Inhibition of PCSK9 with evolocumab in homozygous familial hypercholesterolaemia (TESLA Part B): a randomised, double-blind, placebo-controlled trial

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Summary

Background **Homozygous familial hypercholesterolaemia is a rare, serious disorder caused by very low or absent plasma clearance of LDL, substantially raised LDL cholesterol, and accelerated development of cardiovascular disease. Conventional lipid-lowering treatments are modestly effective. Evolocumab, a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 (PCSK9), reduced LDL cholesterol by 16% in a pilot study. We now report results with evolocumab in a randomised, double-blind, placebo-controlled phase 3 trial.**

Methods **This randomised, double-blind, placebo-controlled phase 3 trial was undertaken at 17 sites in ten countries in North America, Europe, the Middle East, and South Africa. 50 eligible patients (aged ≥12 years) with homozygous familial hypercholesterolaemia, on stable lipid-regulating therapy for at least 4 weeks, and not receiving lipoprotein apheresis, were randomly allocated by a computer-generated randomisation sequence in a 2:1 ratio to receive subcutaneous evolocumab 420 mg or placebo every 4 weeks for 12 weeks. Randomisation was stratified by LDL cholesterol at screening (<11 mmol/L or ≥11 mmol/L) and implemented by a computerised interactive voice-response system. Patients, study personnel, and the funder were masked to treatment and to the efficacy results by the central laboratory not returning LDL cholesterol or any lipid results to the clinical sites after the baseline visit. The primary endpoint was percentage change in ultracentrifugation LDL cholesterol from baseline at week 12 compared with placebo, analysed by intention-to-treat. This trial is registered with ClinicalTrials.gov, number NCT01588496.**

Findings **Of the 50 eligible patients randomly assigned to the two treatment groups, 49 actually received the study drug and completed the study (16 in the placebo group and 33 in the evolocumab group). Compared with placebo, evolocumab significantly reduced ultracentrifugation LDL cholesterol at 12 weeks by 30·9% (95% CI −43·9% to −18·0%; p<0·0001). Treatment-emergent adverse events occurred in ten (63%) of 16 patients in the placebo group and 12 (36%) of 33 in the evolocumab group. No serious clinical or laboratory adverse events occurred, and no anti-evolocumab antibody development was detected during the study.**

Interpretation **In patients with homozygous familial hypercholesterolaemia receiving stable background lipid-lowering treatment and not on apheresis, evolocumab 420 mg administered every 4 weeks was well tolerated and significantly reduced LDL cholesterol compared with placebo.**

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See **Online** for appendix

Introduction

Homozygous familial hypercholesterolaemia is a rare but devastating genetic disorder characterised by very low plasma LDL clearance, substantially raised LDL cholesterol, and early cardiovascular morbidity and mortality.[1] More than 95% of patients have mutations in both LDL receptor alleles. Mutations in apolipoprotein B, proprotein convertase subtilisin/kexin type 9 (PCSK9), or, rarely, the LDL receptor adapter protein (called autosomal recessive hypercholesterolaemia) also occur. True genetic homozygotes have identical mutations in both alleles of the affected gene, but most patients are compound heterozygotes with two different LDL receptor mutations.[2,3] Residual LDL receptor activity, originally established through comparisons of LDL uptake in cultured fibroblasts of patients with homozygous familial hypercholesterolaemia versus that of control fibroblasts, classified patients with less than 2% of LDL uptake as receptor negative and those with 2–25% of normal uptake as receptor defective.[4] The residual LDL receptor activity is associated with the severity of LDL cholesterol elevation and propensity for early cardiovascular disease.[1,5] In homozygous familial hypercholesterolaemia, high doses of simvastatin, atorvastatin, or rosuvastatin reduce mean LDL cholesterol by 22–25%,[6,7] and ezetimibe achieves an additional 20% reduction.[8] Thus, conventional drug therapy can reduce LDL cholesterol by 40–45% on average and is the keystone of treatment in affected patients. Recently, two drugs—mipomersen (which inhibits apolipoprotein B synthesis) and lomitapide (which inhibits microsomal triglyceride transfer protein)—have been approved (both by the US Food and Drug Administration in the USA, but only lomitapide by the European Medicines Agency for marketing in Europe)

with an orphan indication for the treatment of homozygous familial hypercholesterolaemia.[9,10] However, despite the reductions achieved by these drugs, LDL cholesterol remains substantially raised in nearly all patients with familial hypercholesterolaemia and LDL apheresis is often used as therapy.[11] Evolocumab (AMG 145), a fully human monoclonal antibody that binds to PCSK9, a protein that has a crucial role in targeting the LDL receptor for degradation, allows increased recycling of LDL receptors and reduces LDL cholesterol by 60% in patients with heterozygous familial hypercholesterolaemia.[12] In an open-label, single-arm pilot study of eight patients with homozygous familial hypercholesterolaemia, evolocumab reduced LDL cholesterol by 16·5%.[13] In this Article, we report the efficacy and safety of evolocumab for patients with homozygous familial hypercholesterolaemia in a larger randomised, double-blind, placebo-controlled phase 3 trial.

Methods

Study design and participants

The Trial Evaluating PCSK9 Antibody in Subjects with LDL Receptor Abnormalities (TESLA) Part B is a randomised, double-blind, placebo-controlled phase 3 trial, undertaken at 17 sites in ten countries in North America, Europe, Middle East, and South Africa. The list of investigators and patient distribution per country are available in appendix pp 2–3 and p 7.

Eligible participants were male or female patients aged 12 years and older with homozygous familial hypercholesterolaemia diagnosed either by genetic analysis or clinical criteria (history of an untreated LDL cholesterol concentration >13 mmol/L plus either xanthoma before 10 years of age or evidence of heterozygous familial hypercholesterolaemia in both parents).[5] Eligibility criteria were fasting LDL cholesterol of 3·4 mmol/L or higher after at least 4 weeks of a stable, low-fat diet and baseline lipid-lowering therapies; fasting triglycerides of 4·5 mmol/L or lower; and bodyweight of 40 kg or greater. Exclusion criteria are listed in the appendix and included lipoprotein apheresis within 8 weeks before screening, or the use of mipomersen or lomitapide within the preceding 5 months.

Institutional review boards at each site approved the study protocol, and all patients provided written informed consent.

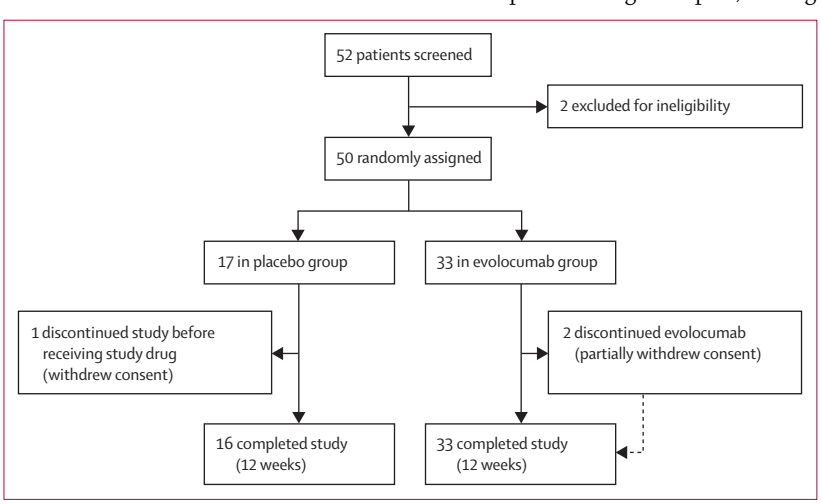

Figure 1: **Trial profile**

Randomisation and masking

Patients were randomly assigned in a 2:1 ratio to subcutaneous injections of evolocumab 420 mg or placebo every 4 weeks. Randomisation was computer generated by the sponsor (Amgen), stratified by LDL cholesterol level at screening (<11 mmol/L or ≥11 mmol/L) and implemented by a computerised interactive voice-response system. Patients, study personnel, and Amgen study staff were masked to treatment assignment. All lipid results were masked by the central laboratory after the baseline visit.

Procedures

Study visits took place at the screening visit, at randomisation (day 1), and at weeks 4, 6, 8, and 12, with optional visits at weeks 2 and 10. The study drug was administered at the investigator site (ie, by the investigator or their staff at the clinic). At each visit, fasting lipids, chemistry, haematology, evolocumab pharmacokinetic parameters, and anti-evolocumab antibodies were assessed and analysed by a central laboratory (Medpace Reference Laboratories [Cincinnati, USA, and Leuven, Belgium] for lipid analyses; Covance Laboratories [Indianapolis, IN, USA, and Geneva, Switzerland] for routine safety analyses; and Millipore [Billerica, MA, USA] for the antibody measurements). Genotyping was done by Progenika Inc (Medford, MA, USA) for all except for one patient for whom local results were used because the local site had documented the defects in this patient by more detailed analysis than that done by Progenika. Allocation of a mutation to LDL receptor activity was done by Progenika with use of an extensive literature base in which the mutation's effect was estimated if a scientific publication included a formal in-vitro assessment of residual LDL receptor activity in genetically homozygous tissue.[14] With use of this conservative metric, LDL receptor functionality was characterised as either defective or negative. If a formal assessment of LDL receptor activity was not available in the published literature, the LDL receptor functionality for that mutation was described as unclassified, irrespective of the potential structural consequences of the mutation on the LDL receptor protein. LDL receptor mutations that have been described as causative of familial hypercholesterolaemia but the function of which has not yet been established or described in published works were grouped as unclassified, or, if no mutation was identified in the LDL receptor or apolipoprotein B gene, as no mutation identified.

Outcomes

The primary endpoint was percentage change from baseline in ultracentrifugation LDL cholesterol at week 12, which was 4 weeks after the final dose of the study drug was administered. Measurement of LDL cholesterol by preparative ultracentrifugation was based on recent studies showing that use of the Friedewald formula to calculate LDL cholesterol underestimates LDL cholesterol substantially when it falls below 1·8 mmol/L, resulting in overestimation of potential LDL cholesterol reductions during treatment.[14] The central lipid laboratory (Medpace Reference Laboratories in Cincinnati, OH, USA, or in Leuven, Belgium) assessed the within-run and between-run precision, as measured by coefficients of variation; within-run precision ranged from 1·4% to 2·6% and between-run precision from 2·0% to 3·3% for LDL cholesterol concentrations of 2·8–4·8 mmol/L. Secondary efficacy endpoints were the absolute change in LDL cholesterol from baseline to week 12 and at the mean of weeks 6 and 12; the percentage change in ultracentrifugation LDL cholesterol at the mean of weeks 6 and 12; the percentage change from baseline at week 12, and at the mean of weeks 6 and 12, in apolipoprotein B, lipoprotein(a), HDL cholesterol, triglycerides, and PCSK9. Exploratory endpoints are listed in the appendix and included the absolute change in high-sensitivity C-reactive protein and pharmacogenetic analyses that focused on inherited genetic variations such as those of the *LDLR* gene to assess the possible correlation to the disease or responsiveness to the treatments. The main safety endpoints were treatment-emergent and serious adverse events, safety laboratory assessments, electrocardiograph parameters, and the development of anti-evolocumab antibodies. Adverse events were classified according to the Medical Dictionary for Regulatory Activities version 16.1.

Statistical analysis

Our planned enrolment of 51 patients (of whom 34 were allocated to evolocumab) had 81% power to detect a treatment effect of around an 18% reduction in LDL cholesterol with a common SD of 20·9% in the evolocumab group over placebo based on a two-sided *t* test at a 0·05 level of significance. We analysed all randomised patients who received at least one dose of study drug for safety and efficacy. We analysed the primary and secondary efficacy endpoints with a repeated measures linear effects model that included terms for treatment group, stratification factor, scheduled visit, and the interaction of treatment with scheduled visit. We did not impute missing values in the repeated measures linear mixed-effects model. If the primary endpoint was significant at the 0·05 level, statistical testing of the secondary and exploratory endpoints followed the Hochberg procedure at a significance level of 0·05. We used descriptive statistics for the safety analyses.

This trial is registered with ClinicalTrials.gov, number NCT01588496.

	Placebo group (n=16)	Evolocumab group (n=33)	All patients (n=49)
Age (years)	32 (14)	30 (12)	31 (13)
Age range (years)	14–57	13–51	13–57
Female sex	8 (50%)	16 (48%)	24 (49%)
Ethnicity*			
White	15 (94%)	29 (88%)	44 (90%)
Asian	1 (6%)	1 (3%)	2 (4%)
Clinically evident coronary artery disease	6 (38%)	15 (46%)	21 (43%)
Previous coronary artery bypass surgery	4 (25%)	8 (24%)	12 (25%)
Aortic valve replacement	3 (19%)	4 (12%)	7 (14%)
Lipid parameters			
LDL cholesterol, ultracentrifugation (mmol/L)	8·7 (3·8)	9·2 (3·5)	9·0 (3·5)
LDL cholesterol, calculated (mmol/L)	8·7 (3·7)	9·2 (3·5)	9·0 (3·6)
Apolipoprotein B (g/L)	2·1 (0·8)	2·1 (0·7)	2·1 (0·7)
Lipoprotein(a) (nmol/L)	128 (80–201)	76 (26–145)	101 (31–146)
Apolipoprotein A1 (g/L)	1·1 (0·4)	1·1 (0·2)	1·1 (0·3)
HDL cholesterol (mmol/L)	1·0 (0·4)	1·0 (0·3)	1·0 (0·3)
Triglycerides (mmol/L)	1·3 (0·7)	1·2 (0·6)	1·2 (0·6)
Free PCSK9 (nmol/L)	9·4 (2·5)	8·9 (2·9)	9·0 (2·7)
Lipid-lowering therapy			
Statin	16 (100%)	33 (100%)	49 (100%)
Atorvastatin	10 (63%)	22 (67%)	32 (65%)
Atorvastatin ≥40 mg/day	10 (63%)	21 (64%)	31 (63%)
Rosuvastatin	6 (38%)	11 (33%)	17 (35%)
Rosuvastatin ≥20 mg/day	5 (31%)	10 (30%)	15 (31%)
Ezetimibe	15 (94%)	30 (91%)	45 (92%)
Genotype			
LDL receptor mutations	14 (88%)	31 (94%)	45 (92%)
True homozygous	7 (44%)	15 (45%)	22 (45%)
Compound heterozygous	7 (44%)	16 (48%)	23 (47%)
Heterozygous	0	1 (3%)	1 (2%)
Apolipoprotein B	2 (13%)	0	2 (4%)
Autosomal recessive hypercholesterolaemia	0	1 (3%)	1 (2%)

Data are mean (SD), range, n (%), or median (IQR). PCSK9=proprotein convertase subtilisin/kexin type 9.*Ethnicity was self-reported and some patients did not answer this question.

Table 1: **Baseline characteristics**

Role of the funding source

Amgen designed the study in collaboration with FJR and EAS 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. An independent data monitoring committee regularly reviewed data prepared by an external biostatistics group. The academic investigators vouch for the accuracy and completeness of the data and analyses as presented, and for the fidelity of this report to the trial protocol. FJR and EAS had the main responsibility for the decision to submit for publication.

Results

Between Feb 17, 2013, and Jan 31, 2014, we screened 52 potential participants and enrolled 50 patients into the study, who were randomly assigned to evolocumab (n=33)

	Placebo group (n=16)	Evolocumab group (n=33)	Treatment difference (95% CI)	p value
LDL cholesterol				
Ultracentrifugation (% change from baseline)				
Week 12 (primary endpoint)	7·9% (−2·7 to 18·5)	−23·1% (−30·7 to −15·4)	−30·9% (−43·9 to −18·0)	<0·0001*
Mean of weeks 6 and 12	4·2% (−5·0 to 13·4)	−25·6% (−32·2 to −19·0)	−29·8% (−40·9 to −18·6)	<0·0001*
Calculated (% change from baseline)				
Week 12	9·0% (−1·5 to 19·6)	−23·1% (−30·8 to −15·4)	−32·1% (−45·1 to −19·2)	<0·0001
Mean of weeks 6 and 12	5·8% (−3·5 to 15·1)	−25·2% (−31·9 to −18·5)	−31·0% (−42·3 to −19·7)	<0·0001
Ultracentrifugation absolute values (mmol/L; change from baseline)				
Week 12	0·5 (−0·5 to 1·6)	−1·9 (−2·6 to −1·1)	−2·4 (−3·7 to −1·1)	0·0004
Mean of weeks 6 and 12	0·2 (−0·7 to 1·1)	−2·1 (−2·7 to −1·5)	−2·3 (−3·3 to −1·2)	<0·0001
Calculated absolute values (mmol/L; change from baseline)				
Week 12	0·6 (−0·4 to 1·6)	−1·9 (−2·6 to −1·1)	−2·5 (−3·7 to −1·2)	0·0002
Mean of weeks 6 and 12	0·3 (−0·6 to 1·2)	−2·0 (−2·6 to −1·4)	−2·3 (−3·4 to −1·2)	<0·0001
Other lipids (% change from baseline)				
Apolipoprotein B				
Week 12	4·0% (−5·6 to 13·5)	−19·2% (−26·1 to −12·2)	−23·1% (−34·8 to −11·5)	0·0007*
Mean of weeks 6 and 12	2·7% (−6·3 to 11·6)	−20·2% (−26·6 to −13·8)	−22·9% (−33·7 to −12·1)	0·0004*
Lipoprotein(a)				
Week 12	2·4% (−8·6 to 13·5)	−9·4% (−17·6 to −1·2)	−11·8% (−25·5 to 1·8)	0·09*
Mean of weeks 6 and 12	−1·4% (−11·1 to 8·2)	−12·7 (−19·8 to −5·6)	−11·3 (−23·1 to 0·6)	0·09*
HDL cholesterol				
Week 12	4·1% (−3·5 to 11·7)	4·0% (−1·6 to 9·5)	−0·1% (−9·4 to 9·2)	0·98
Mean of weeks 6 and 12	6·1% (0·7 to 11·5)	7·4% (3·4 to 11·3)	1·3% (−5·4 to 7·9)	0·70
Triglycerides				
Week 12	−1·7% (−14·1 to 10·6)	−1·4% (−10·6 to 7·8)	0·3% (−14·9 to 15·6)	0·97
Mean of weeks 6 and 12	1·0% (−9·9 to 11·8)	−2·3% (−10·2 to 5·6)	−3·3% (−16·5 to 10·0)	0·62
Other parameters				
PCSK9 (% change from baseline)				
Week 12	10·6% (−5·1 to 26·2)	−29·1% (−40·6 to −17·7)	−39·7% (−59·0 to −20·4)	0·0002
Mean of weeks 6 and 12	8·9% (−1·2 to 19·0)	−59·8% (−67·2 to −52·4)	−68·7% (−81·1 to −56·3)	<0·0001
hsCRP (nmol/L)				
Change from baseline at week 12	−0·95 (−4·76 to 0·95)	−0·19 (−3·81 to 2·86)	8·57 (−11·43 to 29·52)	NA

Data are least-squares mean (95% CI) except for hsCRP data, which are median (IQR). Least-squares mean is from the repeated measures model, which includes treatment group, stratification factor, scheduled visit, and interaction of treatment with scheduled visits as covariates for all endpoints. PCSK9=proprotein convertase subtilisin/kexin type 9. hsCRP=high-sensitivity C-reactive protein. NA=not applicable. *Multiplicity adjustments following the Hochberg procedure were used to control for overall significance at the 0·05 level of significance for the primary and secondary endpoints.

Table 2: **Efficacy outcomes**

or placebo (n=17). One patient in the placebo group withdrew consent and did not receive the study drug and was therefore excluded from efficacy and safety analyses. All 49 randomly assigned patients who received the study drugs completed the trial (figure 1). Two patients allocated to the evolocumab group discontinued the study drug because of partial withdrawal of consent; one patient received study drug at day 1 but had no post-baseline lipid assessment during the study, and the other patient discontinued the study drug after two doses (day 1 and week 4) and had their final lipid assessment at week 8. Both patients completed 12 weeks of follow-up in the study and were included in safety and efficacy analyses.

Baseline characteristics were well balanced between the two groups (table 1 and appendix pp 7–8). Genotyping confirmed homozygous familial hypercholesterolaemia in all patients who had met clinical diagnostic criteria, except for one who was a genetic heterozygote (table 1); this patient was still included in our intention-to-treat analyses. LDL receptor mutations in both alleles were recorded in 45 of 48 (94%) patients, with 22 patients having the same mutation in both alleles (true homozygous familial hypercholesterolaemia) and 23 having different mutations in each LDL receptor allele (compound heterozygous familial hypercholesterolaemia). Appendix pp 9–10 shows the mutations and associated receptor function; one patient had a mutation in both apolipoprotein B alleles (homozygous familial defective apolipoprotein B); one patient was a triple heterozygote with mutations in both apolipoprotein B alleles and one LDL receptor allele; and one patient had autosomal recessive hypercholesterolaemia. All patients were given

statins at baseline, with 46 of 49 (94%) receiving high-intensity statin therapy (atorvastatin ≥40 mg daily or rosuvastatin ≥20 mg daily) and 45 (92%) receiving ezetimibe. Despite their existing treatment, the mean baseline ultracentrifugation LDL cholesterol in the participants was 9·0 mmol/L (SD 3·5) (table 1). Clinically evident coronary artery disease was present in 21 (43%) of 49 patients, with 12 (25%) having undergone coronary artery bypass surgery and seven (14%) aortic valve replacement. Cerebrovascular or peripheral artery disease was present in four (8%) of 49 patients.

For the primary outcome of percentage change in ultracentrifugation LDL cholesterol from baseline to week 12, the least-squares mean percentage reduction in LDL cholesterol from baseline at week 12 was 23·1% (95% CI −30·7 to −15·4) in the evolocumab group, corresponding to a reduction versus placebo of 30·9% (−43·9 to −18·0; adjusted p<0·0001) (table 2). Appendix p 15 shows individual patient responses. The least-squares mean absolute reduction in LDL cholesterol with evolocumab versus placebo at week 12 was 2·4 mmol/L (95% CI −3·7 to −1·1). The least-squares mean percentage reduction in LDL cholesterol from baseline at weeks 6 and 12 was 25·6% (95% CI −32·2 to −19·0) in the evolocumab group, corresponding to a treatment reduction of 29·8% (−40·9 to −18·6) compared with placebo (adjusted p<0·0001). Reductions recorded at week 4 were sustained throughout the study (figure 2). In the seven adolescent patients younger than 18 years of age receiving evolocumab, the least-squares mean reduction in LDL cholesterol from baseline at week 12 was 26·0% (95% CI −49·9 to −2·2).

In a prespecified analysis, we analysed LDL cholesterol responses according to LDL receptor function (table 3); pre-treatment and post-treatment LDL cholesterol concentrations are shown in appendix pp 11–12. Excluding the patient who was a genetic heterozygote, the 28 patients with a receptor-defective mutation in one or both alleles who received evolocumab had a significant reduction in ultracentrifugation LDL cholesterol versus placebo (table 3). In ad-hoc analyses, the patients with two defective mutations (all c.681C>G [Asp227Glu]) receiving evolocumab also had a significant reduction in ultra-centrifugation LDL cholesterol at week 12 compared with those receiving placebo (table 3), whereas those with one defective and one negative mutation had a smaller, but still significant, mean reduction versus placebo (table 3). The interaction between these two populations was not significant (p=0·075). The 22 patients in the evolocumab group with LDL receptor mutations in both alleles of which one or both were unclassified had a mean reduction of 17·9% (95% CI −36·0 to 0·3) from baseline at week 12, which is a non-significant treatment reduction versus placebo of 21·7% (−50·6 to 7·3; p=0·13) (table 3).

The patient with LDL receptor-negative mutations in both alleles and the patient with autosomal recessive homozygous familial hypercholesterolaemia did not respond to evolocumab treatment—the LDL cholesterol levels of both these patients increased slightly (by 3–10%) compared with baseline. The two patients with apolipoprotein B mutations were randomly assigned to placebo. Notably, patients with the same LDL receptor mutations showed variable responses to evolocumab, as exemplified by eight patients homozygous for the most common mutation in the study cohort, the c.681c>G (Asp227Glu) LDL receptor mutation, in whom the reduction in LDL cholesterol with evolocumab at week 12 varied between 7% and 56% (appendix p 12).

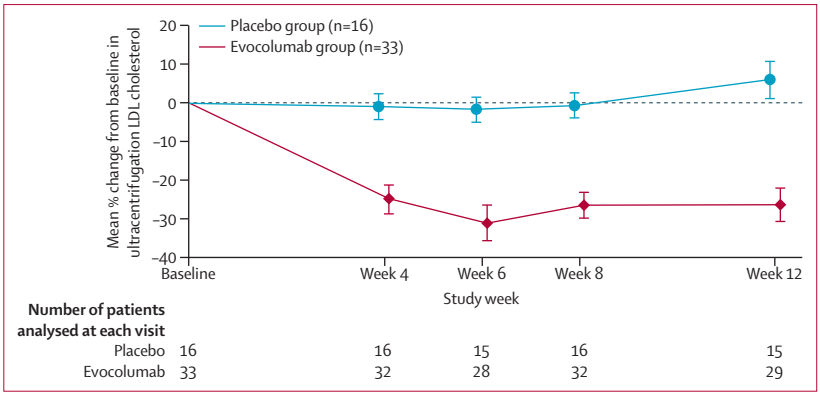

Figure 2: **Mean percentage change in ultracentrifugation LDL cholesterol concentration from baseline to week 12**
Vertical lines represent standard error around the mean. The plot is based on observed values and no imputation was used for missing values. Number of patients represents those analysed for this endpoint at each visit.

The overall results for all participants showed that evolocumab treatment led to a significant least-squares mean reduction in apolipoprotein B at week 12 versus placebo (table 2). However, compared with placebo, evolocumab did not significantly reduce lipoprotein(a) (table 2). In a prespecified analysis—again excluding the patient who was genetically heterozygous—evolocumab-treated patients with LDL receptor mutations in both alleles of which at least one was defective had significant reductions in both apolipoprotein B and lipoprotein(a) (table 3). In patients with known LDL receptor activity, treatment with evolocumab versus placebo resulted in a numerically greater least-squares mean reduction in apolipoprotein B at week 12 in patients with receptor-defective mutations in both LDL alleles when compared with those with one defective and one negative mutation (table 3). Lipoprotein(a) reduction correlated significantly with LDL cholesterol reduction at week 12 in all patients (Spearman correlation coefficient 0·60, p<0·0001) and in patients with LDL receptor mutations in both alleles of which at least one was defective (Spearman correlation coefficient 0·63, p=0·0005). No differences between the two treatment groups were recorded in HDL cholesterol or triglycerides at week 12 (table 2). Other lipid parameters are shown in appendix p 13.

Median changes in high-sensitivity C-reactive protein from baseline to week 12 were −0·19 nmol/L (95% CI −3·81 to 2·86) in the evolocumab group and −0·95 nmol/L

	Placebo group (n=16)	Evolocumab group (n=33)	Treatment difference (95% CI)	p value*
All patients				
Patients (n)	16 patients	33 patients		
Ultracentrifugation LDL cholesterol	7·9% (–2·7 to 18·5)	–23·1% (–30·7 to –15·4)	–30·9% (–43·9 to –18·0)	<0·0001
Apolipoprotein B	4·0% (–5·6 to 13·5)	–19·2% (–26·1 to –12·2)	–23·1% (–34·8 to –11·5)	0·0002
Lipoprotein(a)	2·4% (–8·6 to 13·5)	–9·4% (–17·6 to –1·2)	–11·8% (–25·5 to 1·8)	0·09
LDL receptor mutations status				
Patients defective in one or both alleles	8 patients	20 patients		
Ultracentrifugation LDL cholesterol	11·2% (0·8 to 21·7)	–29·6% (–36·5 to –22·7)	–40·8% (–53·4 to –28·3)	<0·0001
Apolipoprotein B	7·1% (–1·7 to 15·9)	–26·2% (–32·0 to –20·4)	–33·3% (–43·8 to –22·8)	<0·0001
Lipoprotein(a)	9·8% (–4·0 to 23·6)	–15·3% (–24·6 to –6·1)	–25·1% (–41·7 to –8·6)	0·0045
Patients with defective/defective status	5 patients	8 patients		
Ultracentrifugation LDL cholesterol	15·1% (–1·2 to 31·3)	–31·8% (–44·9 to –18·8)	–46·9% (–68·0 to –25·7)	0·0006
Apolipoprotein B	8·9% (–4·4 to 22·2)	–29·5% (–40·2 to –18·8)	–38·4% (–55·7 to –21·0)	0·0006
Lipoprotein(a)	9·8% (–9·3 to 28·9)	–10·0% (–25·4 to 5·3)	–19·8% (–44·8 to 5·1)	0·11
Patients with defective/negative status	3 patients	6 patients		
Ultracentrifugation LDL cholesterol	3·5% (–10·6 to 17·5)	–21·0% (–30·7 to –11·2)	–24·5% (–41·6 to –7·3)	0·0128
Apolipoprotein B	4·8% (–3·2 to 12·9)	–17·6% (–23·1 to –12·1)	–22·4% (–32·1 to –12·6)	0·0013
Lipoprotein(a)	9·1% (7·1)†	–14·7% (7·7)†	–23·8%†	NC†
Patients with unclassified mutation status‡	6 patients	16 patients		
Ultracentrifugation LDL cholesterol	3·8% (–20·7 to 28·3)	–17·9% (–36·0 to 0·3)	–21·7% (–50·6 to 7·3)	0·13
Apolipoprotein B	–0·2% (–21·3 to 20·9)	–16·4% (–32·3 to –0·6)	–16·2% (–41·5 to 9·0)	0·19
Lipoprotein(a)	–2·0% (–21·7 to 17·8)	–5·4% (–21·7 to 10·8)	–3·5% (–27·7 to 20·8)	0·77
Patients with negative/negative mutation status	0 patients	1 patient		
Ultracentrifugation LDL cholesterol	..	10·3%	..	..
Apolipoprotein B	..	9·3%	..	..
Lipoprotein(a)	..	38·0%	..	..
Patients with LDL receptor heterozygous status	0 patients	1 patient	..	..
Ultracentrifugation LDL cholesterol	..	–55·7%	..	..
Apolipoprotein B	..	–50·2%	..	..
Lipoprotein(a)	..	–58·3%	..	..
Other gene mutations				
Patients with apolipoprotein B mutation	2 patients	0 patients		
Ultracentrifugation LDL cholesterol	–10·8% and 13·1%	..	..	..
Apolipoprotein B	–2·3% and 0·8%	..	..	..
Lipoprotein(a)	–22·3% and –9·1%	..	..	..
Patients with autosomal recessive hypercholesterolaemia	0 patients	1 patient		
Ultracentrifugation LDL cholesterol	..	3·5%	..	..
Apolipoprotein B	..	11·5%	..	..
Lipoprotein(a)	..	13%	..	..

Data are least-squares mean (95% CI) for groups with sufficient data; otherwise data are actual value at week 12 or as specified. Least-squares mean is from the repeated measures model, which includes treatment group, stratification factors, scheduled visit, and the interaction of treatment with scheduled visit as covariates. NC=not calculated.*Multiplicity adjustments following the Hochberg procedure were used to control for overall significance at the 0·05 level of significance for the primary and secondary endpoints. †Mean (SE)—treatment difference and p value were not calculated through the repeated measures model because of insufficient patient numbers. ‡Function of one or both LDL receptor mutations is unknown (includes six patients from the group with LDL receptor defective mutation in one or both alleles).

Table 3: **Percentage change from baseline at week 12 in lipid parameters according to receptor mutation status**

(95% CI –4·76 to 0·95) in the placebo group (table 2). Amounts of unbound PCSK9 were reduced by 32·1% from baseline at week 4 (an interval of 4 weeks after evolocumab administration) and by 90·1% at week 6 (2 weeks after evolocumab administration; appendix pp 14, 16). We noted a weak correlation between PCSK9 levels and LDL cholesterol reduction at week 12 in all patients (Spearman correlation coefficient 0·28, p=0·07) and in patients with LDL receptor mutations in both alleles of which at least one was defective (Spearman correlation coefficient 0·33, p=0·10).

Treatment-emergent adverse events occurred in ten (63%) of 16 patients in the placebo group and 12 (36%) of 33 patients in the evolocumab group (table 4). No serious adverse events were reported. The most frequent adverse events in the evolocumab group were upper respiratory

	Placebo group (n=16)	Evolocumab group (n=33)
Treatment-emergent adverse events		
Any	10 (63%)	12 (36%)
Serious	0	0
Leading to discontinuation of study drug	0	0
Deaths	0	0
Frequent treatment-emergent adverse events*		
Upper respiratory tract infection	1 (6%)	3 (9%)
Influenza	0	3 (9%)
Gastroenteritis	0	2 (6%)
Nasopharyngitis	0	2 (6%)
Nausea	2 (13%)	0
Musculoskeletal pain	0	1 (3%)
Potential injection-site reactions†	1 (6%)	0
Neurocognitive events‡	0	0
Abnormal laboratory tests		
Creatine kinase >5×ULN	1 (6%)	1 (3%)
Creatine kinase >10×ULN	0	1 (3%)
ALT or AST >3×ULN	1 (6%)	2 (6%)
Antibodies		
Binding	0	0§
Neutralising	0	0

ALT=alanine aminotransferase. AST=aspartate aminotransferase. ULN=upper limit of normal. *Reported in at least one patient in either or both treatment groups. †Searched with use of high-level term grouping, which includes injection-site rash, inflammation, pruritus, reaction, or urticaria. ‡Searched with use of high-level grouping, which includes deliria (including confusion), cognitive and attention disorders and disturbances, dementia and amnestic disorders, disturbances in thinking and perception, and mental impairment disorders. §Excludes one patient who had a positive binding antibody test at baseline and negative antibody testing at all other study assessments.

Table 4: **Adverse events**

tract infection (three patients [9%]) and influenza (three patients [9%]), whereas in the placebo group nausea was the most common adverse event (two patients [13%]). No patient discontinued study drug because of an adverse event. Creatine kinase increase to more than ten-times the upper limit of normal was recorded at a single routine blood draw in one patient on evolocumab, which was associated with vigorous physical activity before the study visit. No creatine kinase elevations were associated with muscle symptoms or were sustained on repeat testing. No injection-site reactions were reported in the evolocumab group. Liver enzyme elevations of more than three-times the upper limit of normal were recorded in two of 33 (6%) patients in the evolocumab group and one of 16 (6%) patients in the placebo group. No anti-evolocumab antibody development was detected during the study.

Discussion

This study, one of the largest and most global randomised trials in homozygous familial hypercholesterolaemia, further expands on the results from our pilot trial[13] showing that PCSK9 inhibition with monthly subcutaneous evolocumab 420 mg reduced LDL cholesterol by 30·9% compared with placebo. In this new study, evolocumab reduced LDL cholesterol by 40·8% in patients with LDL receptor mutations in both alleles of which at least one was defective. The patient who met the clinical entry criteria for homozygous familial hypercholesterolaemia but was subsequently found to have a single genetic mutation had a reduction in LDL cholesterol of 55·7%, which is consistent with previous studies of heterozygous patients.

Based on an intention-to-treat analysis, the overall mean decrease in LDL cholesterol with evolocumab versus placebo was similar to the 21·4% reduction achieved in the phase 3 efficacy trials with the apolipoprotein B antisense drug, mipomersen, and the 40% decrease with lomitapide, an inhibitor of microsomal triglyceride transfer protein, in this patient population.[15,16] Although both these trials were of 26 weeks' duration, the length of the studies was based on the long period needed for the drugs to reach a stable maximum reduction in LDL cholesterol, which occurred between weeks 18 and 20 for both drugs, to allow roughly 6 weeks of LDL cholesterol stability before measurement of the primary endpoint.[15,17] This situation contrasts with our present trial of evolocumab in which the reduction in LDL cholesterol was achieved by 4 weeks and remained stable throughout the 12 weeks of the trial. Thus, for the purposes of comparing LDL cholesterol-reducing efficacy, the timepoints for assessment of the primary endpoint were consistent. Both mipomersen and lomitapide have recently been approved solely for the treatment of homozygous familial hypercholesterolaemia, although because of side-effects they are prescribed only under a risk assessment and mitigation programme.[9,10] In this 12-week trial, evolocumab was not associated with the injection-site reactions recorded in the 26-week trials with mipomersen, the gastrointestinal side-effects reported with lomitapide, or the significant hepatic transaminase elevation that has occurred with both drugs.[15,17] Longer term efficacy and safety of up to 72 weeks in 23 patients has been reported for lomitapide, with fairly consistent LDL cholesterol reductions as recorded at week 26.[17] Although the long-term efficacy and safety of evolocumab in homozygous patients with familial hypercholesterolaemia has not been studied, the drug has now been assessed in more than 6000 patients in phase 2 and 3 studies of 12–52 weeks' duration with similar good tolerability and safety, and sustained LDL reductions.[18–23]

Assessment of response based on LDL receptor function has not been done systematically in previous trials of homozygous familial hypercholesterolaemia (panel). This assessment was undertaken in the evolocumab pilot study, which showed LDL cholesterol reductions in six LDL receptor-defective patients but no response in two receptor-negative patients.[13] This trial shows that genetic information provides incremental

Panel: **Research in context**

Systematic review

A search of PubMed between Jan 1, 1985, and June 20, 2013, for original research articles published in English with the terms: "homozygous familial hypercholesterolaemia" and "trials with monoclonal antibodies to PCSK9" yielded only the previous pilot study referred to in this report. However, the search yielded treatments for homozygous familial hypercholesterolaemia, including statins, ezetimibe, partial ileal bypass, portacaval shunt, liver transplantation, LDL apheresis, mipomersen, and lomitapide. Despite all previous treatments, patients usually do not achieve optimum LDL cholesterol levels.

Interpretation

We report the results of the first randomised, placebo-controlled trial of the PCSK9 inhibitor, evolocumab, in patients with homozygous familial hypercholesterolaemia. Additionally, this study is the first trial of any therapeutic LDL cholesterol-lowering agent that related response to the underlying genetic mutation causing homozygous familial hypercholesterolaemia. Evolocumab administered 420 mg every 4 weeks was well tolerated and yielded an overall 30·9% mean reduction in LDL cholesterol compared with placebo. The response to evolocumab was related to the underlying genetic defect causing homozygous familial hypercholesterolaemia, ranging from a 40·8% mean reduction in LDL cholesterol compared with placebo in the 57% (28/49) of patients with either one or two defective LDL receptor mutations to no response in patients with two negative or suspected negative mutations. These findings are important because they provide clinicians with evidence that PCSK9 inhibition with evolocumab is an effective LDL cholesterol-lowering therapy in homozygous familial hypercholesterolaemia, attaining cholesterol reductions that are similar to those reported with two drugs recently approved as orphan therapies for this disorder.

insight into homozygous familial hypercholesterolaemia and possible response to treatment. Therefore, in a clinical trial setting, to obtain genetic confirmation for homozygous familial hypercholesterolaemia is valuable and could be useful to select patients for evolocumab treatment in clinical practice. Trials with statins and mipomersen have suggested that patients with receptor-defective status responded better than did those with receptor-negative status.[6,15] The present trial confirms that the response to evolocumab correlates with the underlying genetic cause of homozygous familial hypercholesterolaemia, with a greater reduction in LDL cholesterol recorded in patients with two LDL receptor-defective mutations than in those with even a single LDL receptor-negative mutation. The 40·8% mean reduction in LDL cholesterol with evolocumab compared with placebo in the 57% of patients with either one or two defective LDL receptor mutations indicates that evolocumab potentially represents an additional effective new lipid-lowering therapy for most patients in this difficult-to-treat population. The genetic defects, and associated LDL receptor activities, identified in the current trial from patients in ten countries and four continents seem to be generalisable to the overall homozygous patient population because they are also consistent with previous studies that have reported genotypes in large homozygous familial hypercholesterolaemia cohorts.[3,24,25] In these studies, most patients had at least one allele associated with defective LDL receptor function, whereas homozygotes with two alleles for negative LDL receptor function are less common.[3,24,25] However, even within the group of genetically confirmed homozygous patients with identical LDL receptor mutations, the LDL cholesterol reductions were variable, suggesting that other factors must be involved in lowering of LDL cholesterol, as has been reported with statin therapy.[26,27] Although the least-squares mean LDL cholesterol reduction with evolocumab in the 16 patients with unclassified receptor defects was 17·9%, responses were highly variable (ranging from −56·6% to 42·9%), suggesting that the identified mutations are probably associated with LDL receptor activity ranging from defective to negative. The patient with the 42·9% increase in LDL cholesterol did not discontinue evolocumab and indicated that their background lipid-lowering therapy had not changed during the study.

Patients enrolled in this trial had raised concentrations of lipoprotein(a), as has been well documented in familial hypercholesterolaemia. Lipoprotein(a) is thought to have an independent role in further increasing the risk of atherosclerosis in these already very-high-risk patients.[28,29] Although the overall reduction in lipoprotein(a) with evolocumab versus placebo did not reach statistical significance, the reduction in those patients with either one or two defective LDL receptors was statistically significant. As has been recently reviewed extensively, the mechanism by which evolocumab, or other monoclonal antibodies to PCSK9, reduce lipoprotein(a) is unclear.[30] The clinical benefit of reducing lipoprotein(a) in homozygous familial hypercholesterolemia, as in other populations, is unknown.

All enrolled patients completed the trial without any serious clinical or laboratory adverse events, although two patients in the evolocumab group stopped the study drug before completion for reasons unrelated to the drug. In this patient population receiving high-dose baseline statin therapy, the rates of treatment-emergent muscle events and liver enzyme and creatine kinase elevations were low and consistent with the many large phase 2 and 3 trials of evolocumab.[18–23]

Our study has several limitations that should be considered. The 12-week duration of treatment, although ideal to assess LDL cholesterol-lowering efficacy, provides a curtailed long-term assessment of safety in this population. Longer term safety in homozygous familial hypercholesterolaemia will be

provided in an open-label long-term extension in which patients from this trial, together with those from the pilot trial and other homozygous patients who did not qualify for this trial, will provide a larger cohort.[31] Additionally, evolocumab is still being assessed for long-term safety in large populations with heterozygous familial hypercholesterolaemia and non-familial hypercholesterolaemia, including a large trial of 22 500 patients to assess cardiovascular outcomes.[32] The trial did not include patients on apheresis, who were specifically excluded to minimise the variability in LDL cholesterol that can occur with the procedure even if lipid assessment is done just before each procedure. Variables that affect the pre-apheresis LDL cholesterol include the interval between procedures and the plasma volume processed in any one procedure. Furthermore, the effects of apheresis on evolocumab clearance had not been defined at the time of study implementation and could have affected bioavailability. Patients on apheresis who were excluded from this trial are being assessed in an open-label trial.[31] The inclusion of eight patients who were genetically homozygous for the same mutation, associated with defective function, in both LDL receptor genes and in whom the mean LDL cholesterol reduction was 47% could potentially confound the generalisability of the overall results to a wider homozygous population. However, the variation in LDL cholesterol within this group was large and they constituted a small proportion of the 28 patients with at least one LDL receptor allele associated with defective function in whom the mean LDL cholesterol reduction was around 41%. We also only studied the effect of evolocumab 420 mg dosed every 4 weeks, which results in less prolonged suppression of free PCSK9 and probably less LDL cholesterol reduction than does the same dose administered every 2 weeks as shown in our pilot trial.[13] When administered at more frequent intervals in the pilot trial, substantially greater PCSK9 suppression could be maintained and the additional reduction in LDL cholesterol in patients with at least one mutation in the LDL receptor associated with defective function was 6%.[13] However, the patients from this trial have entered into an open-label, long-term extension in which the frequency of dosing can be increased to every 2 weeks.[31] Finally, the effect of administering evolocumab to patients already being given either mipomersen or lomitapide is unknown in terms of both efficacy and safety.

The strengths of the trial are its size and the global diversity of sites and genetic mutations, which enhance its generalisability to the overall homozygous familial hypercholesterolaemia population. It was also placebo controlled and, for the first time in any therapeutic trial in homozygous familial hypercholesterolaemia, assessed LDL cholesterol response based on underlying genetic mutations, which should allow for more targeted use of evolocumab in this population.

In conclusion, in patients with homozygous familial hypercholesterolaemia not receiving apheresis, monthly evolocumab 420 mg administered with stable background statin therapy with or without ezetimibe significantly reduced LDL cholesterol levels by 31% and apolipoprotein B by 23% compared with placebo at 12 weeks. In most patients, in whom at least one mutation in the LDL receptor was associated with defective activity, the LDL cholesterol reduction was 41%. Evolocumab was well tolerated and offers an effective additional option to reduce LDL cholesterol as part of the clinical management of these patients.

Contributors

Amgen (NH, RS, and SMW) designed the study in collaboration with the academic investigators FJR and EAS. FJR, DJB, and GKH were key clinical site investigators for this trial. FJR participated in study design, data review and interpretation, initial draft of the report, and ongoing review and revisions. DJB and GKH contributed to data collection and report review. NH participated in study design, data review and interpretation, report review, and revisions. FX was the study statistician, and was therefore responsible for the study's statistical plan, and participated in data analysis and interpretation. RS participated in study design, data interpretation, and report review. SMW participated in study design, study oversight, data review and interpretation, and report review and revisions. EAS contributed to study design, data review and interpretation, the initial draft of the report, and ongoing review and revisions. This report was mainly written by FJR and EAS, with assistance from NH and SMW, and was critically reviewed 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. NH, FX, RS, and SMW are employees of Amgen and have received Amgen stock or stock options. RS and SMW are listed as co-inventors on a use patent for PCSK9 monoclonal antibodies in homozygous familial hypercholesterolaemia. EAS has been a consultant to, and participated in symposia sponsored by, Amgen, Regeneron, Sanofi, Roche, and BMS related to PCSK9 inhibitors. He is listed as co-inventor on a use patent for PCSK9 monoclonal antibodies in homozygous familial hypercholesterolaemia but has not received, and will not receive, financial compensation. His institution has received research funding related to PCSK9 inhibitor clinical trials from Amgen, Regeneron/Sanofi, BMS, Genentech/Roche, Pfizer, and Lilly, and funding related to mipomersen from ISIS and Genzyme.

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