 2 weeks (n=54)	Evolocumab 140 mg every 2 weeks (n=110)	Placebo monthly (n=55)	Evolocumab 420 mg monthly (n=110)
Age (years)	51·1 (14·2)	52·6 (12·3)	46·8 (12·1)	51·9 (12·0)
Female sex	25 (46%)	44 (40%)	24 (44%)	46 (42%)
Coronary artery disease	16 (30%)	38 (35%)	10 (18%)	39 (35%)
LDL cholesterol at baseline (mmol/L)*	3·9 (0·9)	4·2 (1·3)	3·9 (1·1)	4·0 (1·1)
LDL cholesterol at screening				
<4·1 mmol/L	35 (65%)	70 (64%)	35 (64%)	70 (64%)
≥4·1 mmol/L	19 (35%)	40 (36%)	20 (36%)	40 (36%)
Other lipid parameters				
Apolipoprotein B (g/L)	1·1 (0·3)	1·2 (0·3)	1·1 (0·2)	1·1 (0·3)
HDL cholesterol (mmol/L)	1·4 (0·4)	1·3 (0·4)	1·3 (0·3)	1·3 (0·4)
Apolipoprotein A1 (g/L)	1·5 (0·3)	1·4 (0·3)	1·3 (0·2)	1·4 (0·3)
Triglycerides (mmol/L)	1·1 (0·8–1·6)	1·3 (1·0–1·8)	1·2 (0·9–1·7)	1·3 (1·0–1·8)
Lipoprotein(a) (nmol/L)	44·0 (24·0–105·0)	77·5 (29·0–205·5)	87·0 (36·0–219·0)	61·0 (17·0–194·0)
hsCRP (nmol/L)	6·5 (3·8–14·1)	8·8 (5·0–18·2)	11·1 (5·0–21·0)	9·9 (5·8–21·7)
Unbound PCSK9 (nmol/L)	6·0 (1·7)	6·4 (2·0)	6·1 (2·0)	6·1 (1·9)

Data are mean (SD), n (%), or median (IQR). hsCRP=high-sensitivity C-reactive protein. PCSK9=proprotein convertase subtilisin/kexin type 9. *Established by the Friedewald formula with reflexive testing through preparative ultracentrifugation when calculated LDL cholesterol was ≤1·0 mmol/L or triglyceride concentrations were ≥4·5 mmol/L.

Table 1: **Baseline characteristics**

Role of the funding source

Amgen designed the study in collaboration with the authors, and was responsible for data collection and analysis. The initial draft of the report was developed by FJR and EAS and editorial assistance was provided by Amgen. The academic investigators vouch for the accuracy and completeness of the data and analyses as presented. FJR and EAS had the main responsibility for the decision to submit for publication.

Results

Of the 415 patients initially screened, 84 were excluded, either because they did not meet the inclusion criteria (the most common reasons were their LDL cholesterol concentration at screening was <2·6 mmol/L or they had hyperthyroidism or hypothyroidism) or because they did not want to enrol in the trial (figure 1). 331 eligible patients were enrolled and randomly assigned to evolocumab 140 mg every 2 weeks (n=111), evolocumab 420 mg monthly (n=110), placebo every 2 weeks (n=55), or placebo monthly (n=55; figure 1). One patient in each of the every-2-weeks dosing groups withdrew consent before treatment and thus did not receive study drug; all remaining analyses were done on the full analysis set of the 329 patients who received at least one dose of study drug. 325 (99%) of these 329 patients completed the week 12 visit. 13 patients (four in the placebo every-2-weeks group [7% of that group] and nine from the evolocumab every-2-weeks group [8% of that group]) were classified as study non-completers because they enrolled into an open-label extension study before completing all assessments associated with this study; however, data from these patients were still included in the intention-to-treat efficacy and safety analyses).

139 (42%) of 331 of enrolled patients were women and 296 (89%) were white. The mean age of patients was 51 years (SD 13); 103 of 331 (31%) had coronary artery disease, and the mean baseline LDL cholesterol concentration was 4·0 mmol/L (SD 1·2). All patients were taking statins; 289 of 331 (87%) were taking high-intensity doses (see appendix for a definition) and 204 of 331 (62%) were also taking ezetimibe. Baseline characteristics were generally similar across the four groups (table 1).

Compared with placebo, evolocumab 140 mg administered every 2 weeks resulted in mean reductions in LDL cholesterol at week 12 of 59·2% (95% CI 53·4–65·1) and reductions at the mean of weeks 10 and 12 of 60·2% (54·5–65·8) (both p<0·0001). Monthly administration of evolocumab 420 mg resulted in mean reductions in LDL cholesterol at week 12 of 61·3%

	Every-2-weeks dosing groups				Monthly dosing groups			
	Placebo every 2 weeks (n=54)	Evolocumab 140 mg every 2 weeks (n=110)	Treatment difference (95% CI)	p value	Placebo monthly (n=55)	Evolocumab 420 mg monthly (n=110)	Treatment difference (95% CI)	p value
LDL cholesterol* (primary endpoint)								
Week 12 change from baseline (%)	−2·0% (−6·9 to 2·9)	−61·3% (−64·7 to −57·8)	−59·2% (−65·1 to −53·4)	<0·0001	5·5% (−0·9 to 12·0)	−55·7% (−60·2 to −51·3)	−61·3% (−69·0 to −53·6)	<0·0001
Change from baseline (mmol/L)	−0·2 (−0·4 to 0·0)	−2·6 (−2·8 to −2·5)	−2·4 (−2·7 to −2·1)	<0·0001	0·1 (−0·2 to 0·4)	−2·3 (−2·4 to −2·1)	−2·4 (−2·7 to −2·0)	<0·0001
Absolute value (mmol/L)	3·8 (0·2)	1·7 (0·1)	NA	..	4·1 (0·2)	1·8 (0·1)	NA	..
Mean of weeks 10 and 12 change from baseline (%)	−1·1% (−5·8 to 3·7)	−61·2% (−64·6 to −57·9)	−60·2% (−65·8 to −54·5)	<0·0001	2·3% (−2·5 to 7·1)	−63·3% (−66·6 to −59·9)	−65·6% (−71·3 to −59·8)	<0·0001
Apolipoprotein B								
Week 12 change from baseline (%)	−0·7% (−5·2 to 3·9)	−49·8% (−53·0 to −46·5)	−49·1% (−54·6 to −43·6)	<0·0001	4·6% (−0·7 to 9·9)	−44·8% (−48·4 to −41·2)	−49·4% (−55·7 to −43·1)	<0·0001
Mean of weeks 10 and 12 change from baseline (%)	−0·2% (−4·3 to 4·0)	−49·6% (−52·5 to −46·7)	−49·4% (−54·3 to −44·5)	<0·0001	2·2% (−1·7 to 6·1)	−52·8% (−55·4 to −50·1)	−55·0% (−59·6 to −50·4)	<0·0001
HDL cholesterol								
Week 12 change from baseline (%)	−1·2% (−4·9 to 2·6)	8·1% (5·4 to 10·7)	9·2% (4·7 to 13·7)	<0·0001	−3·7% (−8·4 to 0·9)	5·4% (2·1 to 8·6)	9·1% (3·5 to 14·7)	0·0016
Mean of weeks 10 and 12 change from baseline (%)	−0·5% (−3·8 to 2·9)	7·9% (5·6 to 10·3)	8·4% (4·4 to 12·4)	<0·0001	−2·9% (−6·5 to 0·8)	6·6% (4·1 to 9·2)	9·5% (5·1 to 13·9)	<0·0001
Apolipoprotein A1†								
Week 12 change from baseline (%)	−1·4% (−5·3 to 2·4)	7·3% (4·5 to 10·0)	8·7% (4·1 to 13·2)	0·0012	1·8% (−2·8 to 6·4)	5·7% (2·6 to 8·8)	3·9% (−1·6 to 9·4)	0·0335
Mean of weeks 10 and 12 change from baseline (%)	−0·3% (−4·2 to 3·5)	7·1% (4·4 to 9·8)	7·4% (2·9 to 12·0)	0·0015	1·6% (−2·1 to 5·3)	6·5% (4·0 to 9·0)	4·9% (0·6 to 9·2)	0·0271
Triglycerides								
Week 12 change from baseline (%)	3·5% (−3·4 to 10·4)	−16·1% (−21·0 to −11·2)	−19·6% (−27·9 to −11·3)	<0·0001	6·4% (−1·8 to 14·6)	−5·1% (−10·7 to 0·5)	−11·6% (−21·4 to −1·7)	0·0214
Mean of weeks 10 and 12 change from baseline (%)	9·1% (3·1 to 15·1)	−13·3% (−17·5 to −9·0)	−22·4% (−29·5 to −15·2)	<0·0001	7·5% (1·1 to 13·9)	−9·3% (−13·7 to −4·8)	−16·7% (−24·4 to −9·1)	<0·0001
Lipoprotein(a)								
Week 12 change from baseline (%)	8·7% (2·2 to 15·1)	−22·9% (−27·5 to −18·3)	−31·6% (−39·3 to −23·9)	<0·0001	6·7% (0·5 to 12·9)	−21·6% (−25·8 to −17·3)	−28·2% (−35·6 to −20·9)	<0·0001
Mean of weeks 10 and 12 change from baseline (%)	7·3% (1·5 to 13·2)	−24·0% (−28·2 to −19·9)	−31·4% (−38·3 to −24·4)	<0·0001	5·4% (−0·5 to 11·2)	−25·7% (−29·7 to −21·6)	−31·0% (−37·9 to −24·1)	<0·0001
Non-HDL cholesterol								
Week 12 change from baseline (%)	−1·4% (−6·1 to 3·3)	−56·2% (−59·6 to −52·8)	−54·8% (−60·5 to −49·1)	<0·0001	5·3% (−0·5 to 11·1)	−49·7% (−53·7 to −45·6)	−55·0% (−62·0 to −48·0)	<0·0001
Mean of weeks 10 and 12 change from baseline (%)	0·2% (−4·3 to 4·7)	−55·8% (−59·0 to −52·6)	−56·0% (−61·4 to −50·6)	<0·0001	2·7% (−1·6 to 7·1)	−57·3% (−60·4 to −54·2)	−60·0% (−65·2 to −54·8)	<0·0001
Ratio of total cholesterol:HDL cholesterol								
Week 12 change from baseline (%)	0·1% (−4·2 to 4·5)	−46·0% (−49·0 to −42·9)	−46·1% (−51·3 to −40·9)	<0·0001	7·1% (0·9 to 13·3)	−38·3% (−42·6 to −34·1)	−45·4% (−52·9 to −38·0)	<0·0001
Mean of weeks 10 and 12 change from baseline (%)	0·9% (−3·2 to 4·9)	−45·7% (−48·6 to −42·9)	−46·6% (−51·4 to −41·8)	<0·0001	−4·1% (−0·1 to 8·4)	−45·0% (−48·0 to −42·1)	−49·2% (−54·2 to −44·1)	<0·0001
Ratio of apolipoprotein B:apolipoprotein A1								
Week 12 change from baseline (%)	1·5% (−3·4 to 6·5)	−52·7% (−56·2 to −49·3)	−54·3% (−60·2 to −48·4)	<0·0001	4·2% (−3·0 to 11·5)	−45·3% (−50·1 to −40·5)	−49·6% (−58·1 to −41·0)	<0·0001
Mean of weeks 10 and 12 change from baseline (%)	0·8% (−3·6 to 5·1)	−52·4% (−55·5 to −49·3)	−53·2% (−58·4 to −48·0)	<0·0001	1·7% (−3·0 to 6·3)	−53·9% (−57·1 to −50·7)	−55·6% (−61·1 to −50·1)	<0·0001
hsCRP‡								
Week 12 change from baseline (%), median (IQR)	9·2% (−19·4 to 35·8)	−7·1% (−28·0 to 39·4)	NA	..	1·4% (−42·5 to 43·8)	4·3% (−30·1 to 73·2)	NA	..
Week 12 change from baseline (nmol/L), median (IQR)	0·29 (−1·24 to 2·67)	−0·48 (−2·76 to 2·29)	NA	..	0·05 (−7·00 to 2·38)	0·29 (−3·62 to 5·81)	NA	..

(Table 2 continues on next page)

	Every-2-weeks dosing groups				Monthly dosing groups			
	Placebo every 2 weeks (n=54)	Evolocumab 140 mg every 2 weeks (n=110)	Treatment difference (95% CI)	p value	Placebo monthly (n=55)	Evolocumab 420 mg monthly (n=110)	Treatment difference (95% CI)	p value
(Continued from previous page)								
Unbound PCSK9‡								
Week 12 change from baseline (%), mean (SE)	1·7% (3·8)	−47·3% (3·0)	NA	..	2·6% (5·2)	−13·5% (4·2)	NA	..
Week 12 change from baseline (nmol/L), mean (SE)	−0·1 (0·2)	−2·9 (0·2)	NA	..	−0·0 (0·3)	−1·0 (0·2)	NA	..

Data are least-squares mean (95% CI) unless otherwise indicated. NA=not available. hsCRP=high-sensitivity C-reactive protein. PCSK9=proprotein convertase subtilisin/kexin type 9. *Established by the Friedewald formula with reflexive testing through preparative ultracentrifugation when calculated LDL cholesterol was ≤1·0 mmol/L or triglyceride concentrations were ≥4·5 mmol/L, adjusted for multiplicity. †Apolipoprotein A1 was not part of multiplicity testing hierarchy. ‡Unadjusted estimates and standard error; p values are versus placebo in the same dosing interval. Analysis was done using a repeated measures model that included terms for treatment group, stratification factors (screening LDL cholesterol <4·1 or ≥4·1 mmol/L and baseline ezetimibe use), scheduled visit, and the interaction of treatment with scheduled visit as covariates.

***Table 2*: Lipid efficacy outcomes at week 12 and at the mean of weeks 10 and 12**

(53·6–69·0) and at the mean of weeks 10 and 12 of 65·6% (59·8–71·3) (both p<0·0001; table 2). Reductions were recorded at 2 weeks, remained consistent through to week 12 (figure 2), and were not related to sex, age, body-mass index, intensity of statin therapy, concomitant use of ezetimibe, or LDL cholesterol at screening (higher or lower than 4·1 mmol/L) (figure 3). At week 12, LDL cholesterol concentration lower than 1·8 mmol/L was achieved by 71 of 104 (68%) patients in the evolocumab 140 mg every-2-weeks group and by 65 of 103 (63%) patients in the evolocumab 420 mg monthly group, compared with one patient (2%) in each of the placebo groups. Similar results were recorded for the mean of weeks 10 and 12 (p<0·0001 for both doses; appendix p 13).

Mean reductions in lipoprotein(a) and apolipoprotein B at week 12 were significantly greater in both evolocumab groups than in the placebo groups (table 2). At week 12, evolocumab 140 mg every 2 weeks significantly reduced triglyceride concentrations compared with placebo, whereas the 420 mg monthly evolocumab dose resulted in a smaller, but still significant, decrease compared with placebo (table 2). Both doses of evolocumab led to significant increases in HDL cholesterol compared with placebo (table 2). Evolocumab had no effect on high-sensitivity C-reactive protein. Table 2 shows the mean percentage changes from baseline at week 12, and at the means of weeks 10 and 12, for other lipids and apolipoproteins.

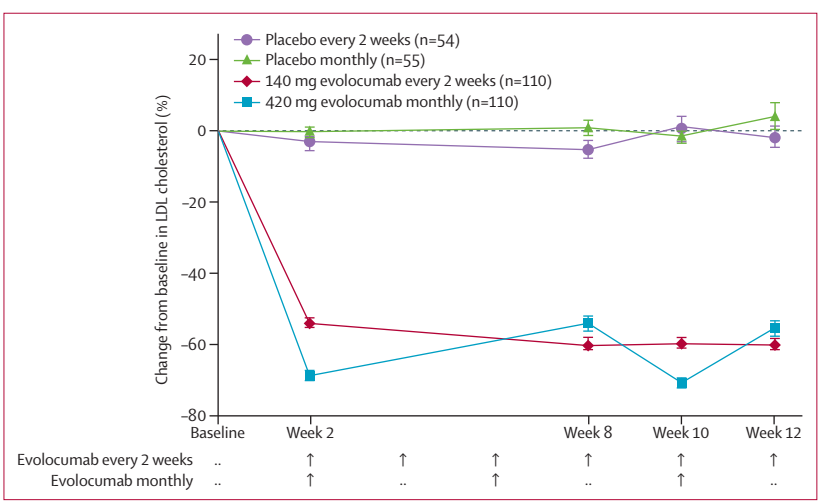

***Figure 2*: Mean percentage change from baseline in LDL cholesterol in the four groups**
The arrows underneath the graph represent timepoints of evolocumab administration. Error bars are standard errors. The percentage change in LDL cholesterol was ascertained by the Friedewald formula, with reflexive testing through preparative ultracentrifugation when calculated LDL cholesterol was 1·0 mmol/L or lower or triglyceride concentrations were 4·5 mmol/L or higher.

Mutations causative of familial hypercholesterolaemia were recorded in 211 of 264 (80%) patients who consented to the genetic analysis. In seven (3%) patients—all of whom were randomly assigned to evolocumab—mutations were recorded in both LDL receptor alleles, which is consistent with either genetic homozygous or compound heterozygous familial hypercholesterolaemia. The mean baseline LDL cholesterol in these patients (5·3 mmol/L [SD 2·8]) was moderately higher than that in the patients with receptor-negative mutations (4·4 mmol/L [1·3]) or receptor-defective mutations (3·9 mmol/L [1·0]) and the prevalence of coronary artery disease was 57% (four of seven patients). Of the 204 patients with a single LDL receptor causative mutation, 75 (37%) overall (40 assigned to evolocumab and 35 assigned to placebo) had alleles associated with defective LDL receptor activity, 66 (32%; 48 evolocumab and 18 placebo) had alleles associated with negative or no activity, and 54 (26%; 38 evolocumab and 16 placebo) had mutations that were unclassified. Nine patients (4%; eight assigned to evolocumab and one assigned to placebo) had mutations in the apolipoprotein B gene. Appendix p 6 shows the baseline characteristics and lipids of the various genotypes.

Appendix p 8 and appendix pp 14–15 show changes in LDL cholesterol based on causative mutations. Compared with placebo, the mean reductions in LDL cholesterol at week 12 in patients with LDL receptor-negative activity

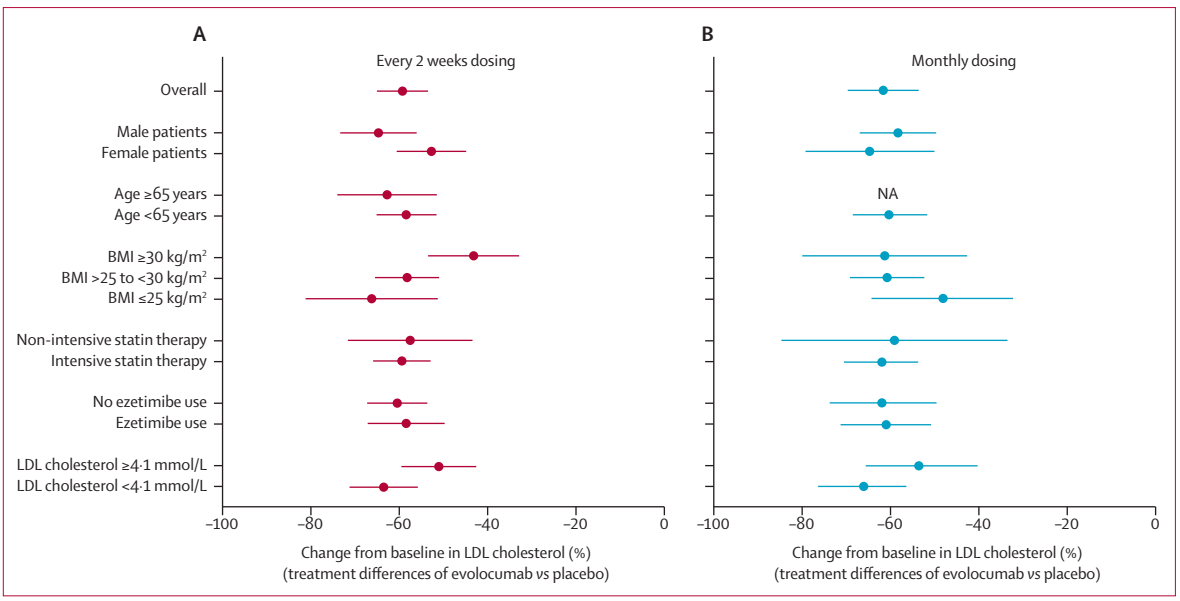

Figure 3: **Treatment differences between evolocumab and placebo in mean percentage changes from baseline in LDL cholesterol at week 12 and the associations with sex, age, BMI, lipid-lowering therapy, and baseline LDL cholesterol concentrations**
(A) Doses administered every 2 weeks. (B) Doses administered monthly. Error bars are 95% CI. BMI=body-mass index. NA=not available.

were 61% (95% CI 45–77) for evolocumab 140 mg administered every 2 weeks and 55% (37–74) for evolocumab 420 mg administered monthly; in patients with receptor-defective activity the reductions were 49% (38–60) and 66% (47–85), respectively; and in patients with unclassified LDL receptor status the reductions were 62% (52–71) and 63% (48–78), respectively (interaction p=0·16 for the every-2-weeks dosing groups and p=0·68 for the monthly dosing groups). At week 12, the reductions in LDL cholesterol with evolocumab compared with placebo in patients in whom no mutation could be identified were 64% (95% CI 38–89) with evolocumab 140 mg every 2 weeks and 43% (28–59) with evolocumab 420 mg monthly, and were similar to those with genetically confirmed familial hypercholesterolaemia (appendix p 8). In 13 patients with the identical mutation c.313+1G>A (the most common LDL receptor mutation identified in the study patients) who were randomly assigned to evolocumab, the reduction in LDL cholesterol ranged from 27% to 83% (data not shown). The mean reductions at week 12 in the seven patients who were either genetic homozygotes or compound heterozygotes were 68% (range 40–82%) with evolocumab 140 mg every 2 weeks and 48% (38–64%) with 420 mg every month. In patients with apolipoprotein B mutations, the mean reductions in LDL cholesterol at week 12 were 51% (range 35–64%) with evolocumab 140 mg every 2 weeks and 50% (36–65%) with 420 mg every month.

The mean reductions in apolipoprotein B at week 12 with evolocumab compared with placebo ranged from 42% to 53% and were similar for both dosing schedules in patients with LDL receptor-negative activity, patients with LDL receptor-defective activity, patients with unclassified LDL receptor status, and patients in whom no mutation could not be identified (appendix p 8) (interaction p=0·88 for the every-2-weeks dosing groups and p=0·70 for the monthly dosing groups). Reductions in lipoprotein(a) ranged from 19% to 45% (appendix p 8), but did not seem to depend on baseline levels or the type of receptor mutation (interaction p=0·44 and p=0·41 for subgroup analysis based on receptor mutation for the every-2-weeks and monthly dosing groups, respectively). Appendix pp 10–11 show the mean percentage changes from baseline in other lipids, apolipoproteins, and high-sensitivity C-reactive protein based on causative mutations.

Rates of adverse events, positively adjudicated cardiovascular events, abnormal laboratory values, neurocognitive events, and anti-evolocumab antibodies were similar to those in previous studies of evolocumab[15,17,23,24] and were similar between the evolocumab and placebo groups (table 3). Nasopharyngitis was reported more frequently in patients who received evolocumab (in 19 of 220 patients [9%]) than in those given placebo (five of 110 patients [5%]), as were muscle-related adverse events (ten patients [5%] in the evolocumab groups *vs* one [1%] in the placebo groups). No serious adverse events led to study drug discontinuation and none of the seven severe events in the evolocumab groups were judged to be related to the study drug. No deaths occurred during the trial.

Discussion

This study, the largest reported global trial of patients with heterozygous familial hypercholesterolaemia with a monoclonal antibody to PCSK9, showed that the addition of evolocumab 140 mg every 2 weeks or

	Placebo every 2 weeks (n=54)	Evolocumab 140 mg every 2 weeks (n=110)	Placebo monthly (n=55)	Evolocumab 420 mg monthly (n=110)
Adverse events				
Any	23 (43%)	61 (55%)	30 (55%)	63 (57%)
Serious*	2 (4%)	3 (3%)	3 (5%)	4 (4%)
Deaths	0	0	0	0
Leading to treatment discontinuation	0	0	0	0
Most common adverse events in patients given evolocumab				
Nasopharyngitis	2 (4%)	8 (7%)	3 (5%)	11 (10%)
Headache	1 (2%)	4 (4%)	3 (5%)	5 (5%)
Contusion	0	5 (5%)	1 (2%)	4 (4%)
Back pain	0	2 (2%)	1 (2%)	6 (5%)
Nausea	0	5 (5%)	1 (2%)	3 (3%)
Positively adjudicated cardiovascular events	0	2 (2%)	0	1 (1%)
Potential injection-site adverse events†	2 (4%)	5 (5%)	2 (4%)	8 (7%)
Neurocognitive adverse events‡	0	0	0	0
Muscle-related adverse events	0	8 (7%)	1 (2%)	2 (2%)
Laboratory results				
ALT or AST >3× ULN (any post-baseline value)	0	0	0	0
Creatine kinase >5× ULN (any post-baseline value)	0	0	2 (4%)	0
Development of anti-evolocumab antibodies				
Binding antibodies	NA	0	NA	0
Neutralising antibodies	NA	0	NA	0

ALT=alanine aminotransferase. AST=aspartate aminotransferase. ULN=upper limit of normal. NA=not applicable. *Defined as an adverse event that was fatal, life-threatening, required admission to hospital or prolonged stay in hospital, or caused persistent or significant disability or incapacity or a congenital anomaly or birth defect. †Reported using high-level term grouping, which includes injection-site rash, inflammation, pruritus, reaction, and urticaria. ‡Defined using the Medical Dictionary for Regulatory Activities high-level group terms deliria (including confusion), cognitive and attention disorders and disturbances, dementia and amnestic disorders, disturbances in thinking and perception, and mental impairment disorders.

Table 3: **Adverse events and laboratory results**

420 mg monthly to existing lipid-lowering therapy achieved similar LDL cholesterol reductions of roughly 60%. Additionally, more than 60% of patients who were given evolocumab at either dosing frequency achieved LDL cholesterol levels lower than 1·8 mmol/L. These results support and expand upon those reported in previous, smaller studies of heterozygous familial hypercholesterolaemia (panel).[15–17]

The identification of mutations in 80% of the 264 patients who consented to be genotyped is exceptionally high for a global and diverse population, compares favourably with the highest identification rate of mutations reported in a single country,[25] and exceeds that of a recent study in patients from UK lipid clinics reported by Talmud and colleagues.[26] In that study, patients with familial hypercholesterolaemia, which was diagnosed as definite or possible on the basis of Simon Broome criteria[19]—similar to our present trial—were genotyped for associated LDL receptor, apolipoprotein B, and PCSK9 mutations. The investigators reported a 78% detection rate in the cohort of 307 patients with a definite diagnosis, and a 33% rate in the Oxford lipid clinic population that included patients with both definite and possible familial hypercholesterolaemia.[26] In our study, roughly 20% of patients had no detectable mutation, whereas in 3% of patients, mutations in both LDL receptor alleles were identified, even though they were thought to have heterozygous familial hypercholesterolaemia on the basis of clinical criteria. This result is consistent with recent findings by Sjouke and colleagues[27] in a Dutch study in which genetic homozygous familial hypercholesterolaemia patients detected by cascade screening had LDL cholesterol levels before lipid-lowering treatment as low as 4·4 mmol/L and 50% of the patients did not meet the traditional clinical criteria for homozygous familial hypercholesterolaemia (LDL cholesterol >13·0 mmol/L). We now show that these genetic homozygous familial hypercholesterolaemia patients respond to evolocumab therapy in a similar way to those with heterozygous familial hypercholesterolaemia, and to a greater degree than that reported in specifically selected patients with homozygous familial hypercholesterolaemia.[28] In the homozygous familial hypercholesterolaemia trial, LDL cholesterol response was related to LDL receptor genotype with diminishing reductions as the number of alleles associated with receptor negative activity increased. Thus, perhaps unexpectedly, the present analysis shows that patients

with receptor-negative mutations respond equally well to treatment as those with defective mutations or those with mutations in apolipoprotein B, which suggests that the response to PCSK9 inhibition with evolocumab in heterozygous familial hypercholesterolaemia depends mainly on upregulation of the non-affected LDL receptor, whereas the mutant receptor has a negligible role. This idea is supported by previous studies that showed similar reductions in LDL cholesterol in patients with heterozygous familial hypercholesterolaemia as those without the disorder when evolocumab was added to existing lipid-lowering therapy.[15–18] Importantly, this trial has clinical implications in that it indicates that the genetic analysis might not be helpful in assessment of response to evolocumab in patients with heterozygous familial hypercholesterolaemia, unlike patients with homozygous familial hypercholesterolaemia, in whom the underlying genetic mutations seem to be very helpful in predicting response to treatment.[28]

Notably, and in agreement with previous studies, the LDL receptor defect seemed to be associated with the baseline LDL cholesterol and presence of coronary artery disease: patients with two LDL receptor defects had higher LDL cholesterol and more coronary artery disease than did those with a single LDL receptor-negative mutation, who in turn had higher LDL cholesterol levels and more coronary artery disease than did those with an LDL receptor-defective mutation, although the sample sizes within these groups were small (between seven and nine patients). The other new finding is the variable—although still good—response in patients with identical receptor mutations, which indicates that, as with statin therapy, other factors must have a role in LDL cholesterol reduction with evolocumab.[7] As previously shown by Talmud and colleagues, a constellation of minor genetic defects, which they termed polygenic familial hypercholesterolaemia, can produce a similar clinical phenotype.[26] Perhaps assessments of these additional defects can assist in further elucidation of determinants of response to treatments that upregulate LDL receptor activity.[26]

Evolocumab was well tolerated and was not associated with any serious treatment-related adverse events. The incidence of adverse events in the evolocumab groups was similar to that in the placebo groups.

Our study has several limitations. The analysis of response based on genotype was post hoc and was not prespecified in the study protocol or analysis plan, and should therefore be viewed as a hypothesis-generating step. The study duration of 12 weeks, although sufficient for assessment of LDL cholesterol-lowering efficacy given that reductions in LDL cholesterol and other lipoproteins were achieved within 2 weeks and remained stable throughout the trial, is not sufficient to assess long-term durability of the reductions or safety. However, two recent 52-week trials with evolocumab in patients with and without heterozygous familial hypercholesterolaemia have shown that the LDL cholesterol reductions recorded at 12 weeks are maintained for at least 52 weeks, with no additional safety or tolerability issues.[23,24] A long-term study to establish the effect of the large LDL cholesterol reduction with evolocumab on cardiovascular outcome is already underway.[29]

In patients with heterozygous familial hypercholesterolaemia, inhibition of PCSK9 with evolocumab resulted in LDL cholesterol reductions of 60% compared with placebo, and achievement of LDL cholesterol concentrations lower than 1·8 mmol/L in more than 60% of patients. The response to evolocumab was unrelated to the underlying genetic mutation. Evolocumab was well tolerated and offers the potential to achieve large reductions in LDL cholesterol in this difficult-to-treat, high-risk patient population.

Panel: Research in context

Systematic review

A PubMed search for original research articles published in English between Jan 1, 1985 and June 20, 2013, with the terms "heterozygous familial hypercholesterolaemia" or "familial hypercholesterolaemia" and "PCSK9-inhibition" yielded three previous studies.[15–17] However, none of these trials enrolled such a large and diverse cohort of patients with heterozygous familial hypercholesterolaemia as our present study. Furthermore, none of these previous smaller studies have assessed the response to PCSK9 inhibition according to function of the mutation causative of heterozygous familial hypercholesterolaemia. Our present study was also the first to compare monthly versus every-2-weeks administration of a PCSK9 inhibitor in this patient group.

Interpretation

We report the results of a large, randomised, placebo-controlled trial of the PCSK9 inhibitor, evolocumab, in patients with heterozygous familial hypercholesterolaemia. Evolocumab administered either 140 mg every 2 weeks or 420 mg monthly was well tolerated and yielded similar and rapid 60% reductions in LDL cholesterol. These findings provide clinicians with evidence that PCSK9 inhibition is an effective LDL cholesterol-lowering treatment in heterozygous familial hypercholesterolaemia, with patients attaining reductions similar in magnitude to those without the disorder, and the response is not related to the underlying genetic cause of familial hypercholesterolaemia.

Contributors

Amgen (RaS, RoS, and SMW) designed the study in collaboration with the academic investigators. FJR, RD, TT, FC, LB, GL, RuS, AGO, DS, GKH, BC, IG-B, and DG were clinical site investigators for this trial. IB was the study statistician. FJR, RaS, SMW, and EAS helped to interpret the results. All other authors contributed to interpretation through discussion of the results. This report was mainly written by FJR and EAS with assistance from RaS and SMW, and was critically reviewed, revised, and subsequently approved by all authors.

Declaration of interests

FJR has received consulting fees from Amgen and Sanofi related to PCSK9 inhibitors and from Genzyme (a Sanofi company) related to apolipoprotein B inhibitors. His institution has received research funding related to PCSK9 inhibitor clinical trials from Amgen and Sanofi and from Isis and Genzyme (a Sanofi company) for trials related to mipomersen. EAS has been a consultant to, and participated in symposia sponsored by, Amgen, Regeneron, Sanofi, Roche, and BMS related to PCSK9 inhibitors. His institution has received research funding related to PCSK9 inhibitor clinical trials from Amgen, Regeneron/Sanofi, BMS, Genentech/Roche, Pfizer, and Lilly related to PCSK9 inhibitors. RaS, IB, RoS, and SMW are employees and minor

stockholders of Amgen. RD has received research grant support and speaker or consulting honoraria from Amgen, Pfizer, Regeneron, Sanofi, and Valeant. AGO has received support for clinical trials from Amgen, AstraZeneca, Karobio, MSD, Pfizer, Roche, and Sanofi-Aventis; and consultation fees from AstraZeneca, Karobio, Merck, Pfizer, and Roche. FC has received modest consulting or advisory fees from Amgen. GL has received consultant or advisory board fees from Janssen, Sanofi-Aventis, and Amgen. DS has received research funding from Amgen, Merck, Amarin, and Eli Lilly; modest honoraria for education programmes from Abbott, AbbVie, AstraZeneca, and Novo Nordisk; and advisory boards fees from Amgen, Abbott, Merck, and Sanofi. GKH has received lecture fees from Amgen, Sanofi, and Genzyme; and consulting fees from Pfizer. BC has received research support from Novo Nordisk and Sanofi; and advisory board fees from Amgen, Genfit, Janssen, Eli Lilly, Novo Nordisk, Merck Sharp and Dohme, and Sanofi. IG-B has received research grants from Bayer HealthCare; honoraria and travel expenses from Genzyme, MSD, Novartis, Novo Nordisk, Pfizer, Ipsen, Bristol-Myers Squibb, Amgen, Sanofi, and Otsuka. DG has received reimbursement for conducting clinical trials from Amgen. TT, LB, and RuS declare no competing interests.

Acknowledgments

We thank Wei Cui and Tim Palmer of Amgen for their assistance with the statistical analyses, and Janice Carlson of Amgen for editorial support.

References

1 Watts GF, Gidding S, Wierzbicki AS, et al. Integrated guidance on the care of familial hypercholesterolaemia from the International FH Foundation. *Int J Cardiol* 2014; **171:** 309–25.

2 Goldstein JL, Hobbs HH, Brown MS. Familial hypercholesterolemia. In: Scriver CR, Beaudet AL, Sly WS, et al, eds. The metabolic and molecular bases of inherited disease, 8th edn. New York: McGraw Hill, 2001: 2863–913.

3 Nordestgaard BG, Chapman MJ, Humphries SE, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. *Eur Heart J* 2013; **34:** 3478–90a.

4 Leigh SE, Foster AH, Whittall RA, Hubbart CS, Humphries SE. Update and analysis of the University College London low density lipoprotein receptor familial hypercholesterolaemia database. *Ann Hum Genet* 2008; **72:** 485–98.

5 Hobbs HH, Brown MS, Goldstein JL. Molecular genetics of the LDL receptor gene in familial hypercholesterolemia. *Hum Mutat* 1992; **1:** 445–66.

6 Usifo E, Leigh SE, Whittall RA, et al. Low-density lipoprotein receptor gene familial hypercholesterolemia variant database: update and pathological assessment. *Ann Hum Genet* 2012; **76:** 387–401.

7 Austin MA, Hutter CM, Zimmern RL, Humphries SE. Familial hypercholesterolemia and coronary heart disease: a HuGE association review. *Am J Epidemiol* 2004; **160:** 421–29.

8 Innerarity TL, Weisgraber KH, Arnold KS, et al. Familial defective apolipoprotein B-100: low density lipoproteins with abnormal receptor binding. *Proc Natl Acad Sci USA* 1987; **84:** 6919–23.

9 Abifadel M, Varret M, Rabès JP, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. *Nat Genet* 2003; **34:** 154–56.

10 Vandrovcova J, Thomas ERA, Atanur SS, et al. The use of next-generation sequencing in clinical diagnosis of familial hypercholesterolemia. *Genet Med* 2013; **15:** 948–57.

11 Neil A, Cooper J, Betteridge J, et al. Reductions in all-cause, cancer, and coronary mortality in statin-treated patients with heterozygous familial hypercholesterolaemia: a prospective registry study. *Eur Heart J* 2008; **29:** 2625–33.

12 Versmissen J, Oosterveer DM, Yazdanpanah M, et al. Efficacy of statins in familial hypercholesterolaemia: a long term cohort study. *BMJ* 2008; **337:** a2423.

13 Stein EA, Ose L, Retterstol K, et al. Further reduction of low-density lipoprotein cholesterol and C-reactive protein with the addition of ezetimibe to maximum-dose rosuvastatin in patients with severe hypercholesterolemia. *J Clin Lipidol* 2007; **1:** 280–86.

14 Pijlman AH, Huijgen R, Verhagen SN, et al. Evaluation of cholesterol lowering treatment of patients with familial hypercholesterolemia: a large cross-sectional study in The Netherlands. *Atherosclerosis* 2010; **209:** 189–94.

15 Raal F, Scott R, Somaratne R, et al. Low-density lipoprotein cholesterol-lowering effects of AMG 145, a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 serine protease in patients with heterozygous familial hypercholesterolemia: the Reduction of LDL-C with PCSK9 Inhibition in Heterozygous Familial Hypercholesterolemia Disorder (RUTHERFORD) randomized trial. *Circulation* 2012; **126:** 2408–17.

16 Dias C, Shaywitz A, Wasserman SM, et al. Effects of AMG 145 on low-density lipoprotein cholesterol levels: results from 2 randomized, double-blind, placebo-controlled, ascending-dose phase I studies in healthy volunteers and hypercholesterolemic subjects on statins. *J Am Coll Cardiol* 2012; **60:** 1888–98.

17 Stein EA, Mellis S, Yancopoulos GD, et al. Effect of a monoclonal antibody to PCSK9 on LDL cholesterol. *N Engl J Med* 2012; **366:** 1108–18.

18 Koren MJ, Scott R, Kim JB, et al. Efficacy, safety, and tolerability of a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 as monotherapy in patients with hypercholesterolaemia (MENDEL): a randomised, double-blind, placebo-controlled, phase 2 study. *Lancet* 2012; **380:** 1995–2006.

19 Scientific Steering Committee on behalf of the Simon Broome Register Group. Risk of fatal coronary heart disease in familial hypercholesterolaemia. *BMJ* 1991; **303:** 893–96.

20 Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; **18:** 499–502.

21 Hochberg Y. A sharper Bonferroni procedure for multiple tests of significance. *Biometrika* 1988; **75:** 800–02.

22 Quade D. Rank analysis of covariance. *J Am Stat Assoc* 1967; **62:** 1187–200.

23 Koren MJ, Giugliano RP, Raal FJ, et al. Efficacy and safety of longer-term administration of evolocumab (AMG 145) in patients with hypercholesterolemia: 52-week results from the Open-Label Study of Long-Term Evaluation Against LDL-C (OSLER) randomized trial. *Circulation* 2014; **129:** 234–43.

24 Blom DJ, Hala T, Bolognese M, et al. A 52-week placebo-controlled trial of evolocumab in hyperlipidemia. *N Engl J Med* 2014; **370:** 1809–19.

25 Besseling J, Kindt I, Hof M, Kastelein JJ, Hutten BA, Hovingh GK. Severe heterozygous familial hypercholesterolemia and risk for cardiovascular disease: a study of a cohort of 14,000 mutation carriers. *Atherosclerosis* 2014; **233:** 219–23.

26 Talmud PJ, Shah S, Whittall R, et al. Use of low-density lipoprotein cholesterol gene score to distinguish patients with polygenic and monogenic familial hypercholesterolaemia: a case-control study. *Lancet* 2013; **381:** 1293–301.

27 Sjouke B, Kusters DM, Kindt I, et al. Homozygous autosomal dominant hypercholesterolaemia in the Netherlands: prevalence, genotype-phenotype relationship, and clinical outcome. *Eur Heart J* 2014; published online Feb 28. DOI:10.1093/eurheartj/ehu058.

28 Raal FJ, Honarpour N, Blom DJ, et al, for the TESLA Investigators. Inhibition of PCSK9 with evolocumab in homozygous familial hypercholesterolaemia (TESLA Part B): a randomised, double-blind, placebo-controlled trial. *Lancet* 2014; published online Oct 2. http://dx.doi.org/10.1016/S0140-6736(14)61374-X.

29 Further cardiovascular outcomes research with PCSK9 inhibition in subjects with elevated risk (FOURIER). http://clinicaltrials.gov/ct2/show/NCT01764633 (accessed Aug 29, 2013).