Effect of the PCSK9 Monoclonal Antibody, AMG 145, in
Homozygous Familial Hypercholesterolemia

Running title: Stein et al.; PCSK9 inhibition in HoFH

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Other treatment
Abstract

Background—Homozygous familial hypercholesterolemia (HoFH), is a rare, serious disorder with substantial reduction in low-density lipoprotein (LDL) receptor function, severely elevated LDL cholesterol, cardiovascular disease, and often death, in childhood. Response to conventional drug therapies is modest. Monoclonal antibodies to proprotein convertase subtilisin/kexin 9 (PCSK9) reduce LDL cholesterol in heterozygous familial hypercholesterolemia. The effect in HoFH is unknown and uncertain. We evaluated the efficacy and safety of AMG 145 in an open-label, single arm, multicenter, dose-scheduling pilot study in patients with HoFH.

Methods and Results—Eight patients with LDL receptor negative or defective HoFH on stable drug therapy were treated with subcutaneous AMG 145 420 mg every 4 weeks for ≥12 weeks, followed by AMG 145 420 mg every 2 weeks for an additional 12 weeks. All patients completed both treatment periods. Mean (range) change from baseline in LDL cholesterol at week 12 was –16.5% (+5.2% to –43.6%; P=0.0781) and –13.9% (+39.9 to –43.3%; P=0.1484) with 4- and 2-week dosing, respectively. No reduction was seen in the 2 receptor-negative patients. Over the treatment periods, mean (SD) LDL cholesterol reductions in the six LDL receptor-defective patients were 19.3% (16) and 26.3% (20) with 4- and 2-week dosing, respectively (P=0.0313 for both values) ranging from 4 to 48% with 2 week dosing. No serious side effects were reported.

Conclusions—This study demonstrates significant and dose related LDL cholesterol lowering with a PCSK9 monoclonal antibody in HoFH patients with defective LDL receptor activity but no reduction in those who were receptor negative.


Key words: familial hypercholesterolemia, PCSK9, low-density lipoprotein cholesterol, Homozygous Familial Hypercholesterolemia
Introduction

Homozygous familial hypercholesterolemia (HoFH) is a rare but serious clinical disorder caused by substantial reduction in low-density lipoprotein (LDL) receptor function.\(^1\) As a result, LDL cholesterol levels are severely elevated, leading to cardiovascular disease, and often death, in childhood.\(^1\) Over 95% of HoFH patients have a mutation in the LDL receptor, less than 4% in apolipoprotein B, and less than 0.5% in proprotein convertase subtilisin/kexin 9 (PCSK9).\(^2,3\) While true genetic HoFH is not uncommon, the majority of patients are compound heterozygotes.\(^4\) The residual LDL receptor activity, either negative (<2% function) or defective (2% to 25% function), is associated with severity of LDL cholesterol elevation and propensity for early cardiovascular disease.\(^1\)

Conventional therapies, such as statins\(^5,6\) and ezetimibe,\(^7\) are the most commonly used drugs for HoFH, and result in LDL cholesterol reductions of 15% to 25%. Patients usually also require LDL apheresis when available.\(^8\) These improvements in LDL cholesterol, particularly with statins, appear to reduce cardiovascular disease morbidity and mortality.\(^9\) Recently two drugs, lomitapide\(^10\) and mipomersen,\(^11\) which both reduce hepatic lipoprotein production and are not dependent on LDL receptor function, have been approved by the US Food and Drug Administration solely for the treatment of HoFH. AMG 145, a fully human monoclonal antibody to PCSK9, significantly reduces LDL cholesterol in heterozygous familial hypercholesterolemia,\(^12\) but as HoFH patients have either no or minimal, LDL receptor function, defined as <2% or between 2-25% of normal respectively, it is uncertain if PCSK9 inhibition would be effective. We evaluated the efficacy and safety of AMG 145 in an open-label, single arm, multicenter, dose-scheduling pilot study in patients with HoFH.

Methods
Patients

The Trial Evaluating PCSK9 Antibody in Subjects with LDL Receptor Abnormalities (TESLA) study allowed for inclusion of patients with HoFH either by genetic confirmation or clinical diagnosis [history of an untreated LDL cholesterol concentration greater than 13 mmol/L (500 mg/dL) plus either xanthoma before 10 years of age or evidence of heterozygous familial hypercholesterolemia in both parents]. Irrespective of prior LDL receptor and apolipoprotein B DNA genotyping, all patients were re-genotyped and based on known defects in LDL receptor function assigned as receptor negative or defective.

At screening male or female patients aged 12 to 65 years and >40 kg were required to be stable on a low-fat diet and existing lipid-lowering therapies (including statins, cholesterol-absorption inhibitors, bile-acid sequestrants, nicotinic acid, or combinations thereof) for at least 4 weeks, with a fasting LDL cholesterol concentration ≥ 3.4 mmol/L (≥ 130 mg/dL) and triglyceride concentration ≤ 4.5 mmol/L (≤ 400 mg/dL). Background lipid-lowering therapy was unchanged during the trial.

Patients receiving LDL apheresis within 8 weeks of the screening visit or scheduled to receive it during the study, or treated with mipomersen or lomitapide within 5 months of screening, were excluded.

Additional exclusion criteria are shown in the Supplementary Appendix.

Study design and oversight

This was an investigator-initiated trial designed jointly with Amgen. The institutional review board at each site approved the protocol, and all patients (and legal guardian if a minor) provided written informed consent prior to entering the trial. Patients were enrolled into an open-label, single arm, multicenter, dose-scheduling pilot study with subcutaneous AMG 145 at a dose of
420 mg every 4 weeks for 12 weeks (Clinical Trials.gov trial NCT01588496), maintained for an additional 12 weeks of treatment at 4-week intervals, and then AMG 145 420 mg was administered every 2 weeks for an additional 12 weeks (Clinical Trials.gov trial NCT01624142) (Figure 1). The 12-week every-4-week dosing maintenance period was necessitated by the protocol amendment process to add every-2-week dosing to the subsequent 12-week phase. Patients meeting eligibility at screening returned within 5 to 10 days for enrollment in the treatment phase (day 1). Subsequent study visits were conducted at weeks 4, 8, and 12 for each treatment phase (Figure 1), with optional laboratory visits at weeks 2 and 10. At clinic visits, assessments included side effects, dietary compliance, concomitant lipid drugs, other prescription drugs, vital signs, physical exam, and 12-lead ECGs. Blood for laboratory testing was obtained under fasting (>10 hours, water only) conditions, and laboratory tests included lipid and safety measurements, anti-AMG 145 antibodies, biomarker sample collection, serum pregnancy testing (females of childbearing potential), and urinalysis. Study drug was administered on site by trained study staff.

**Efficacy and safety evaluations**

The primary efficacy end point was percentage change from baseline in LDL cholesterol by ultracentrifugation at week 12 of each of the 4- and 2-week treatment periods. Secondary efficacy end points included absolute change and percentage change from baseline in non-high-density lipoprotein (non-HDL) cholesterol, apolipoprotein B, apolipoprotein A1, lipoprotein (a), HDL cholesterol, and PCSK9, and the proportion of patients with a response (defined as a 15% or greater reduction in LDL cholesterol from baseline). The primary safety end point was the incidence of treatment-emergent adverse events; other safety end points included the incidence of anti-AMG 145 antibodies, laboratory abnormalities, and changes in ECG parameters. Adverse
events were coded using the Medical Dictionary for Regulatory Activities (MedDRA), version 15.1. An independent Data Monitoring Committee regularly reviewed data from this and other ongoing AMG 145 studies, prepared by an external biostatistical group.

**Laboratory methods**

All lipid and apolipoprotein analyses, including measurement of LDL cholesterol by preparative ultracentrifugation, were performed in a Center for Disease Control and Prevention Part III standardized central lipid laboratory, and safety testing was conducted in a College of American Pathology accredited central laboratory as previously described.12 Free PCSK9 measurements were performed by enzyme-linked immunosorbent assay.12 All patients were genotyped by Progenika Inc. (Medford, MA, USA) to identify or confirm mutations in LDL receptor or apolipoprotein B genes.

**Statistical analysis**

The analyses of baseline demographics, lipid parameters, efficacy, and safety end points included data from all enrolled patients. The baseline for lipid parameters was the average of screening and Day 1 values. In addition to the change from baseline at week 12, the mean percentage change and absolute change in mmol/L from baseline over each treatment period were assessed using data from weeks 4, 8 and 12. Statistical analyses in this open-label, single arm study are descriptive in nature. No statistical inference or missing value imputation was performed. All efficacy end points for the initial 12-week period of 4-week dosing and the 12 weeks of 2-week dosing were summarized with descriptive statistics. Significance differences were tested using the signed-rank test. Safety end points were reported over the entire duration of the trial as patient incidence. Summary statistics reported for continuous variables include the number of patients, mean, median with interquartile range, standard deviation (SD), and
minimum and maximum. For categorical variables, the frequency and percentage are reported.

Results

Patient characteristics

The first patient was screened on 5 March 2012 and the last patient completed the trial on 9 April 2013. The eight patients were from two sites, Johannesburg, South Africa, and Cincinnati, OH, USA. All patients had LDL-receptor mutations confirmed in both alleles (Table 1). Demographic and baseline characteristics are shown in Supplementary Table S1 and additional details on cardiovascular history and baseline lipid therapy shown for each patient in supplementary Table S2. All patients were Caucasian, with a mean age 34.3 years (range 14 to 54), six were male and six had clinical or angiographic evidence of coronary artery disease. All patients were receiving at a minimum both ezetimibe and intensive statin therapy at baseline. The LDL receptor activity of 6 patients was consistent with defective status and two with being negative, both of which were consistent their prior skin fibroblasts measurements. The mean (range) LDL cholesterol by ultracentrifugation at baseline was 11.4 (5.6 to 14.6) mmol/L [441.7 (218 to 563) mg/dL] (Table 2) with individual LDL cholesterol values shown in Supplementary Figure S1.

Efficacy Outcomes

LDL cholesterol

Changes and percentage changes in lipid-related parameters are shown in Table 2. At week 12 of every-4-week treatment, the mean LDL cholesterol by ultracentrifugation decreased from baseline by 17% (1.8 mmol/L [70.6 mg/dL]), with a range from +5% to -44% (+0.6 to -5.9 mmol/L [+23 to -228 mg/dL]), P=0.0781. Individual responses are shown as percent change versus baseline in Figure 2 Panel A and Supplementary Figure S2 Panel A, with four patients
experiencing a reduction in LDL cholesterol of $\geq 15\%$, and reductions in three patients $\geq 30\%$. The two patients with negative LDL receptor activity did not demonstrate reductions in LDL cholesterol. Absolute changes in mmol/L for each patient are shown in Supplementary Figure S1.

After 12 weeks of every-2-week treatment, mean LDL cholesterol decrease from baseline was $14\%$ (1.6 mmol/L [60.8 mg/dL]), $P=0.1484$. Again, no LDL cholesterol reduction was seen in the two LDL receptor-negative patients, but a greater reduction occurred over the 12-week treatment period in the six patients with receptor defective function (Table 3, Figure 2 Panel A, and Supplementary Figure S2 Panel C). Mean (SD) LDL cholesterol reductions, averaged over the 12 weeks of treatment, in the receptor-defective patients were $19.3\%$ (16)%, $P=0.0313$ and $26.3\%$ (20)%, $P=0.0313$ with 4- and 2-week dosing, respectively (Table 3 and Supplementary Figure S2 Panels B and D). Within the LDL receptor defective group LDL cholesterol changes ranged +2% to -43% with 4 week dosing and from -4 to -48% with 2 week dosing (Supplementary Figure S2 Panels C and D).

Additional efficacy outcomes

The changes from baseline at week 12 in apolipoprotein B with 4- and 2-week dosing are shown in Table 2 and were consistent with those seen in LDL cholesterol. The mean (SD) change in lipoprotein (a) was $-11.7$ (11)% and $-18.6$ (12)% with 4- and 2-week dosing, respectively, and did not appear to be related to LDL receptor activity (Table 3, Figure 2 Panel B). Triglycerides, HDL-cholesterol, and apolipoprotein A1 were essentially unchanged with either dosing schedule (Table 2). Mean (SD) reductions in free PCSK9 at week 12 following every 4- and 2-week treatment with AMG 145 420 mg were $22.7$ (37)% and $87.6$ (8)%, respectively (Table 2, Figure 2 Panel C).

Safety
During the study, six of the eight patients reported adverse events, all of which were considered not serious and unrelated to treatment by the investigator (Supplementary Table S3). Antibodies to AMG 145 were not detected during treatment. No patients had creatine kinase elevations greater than five times the upper limit of normal or liver enzymes (alanine aminotransferase or aspartate aminotransferase) greater than three times the upper limit of normal (Supplementary Table S3).

Discussion

This first study of PCSK9 inhibition in HoFH patients demonstrates that additional LDL cholesterol reduction is achievable in LDL receptor defective patients when AMG 145 is added to high dose statin and ezetimibe. Although the study included only two patients who were receptor negative neither experienced LDL cholesterol reduction even with dosing every 2 weeks and nearly 90% reduction in plasma PCSK9. However the mean decrease in all 8 patient of 17% (1.8 mmol/L [70.6 mg/dL]) at week 12 with AMG 145 420 mg every 4 weeks compares favorably with reductions achieved with statins in this population. This proof-of-concept trial of eight patients is larger than the initial proof-of-concept trials in HoFH for lomitapide and mipomersen and is as large as the statin trials. The study with simvastatin enrolled 12 homozygous patients, with eight randomized to 80 mg/day and four patients to 40 mg/day for 9 weeks, and reported LDL cholesterol reductions of 14% and 25% respectively. The homozygous rosvastatin trial (n=21) reported mean LDL cholesterol reductions from baseline after crossover treatment with rosvastatin 80 mg/day and atorvastatin 80 mg/day of 19% and 18%, respectively. A 12-week trial in 50 homozygous patients comparing ezetimibe 10 mg added to 40 mg/day of statin to increasing the statin to 80 mg/day reported a reduction of 20.7%
versus 6.7% respectively. The phase three trial with mipomersen, an apolipoprotein B synthesis inhibitor, randomized 51 homozygous patients on stable maximal drug therapy, but not on LDL apheresis, to subcutaneous mipomersen 200 mg/week or placebo for 26 weeks. The mean percentage reduction in LDL cholesterol from baseline was 24.7% (placebo reduction 3.3%) from a baseline of 11.4 mmol/L (440 mg/dL). The most effective reductions in LDL cholesterol with drug therapy in HoFH, a mean of 50% decrease after 26 weeks, have been reported in a phase three trial with the microsomal triglyceride transfer protein inhibitor, lomitapide. The open-label trial enrolled patients on background drug therapy, including 18 also on LDL-apheresis, and reported results on the 23 patients of 29 patients who completed up to 78 weeks of therapy.

Assessment of response based on LDL receptor function has not been systematically performed in prior HoFH trials, although with statins and mipomersen it has been suggested that patients with receptor defective status responded better than those who were receptor-negative. In the current trial, there was no LDL cholesterol response seen in the two LDL receptor negative patients. While this may have been anticipated, LDL cholesterol reductions have been reported in negative patients with statins. However a significant (p<0.05) reduction in LDL cholesterol was seen over the 12 weeks of treatment in the six receptor defective patients which averaged 19% (2.1 mmol/L [81.5 mg/dL]) and 26% (3 mmol/L [115 mg/dL]) with 4- and 2-week dosing, respectively. The additional LDL cholesterol reduction of 7% (average of weeks 4, 8, and 12) with more frequent dosing in these patients is similar to that reported with a doubling of statin dosage. Of interest is that two LDL receptor defective subjects with identical mutations (Table 1 - subjects #6 and #7) and very similar baseline LDL cholesterol levels had markedly different responses (Supplementary Figure S1) with patient 7 showing the largest response and
patient 6 the least response in this receptor subgroup. The heterogeneity in response in HoFH patients with the identical mutations (FH Afrikaner-1) has also been reported with mipomersen, which showed LDL cholesterol reductions ranging from -0.8% to -47.3%. Trials with AMG 145 in larger numbers of patients with the same mutations will hopefully yield additional information as to the response differences.

While the percentage reductions in HoFH LDL receptor-defective patients with AMG 145 are lower than in non HoFH patient populations in prior trials, the mean absolute reductions in LDL cholesterol with 4-week dosing of 2.1 mmol/L (81.5 mg/dL) are similar to the reductions seen in non-FH patients, while the reduction of 3 mmol/L (115 mg/dL) with 2-week dosing exceeds that seen in heterozygous FH and all other PCSK9 monoclonal antibody trials.12-18-20

The reduction in LDL cholesterol achieved with AMG 145 420 mg Q2W dosing in the LDL receptor-defective subjects is very similar to the reduction seen with mipomersen (26% versus 24.7%), from an almost identical baseline LDL cholesterol of 11.4 mmol/L (440 mg/dL). Comparison with lomitapide is somewhat more complicated, as the baseline mean LDL cholesterol in the lomitapide trial was significantly lower than in all prior HoFH trials at 8.7 mmol/L (335 mg/dL), and the trial reported results only in those completing 26, 52 and 78 weeks with mean LDL cholesterol reductions of 50%, 44% and 38% respectively. Thus the mean absolute reductions in LDL cholesterol at 26, 52 and 78 weeks were 4.4 mmol/L, 3.8 mmol/L and 3.3 mmol/L respectively. While the percentage reductions reported with lomitapide were superior to those seen with AMG 145 420 mg dosed every 2 weeks, the absolute reductions in the LDL receptor-defective subjects of 3.0 mmol/L approached those seen with longer-term lomitapide therapy.
In terms of relevancy for all HoFH patients, it is important to note that in two large studies\(^9,^{21}\) of over 200 HoFH patients, 70 to 75% had mutations consistent with defective LDL receptor function, with approximately 15% receptor negative and the remainder unknown. If the response seen in this trial is confirmed, AMG 145 may offer an additional therapeutic option for a large number of these patients. In addition the lack of LDL cholesterol response in LDL receptor negative patients should be confirmed in a larger cohort as there were only two such patients in this trial.

Of additional interest was the high baseline levels of PCSK9, well above those recently reported in a larger cohort of homozygous and heterozygous familial hypercholesterolemia patients,\(^21\) which required higher doses and more frequent dosing with AMG 145 to reduce PCSK9 levels to those achieved in prior trials with AMG 145.\(^12\) Despite the greater, almost 90%, decrease in free PCSK9 levels, there was no LDL cholesterol reduction seen in LDL receptor-negative patients, while the additional reductions seen in receptor-defective patients suggest that 2-week dosing may be more optimal for these patients.

The importance of the elevated lipoprotein (a) observed in homozygous patients is uncertain but it has been reported to contribute to accelerated cardiovascular disease in heterozygous familial hypercholesterolemia.\(^22\) The elevated lipoprotein (a) levels at baseline in the current study were reduced by 11.7% and 18.6% with every-4- and every-2-week dosing of AMG 145, respectively. Interestingly, patients with LDL receptor-negative function appeared to experience a reduction in lipoprotein (a); although based on only two patients, this finding needs to be validated. Reductions in lipoprotein (a) in homozygotes were reported with mipomersen, but no significant reductions were seen with statins, ezetimibe, or longer term treatment with lomitapide.\(^5,\)\(^7,^{16}\) This effect with AMG 145 on lipoprotein (a), although not well understood, is
consistent with that seen in non-homozygote patients.\textsuperscript{12, 23} The effects on HDL cholesterol and apolipoprotein A1 are also consistent with prior trials of AMG 145 and contrast with lomitapide, which significantly reduced HDL cholesterol and its associated apolipoprotein A1 by 12\% and 14\%, respectively.\textsuperscript{16}

The mechanism for LDL cholesterol reduction appears to be consistent with further upregulation of residual LDL receptor function, as exemplified by the lack of response in those patients with minimal or no LDL receptor activity. The large variation in response in the two genetically homozygous patients with receptor defective function and identical mutations is puzzling. It is possible that other minor modifying genes contribute to the variability and hopefully larger trials will assist in elucidating these differences. It is also possible that contributions to cholesterol excretion via an alternate pathway in the gut may be involved.

Recently Le May and colleagues\textsuperscript{24} demonstrated transintestinal cholesterol excretion (TICE) in human intestine confirming the findings previously described in mice, where this mechanism accounts for about 30\% of total intestinal cholesterol excretion. They also showed that the delivery of cholesterol for excretion was dependent on the LDL receptor but that an independent pathway for excretion also existed. Using LDL receptor knockout mice, Le May and colleagues showed a 40\% increase in TICE, suggesting such a mechanism could play a role in HoFH.\textsuperscript{24}

This trial, while small, assessed the safety and tolerability over a period of 36 weeks (Figure 1) including 12 weeks of every-2-week administration of AMG 145 420 mg, a dose only administered every 4 weeks in prior trials.\textsuperscript{12, 23, 25, 26} All enrolled patients completed the trial without any significant clinical or laboratory adverse experiences. Injection site reactions were minimal and no different in frequency from those reported in the large phase 2 trials in non-homozygote patients.\textsuperscript{12, 23, 25, 26} The low incidence of injection site reactions with AMG 145
contrasts with the other subcutaneously administered drug for HoFH, mipomersen, where 76% of patients reported injection site reactions, and 18% (6/34) discontinued therapy in the first 26 weeks. In the homozygote lomitapide trial, 21% (6/29) of patients discontinued therapy within 26 weeks, and approximately 80% of patients reported diarrhea, 65% nausea, 35% vomiting, and 28% abdominal pain. Administration of AMG 145, including 420 mg every 2 weeks, was not associated with elevated hepatic transaminases, a frequent finding with both mipomersen and lomitapide therapy. In the 26-week trials, hepatic transaminase elevations greater than 3 times the upper limit of normal were reported in 12% (4/34) of those on mipomersen and 34% (10/29) of those on lomitapide. These increases were associated with significant hepatic fat accumulation, and are consistent with similar findings seen in non-homozygote patients treated with these drugs. The long-term impact of these side effects is not known and it is unlikely that large and long enough trials in any patient population with either drug will ever effectively answer this issue. Due to the risk of hepatotoxicity, both mipomersen and lomitapide are available only through a restricted program under a Risk Evaluation and Mitigation Strategy. AMG 145 administration has already been reported in over 900 patients in the phase 2 program with no increase in hepatic transaminases. The safety will be further assessed in a large phase 3 program, including a large cardiovascular outcome trial involving more than 22,500 high-risk patients. This extensive safety program will presumably also provide reassurance for homozygote patients.

The current trial was a proof-of-concept, open-label study of 8 patients and thus as such has a number of limitations. However based on the results, a larger, double-blind, randomized and placebo-controlled trial of AMG 145 in HoFH has commenced.

This study demonstrates for the first time that LDL cholesterol lowering is achievable
with a PCSK9 monoclonal antibody in homozygous familial hypercholesterolemia patients specifically those with receptor defective status. An ongoing larger, placebo controlled study will be able to better assess PCSK9 targeting therapy in HoFH patients and could provide more insight for efficacy in LDL receptor negative patients as well.

**Acknowledgments:** We thank the patients who participated in the study, the research professionals at the clinical centers, and Thomas Liu, PhD, Patric Nelson, MPH, MBA, and Moetaz Albizem, MD, for statistical, clinical, and operational support respectively. We also thank Sue Hudson, BA, on behalf of Amgen Inc. and Meera Kodukulla, PhD, of Amgen Inc. for editorial support.

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**Conflict of Interest Disclosures:** Dr Stein has received consulting fees from Amgen Inc., Adnexus Therapeutics/BMS, Genentech/Roche, and Regeneron/Sanofi related to PCSK9 inhibitors, and his institution has received research funding related to PCSK9 clinical trials from Amgen Inc., Alnylam, BMS, Genentech/Roche, and Regeneron/Sanofi. Dr Raal has received consulting fees from Amgen Inc., and Sanofi related to PCSK9 inhibitors, and his institution has received research funding related to PCSK9 inhibitor clinical trials from Amgen Inc. and Sanofi. Drs Honarpour, Wasserman, and Scott, and Mr Xu are employees of Amgen Inc. and have received Amgen stock/stock options.

**References:**


30. Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk (FOURIER)

31. Trial Evaluating PCSK9 Antibody in Subjects With LDL Receptor Abnormalities (TESLA)
Table 1. Patient Genotypes

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mutation Allele 1 (Estimated LDLR Function)</th>
<th>Mutation Allele 2 (Estimated LDLR Function)</th>
<th>Overall LDLR Function</th>
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<tbody>
<tr>
<td>Patient 1</td>
<td>Asp266Glu (15%–30%)</td>
<td>Asp266Glu (15%–30%)</td>
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<td>Patient 2</td>
<td>1187–10 G&gt;A* (Not determined)</td>
<td>Asp266Glu (15%–30%)</td>
<td>Receptor defective</td>
</tr>
<tr>
<td>Patient 3</td>
<td>Asp224Asn (&lt;2% )</td>
<td>Cys296Tyr (Not determined)</td>
<td>Negative†</td>
</tr>
<tr>
<td>Patient 4</td>
<td>Deletion Exon 4–18 (Not determined)</td>
<td>Cys197Gly (Not determined)</td>
<td>Negative†</td>
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<tr>
<td>Patient 5</td>
<td>Asp221Gly (&lt;2%)</td>
<td>Asp227Glu (5%–15%)</td>
<td>Receptor defective</td>
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<tr>
<td>Patient 6‡§</td>
<td>Asp227Glu (5%–15%)</td>
<td>Asp227Glu (5%–15%)</td>
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<tr>
<td>Patient 7‡§</td>
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<td>Asp227Glu (5%–15%)</td>
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<td>Patient 8</td>
<td>Asp175Asn (Not determined)</td>
<td>Asp227Glu (5%–15%)</td>
<td>Receptor defective</td>
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</table>

LDLR, Low-density lipoprotein receptor.

* Mutation at splice acceptor site 10 nucleotides upstream of the first nucleotide of exon 9, 1187.
† Confirmed by fibroblast culture.
‡ True homozygous patient.
§ Patients share the same genotype.
### Table 2. Efficacy Outcomes (Overall).

<table>
<thead>
<tr>
<th>Parameter*</th>
<th>Baseline Value</th>
<th>AMG 145 (N = 8)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Change from baseline</td>
<td>Percentage change from baseline (%)</td>
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<tr>
<td><strong>Week 12, Every-4-Week Dosing</strong></td>
<td><strong>Average Week 4, 8, 12, Every-4-Week Dosing</strong></td>
<td><strong>Week 12, Every-2-Week Dosing</strong></td>
</tr>
<tr>
<td>LDL cholesterol (ultracentrifugation), mmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>11.4 (2.9)</td>
<td>9.6 (3.7)</td>
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<tr>
<td>Range</td>
<td>5.6 to 14.6</td>
<td>4.9 to 14.6</td>
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<tr>
<td>Calculated LDL cholesterol, mmol/L</td>
<td>11.6 (3.0)</td>
<td>9.6 (3.7)</td>
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<td>HDL cholesterol, mmol/L</td>
<td>0.9 (0.2)</td>
<td>0.9 (0.3)</td>
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<tr>
<td>Apolipoprotein B, g/L</td>
<td>2.7 (0.5)</td>
<td>2.3 (0.6)</td>
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<tr>
<td>Apolipoprotein A1, g/L</td>
<td>1.0 (0.2)</td>
<td>1.0 (0.1)</td>
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<td>Triglycerides, mmol/L</td>
<td>1.3 (0.7)</td>
<td>1.1 (0.6)</td>
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<tr>
<td>Lipoprotein (a), nmol/L</td>
<td>246.5 (61.5 to 276.0)</td>
<td>170.6 (116.5)</td>
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<tr>
<td>Free PCSK9, nmol/L</td>
<td>8.31 (1.68)</td>
<td>6.21 (2.90)</td>
</tr>
</tbody>
</table>

Conventional unit conversion factors: To convert values for cholesterol to mg/dL, divide by 0.0259. To convert values for free PCSK9 to ng/mL, multiply by 72. SD, standard deviation; LDL, low-density lipoprotein; HDL, high-density lipoprotein; PCSK9, proprotein convertase subtilisin/kexin type 9. Values are mean (SD) unless otherwise stated. * Signed-rank test. † Median (interquartile range).
Table 3. Efficacy Outcomes Based on Mutation Status

<table>
<thead>
<tr>
<th>Mutation Status</th>
<th>Week 12, Every-4-Week Dosing</th>
<th>Week 12, Every-2-Week Dosing</th>
<th>Average of Week 4, 8, and 12, Every-4-Week Dosing</th>
<th>Average of Week 4, 8, and 12, Every-2-Week Dosing</th>
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<tbody>
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<td></td>
<td>UC LDL-C</td>
<td>Apolipoprotein B</td>
<td>Lipoprotein (a)*</td>
<td>UC LDL-C</td>
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<td>Defective LDL receptor (n=6)</td>
<td>−22.9 (17.5)</td>
<td>−18.3 (14.9)</td>
<td>−10.0 (11.5)</td>
<td>−23.6 ()</td>
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<tr>
<td>Negative LDL receptor (n=2)</td>
<td>2.6 (3.7)</td>
<td>−4.5 (3.5)</td>
<td>−16.8 (8.0)</td>
<td>15.3 (34.7)</td>
</tr>
<tr>
<td></td>
<td>Average of Week 4, 8, and 12, Every-4-Week Dosing</td>
<td>Average of Week 4, 8, and 12, Every-2-Week Dosing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Defective LDL receptor (n=6)</td>
<td>−19.3 (15.5)</td>
<td>−18.0 (13.1)</td>
<td>−10.0 (11.5)</td>
<td>−26.3 (20.4)</td>
</tr>
<tr>
<td>Negative LDL receptor (n=2)</td>
<td>4.4 (10.3)</td>
<td>1.4 (5.6)</td>
<td>−16.8 (8.0)</td>
<td>11.0 (23.6)</td>
</tr>
</tbody>
</table>

SD, standard deviation; UC, ultracentrifugation; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein cholesterol. * Lipoprotein (a) was only collected at week 12 for every-4-week dosing. † Signed-rank test.
Figure Legends:

**Figure 1.** Study design. Eight patients with genetically confirmed HoFH on stable drug therapy were enrolled. SC, subcutaneous; LDL-C, low-density lipoprotein cholesterol.

**Figure 2.** Efficacy of AMG 145 in the treatment of patients with HoFH. Panel (A): Percentage change from baseline in LDL cholesterol by ultracentrifugation, week 4, 6, 8, and 12 of the 4-week dosing period, and week 4, 8, and 12 of the 2-week dosing period (N=8); as shown, data for patient 2 was missing at week 8 of the 2-week dosing period and data for patient 4 was missing at week 0 of the 2-week dosing period. Panel (B): Percentage change in lipoprotein (a) from baseline and week 12 of the 4-week dosing period and weeks 4, 8, and 12 of the 2-week dosing period; as shown, data for patient 4 was missing at week 0 of the 2-week dosing period. Panel (C): PCSK9 levels by patient, baseline, week 4, 6, 8, and 12 of the 4-week dosing period and week 4, 8, and 12 of the 2-week dosing period; as shown, data patient 4 was missing at week 0 of the 2-week dosing period. UC, ultracentrifugation; LDL-C, low-density lipoprotein cholesterol; PCSK9, proprotein convertase subtilisin/kexin 9; LDLR, low-density lipoprotein receptor.
Figure 1

Screening period:
Fasting LDL-C 5 to 10 days before Day 1

AMG 145 420 mg SC Every 4 weeks N=8

Dosing every 4 weeks

Day 1 2* 4 6 8 10* 12

Study Week

Dosing every 2 weeks

Day 1 2* 4 6 8 10* 12

Study Week

↓ Administration of AMG 145 420 mg

* Week 2 and week 10 study visits are optional.
Red rectangles indicate prespecified cutoff dates for efficacy and safety analyses.
An unconnected line indicates a missing value between two timepoints. A dashed line indicates time between the two dosing periods of the study.

*Defective LDLR function; †Negative LDLR function
Figure 2B

An unconnected line indicates a missing value between two timepoints. A dashed line indicates time between the two dosing periods of the study.

*Defective LDLR function; †Negative LDLR function
An unconnected line indicates a missing value between two timepoints. A dashed line indicates time between the two dosing periods of the study.

*Defective LDLR function; †Negative LDLR function

Figure 2C