Role of PCSK9 Inhibitors in Patients with Familial Hypercholesterolemia

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Patients with familial hypercholesterolemia (FH) are at high or very high risk for cardiovascular disease. Those with heterozygous FH (HeFH) often do not reach low-density lipoprotein cholesterol (LDL-C) targets with statin and ezetimibe therapy, and those with homozygous FH (HoFH) usually require additional lipid-modifying therapies. Drugs that inhibit proprotein convertase subtilisin/kexin type 9 (PCSK9) offer a novel approach to reduce LDL-C. The monoclonal antibodies, alirocumab and evolocumab, given by subcutaneous injection every 2 or 4 weeks produce reductions in LDL-C of 50% to 60% in patients with HeFH, allowing many of them to achieve their LDL-C goals. Patients with HoFH show a reduced and more variable LDL-C response, which appears to depend on residual LDL receptor activity, and those with receptor-negative mutations may show no response. Inclisiran is a long-acting small interfering RNA therapeutic agent that inhibits the synthesis of PCSK9. Subcutaneous doses of 300 mg can reduce LDL-C by more than 50% for at least 6 months and the responses in HeFH and HoFH patients are similar to those achieved with monoclonal antibodies. These PCSK9 inhibitors are generally well tolerated and they provide a new opportunity for effective treatment for the majority of patients with FH.

Keywords: Alirocumab; Evolocumab; Hydroxymethylglutaryl-CoA reductase inhibitors; Hyperlipoproteinemia type II; PCSK9 protein, human; RNA, small interfering

INTRODUCTION

Familial hypercholesterolemia (FH) is a genetic autosomal co-dominant condition causing high levels of low-density lipoprotein cholesterol (LDL-C), which predispose individuals to premature atherosclerotic cardiovascular disease (ASCVD) and especially coronary heart disease (CHD) [1]. Heterozygous FH (HeFH) was originally thought to occur in about 1/500 people, but recent estimates suggest the prevalence is about 1/200 to 1/300 in most populations [2]. The phenotype of HeFH is quite variable and many people with the condition are not aware of it unless they undergo genetic testing or until they suffer an ischemic event [3,4].

Patients with homozygous FH (HoFH) also exhibit a variable phenotype, but are generally easier to recognize as they usually present the clinical features of tendon xanthomas and cutaneous

xanthomas in childhood and typically develop very early CHD unless suitable interventions are implemented from an early age [5]. The prevalence of autosomal dominant HoFH in the Netherlands was estimated to be about 1/300,000 in a report in 2015 [6].

DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLEMIA

Clinical criteria have often been used for the diagnosis of FH [7-9]. These include the Simon Broome Register Group criteria, the Make Early Diagnosis to Prevent Early Deaths (MEDPED) criteria, and the Dutch Lipid Clinic Network criteria. Recently, the American Heart Association (AHA) developed revised criteria [10]. The Japan Atherosclerosis Society has also developed their own criteria, which include measurements of the Achilles tendon thickness as an objective assessment of tendon involvement [11].

It is desirable to confirm the diagnosis of FH with genetic testing to optimize CHD risk assessment and facilitate cascade screening [12]. Monogenic FH most often occurs due to missense loss-of-function (LOF) mutations in the low-density lipoprotein receptor gene (*LDLR*) and over 2,300 different *LDLR* mutations have been identified and listed in the ClinVar database in 2018 [13]. The next most commonly affected gene is *APOB*, which encodes apolipoprotein B (apoB); mutations in this gene are often associated with a somewhat less severe phenotype that is also referred to as familial defective apoB [3,10]. The frequency of different mutations varies in different parts of the world and some common mutations in the *LDLR* and *APOB* genes have been described in Asian countries [14,15]. Common mutations occur in some regions as a founder effect causing an increased local population prevalence of FH [16].

An autosomal recessive form of FH caused by LOF mutations in the LDL receptor adaptor protein 1 gene (*LDLRAP*) was described in 2003 [17]. The clinical phenotype varies but may be less severe than that of classic dominant HoFH caused by true homozygous or compound heterozygous *LDLR* mutations. More recently, researchers have identified another autosomal recessive genetic cause involving lysosomal acid lipase deficiency with mutations in the lipase A gene (*LIPA*), which results in a wide spectrum of phenotypes including cholesterol ester storage disease and Wolman disease [18,19].

Proprotein convertase subtilisin/kexin type 9 (PCSK9) was discovered as the product of the third gene found to cause autosomal dominant FH due to gain-of-function (GOF) mutations in *PCSK9* [20]. PCSK9 was recognized to be the same as the secretory proprotein convertase, which had been called neural apoptosis-regulated convertase 1 (NARC-1) [21]. This discovery provided a new target for treatment to reduce LDL-C levels. This is particularly relevant for patients with FH, as many of those with HeFH do not reach LDL-C targets with the maximum tolerated statin therapy or even with the addition of ezetimibe [22].

Many people thought to have FH may have a high polygenic score rather than monogenic dominant or recessive conditions. In a cohort of 313 individuals with severe hypercholesterolemia, defined as LDL-C >5.0 mmol/L (>194 mg/dL), monogenic FH-causing mutations were present in 47.3% of individuals and monogenic mutations increased to 53.7% when copy number variations were included; the percentage with a genetic component further increased to 67.1% when individuals with extreme polygenic scores were included [23]. Polygenic contributions to LDL-C also explain some of the heterogeneity in clinical presentation and ASCVD risk for individuals with FH [24].

GOALS AND LIPID-LOWERING TREATMENTS FOR FAMILIAL HYPERCHOLESTEROLEMIA

In the 2019 European Society of Cardiology (ESC)/European Atherosclerosis Society (EAS) guidelines, patients with FH without additional risk factors are considered high risk and the LDL-C treatment goal is <1.8 mmol/L (<70 mg/dL), along with a reduction of ≥50% from baseline, whereas those with ASCVD or another major risk factor are considered very high risk and the LDL-C treatment goal is <1.4 mmol/L (<55 mg/dL) [25]. The 2018 Korean guidelines for the management of dyslipidemia recommend a target LDL-C <100 mg/dL for FH patients without CHD or other major risk factors and <70 mg/dL when CHD or other major risk factors are present [9].

The risk for ASCVD in FH is influenced by the usual cardiovascular risk factors including lipoprotein(a) (Lp(a)) [26]. Lp(a) levels are raised in FH and are an important predictor of cardiovascular disease independent of the type of LDL receptor mutation [27]. Marked elevation of Lp(a) may be present in 30% to 50% of patients with FH, and the cholesterol content of Lp(a) contributes to LDL-C measurements and may influence the LDL-C threshold diagnostic criteria for FH used in clinical algorithms [28]. Statins tend to increase Lp(a) [29]; therefore, having other treatments that reduce it may be an advantage and some FH patients may need specific treatment to target Lp(a).

In a study of HeFH patients in the Netherlands, statin treatment reduced the risk of myocardial infarction to a level that was not significantly different from that of an age-matched sample from the general population [30]. A 20-year follow-up study of statin therapy in children in the Netherlands showed that early initiation of treatment in FH patients can considerably reduce the risk of ASCVD [31]. However, another study of HeFH patients in the Netherlands found that only about half of them reached a target LDL-C of <3 mmol/L and only 21% achieved a target of <2.5 mmol/L [22]. Many patients were not on maximum statin doses and only 54% of patients were on ezetimibe. The main reason for patients not reaching the LDL-C targets appeared to be inertia of the treating physician.

In a prospective study of HeFH patients in Korea, the LDL-C target of <100 mg/dL was achieved by 28% of patients and 47% achieved a ≥50% LDL-C reduction at 12 months with high-intensity statin regimens or combination therapy; according to that study, pretreatment LDL-C was a major determinant of the response [32].

Lipid-lowering therapy, predominantly with statins, has improved the prognosis of patients with HoFH [33], but they usually require additional lipid-modifying therapy such as lomitapide, and adjunctive lipoprotein apheresis is often utilized when available [5]. Having a highly effective treatment to inhibit PCSK9, such as the fully human monoclonal antibodies (mAbs), alirocumab and evolocumab, can enable many more patients with FH to achieve the recommended LDL-C goals [34,35].

PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9

Numerous studies have investigated the role of PCSK9 in lipid metabolism and the effects of genetic variations in *PCSK9*. Two nonsense mutations—c.426C>G (rs67608943, p.Y142X) and c.2037C>A (rs28362286, p.C679X) in *PCSK9*—were found to be common in African Americans and were associated with a 40% reduction in plasma levels of LDL-C [36]. Further studies showed that these nonsense mutations in *PCSK9* were associated with a 28% reduction in mean LDL-C and an 88% reduction in the risk of CHD in African Americans, while the p.R46L (rs11591147, c.137G>T, p.Arg46Leu) variation in *PCSK9* that was more common in white subjects was associated with a 15% reduction in LDL-C and with a 47% reduction in the risk of CHD over a 15-year interval [37].

An analysis of three studies from Denmark also showed that the *PCSK9* R46L variant was associated with a modest reduction of LDL-C levels and a more marked reduction of CHD risk compatible with a lifetime exposure to lower LDL-C [38]. The reported variants in *PCSK9* present a spectrum of hypercholesterolemia or hypocholesterolemia phenotypes [39].

It was shown in several studies that PCSK9 induces the degradation of the LDLR and related receptors by acting as a chaperone that binds the LDLR during receptor-mediated endocytosis and then directs it for lysosomal degradation (Fig. 1) [40-42]. The enzymatic activity of PCSK9 is not involved in the process. Statins upregulate PCSK9 by causing intracellular sterol depletion and increasing levels of the sterol-regulatory element binding protein-2 (SREBP-2), which activates transcription of
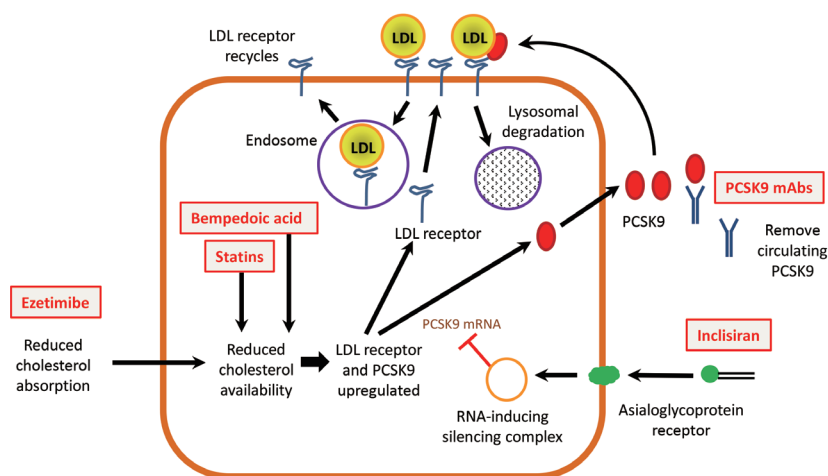
Fig. 1. Action of proprotein convertase subtilisin/kexin type 9 (PCSK9) monoclonal antibodies and inclisiran. LDL, low-density lipoprotein; mAbs, monoclonal antibodies.

PCSK9 as well as the LDLR [43-45]. When the dose of statin is increased, the increase in levels of PCSK9 attenuates the increased expression of the LDLR, which is thought to account for the limited additional LDL-C lowering effect of about 6% when statin doses are doubled [46].

PCSK9 levels were elevated in untreated FH patients, particularly in those with HoFH, and high-dose statin therapy further increased PCSK9 levels in these patients [47]. Moreover, adding ezetimibe to statin resulted in additional increases in PCSK9 levels [48].

PCSK9 MONOCLONAL ANTIBODIES

The mAbs developed to target PCSK9 include alirocumab and evolocumab, which are fully human mAbs, and bococizumab which is a humanized mAb that might be expected to have some risk of immunogenicity [49]. Indeed, the development of bococizumab was discontinued when it was seen that there was an attenuation of LDL-C lowering over time as well as higher rates of injection-site reactions in the Studies of PCSK9 Inhibition and the Reduction of vascular Events (SPIRE) trials [50,51].

In two phase II studies in which bococizumab was given intravenously every 4 weeks for 12 weeks in various doses to subjects with primary hypercholesterolemia on high doses of statin, 15% of subjects had genetic variants associated with FH and they responded in a similar way to subjects who did not carry these variants [52]. In the SPIRE program, 1,578 patients with FH were compared with 15,959 patients without FH selected for comparable lipid levels who had been randomized to bococizumab 150 mg subcutaneously (SC) every 2 weeks (Q2W) or to matching placebo and followed over a median period of 11.2 months [53]. The FH and non-FH patients both had reductions in LDL-C of 55% with bococizumab, but the proportion of patients developing antidrug antibodies was higher among those with FH than among those without FH (43% vs. 36%, $P<0.001$).

Despite the attenuation of LDL-C lowering over time with bococizumab, the SPIRE-2 trial, which enrolled higher risk patients with LDL-C ≥100 mg/dL, showed a significant 21% reduction in major cardiovascular events over a median of 10 months illustrating the greater benefit of PCSK9 inhibition in higher-risk patients [50].

Evolocumab
Evolocumab is an immunoglobulin G 2 (IgG2)-isotype fully human mAb developed for use at doses of 140 mg SC Q2W or 420 mg SC monthly (QM), which have similar effects on LDL-C reduction [54,55]. It was approved by the U.S. Food and Drug Administration (FDA) in 2015 as an adjunct to diet and maximally tolerated statin therapy for the treatment of adults with HeFH or clinical ASCVD, who require additional lowering of LDL-C and as adjunct to diet and other LDL-lowering therapies (e.g., statins, ezetimibe, LDL apheresis) in patients with HoFH who require additional lowering of LDL-C. The effects of evolocumab in studies in patients with HeFH and HoFH are shown in Table 1.

Evolocumab was tested in 167 patients with HeFH diagnosed by the Simon Broome criteria with LDL-C ≥2.6 mmol/L (100 mg/dL) despite statin therapy with or without ezetimibe in the phase 2 Reduction of LDL-C with PCSK9 Inhibition in Heterozygous Familial Hypercholesterolemia Disorder (RUTHERFORD) study [56]. The HeFH patients were randomized to receive evolocumab 350 or 420 mg or placebo SC QM and at week 12. The mean±standard deviation (SD) LDL-C reduction was 42.7%±2.9% with 350 mg and 55.2%±2.9% with 420 mg evolocumab compared with an increase of 1.1%±2.9% with placebo ($P<0.001$ for both dose groups) (Table 1). There was some diminution in the LDL-C lowering effect between 2 and 4 weeks after giving evolocumab, even with the 420 mg dose; therefore, these changes at week 12 (i.e., 4 weeks after the last dose) may not be the maximum effect [57].

In the phase 3 RUTHERFORD-2 study, 331 eligible patients meeting the clinical criteria for HeFH and fasting LDL-C ≥2.6 mmol/L on stable lipid-lowering therapy for at least 4 weeks, were randomized to evolocumab, given SC either 140 mg Q2W or 420 mg QM, or a matching placebo [58]. The reduction in mean LDL-C with evolocumab compared to placebo at the mean of weeks 10 and 12 was 60.2% (95% confidence interval [CI], 54.5% to 65.8%) in 111 subjects with the 140 mg dose Q2W and 65.6% (95% CI, 59.8% to 71.3%) in 110 subjects with the 420 mg dose QM. The reductions in LDL-C tended to be greater at week 10 than at week 12 with the 420 mg QM dose. The drug was well tolerated.

In a small retrospective study of patients with hypercholesterolemia who were treated with evolocumab 140 mg SC Q2W, 32 patients with monogenic HeFH had similar reductions in LDL-C compared with seven patients with polygenic hypercholesterolemia (63.9%±16.0% and 67.7%±20.7%, respectively; no significant difference) [59].

In the recent Trial Assessing Efficacy, Safety and Tolerability of Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) Inhibition in Paediatric Subjects With Genetic Low-Density Lipo-

Table 1. Changes in LDL-C in Studies with Evolocumab in Patients with Familial Hypercholesterolemia

Study, duration, and reference	Patients, number and type	Treatment	Change in LDL-C, %[a]
RUTHERFORD, 12 weeks, Raal et al. (2012) [56]	56 HeFH	Placebo Q4W	1.1±2.9
	55 HeFH	E 350 mg Q4W	−42.7±2.9
	56 HeFH	E 420 mg Q4W	−55.2±2.9
RUTHERFORD-2, 12 weeks, Raal et al. (2015) [58]	54 HeFH	Placebo Q2W	−1.1 (−5.8 to 3.7)
	110 HeFH	E 140 mg Q2W	−61.2 (−64.6 to −57.9)
	55 HeFH	Placebo QM	2.3 (−2.5 to 7.1)
	110 HeFH	E 420 mg QM	−63.3 (−66.6 to −59.9)
HAUSER-RCT, 24 weeks, Santos et al. (2020) [60]	53 HeFH[b]	Placebo QM	−5.9 (−11.1 to −0.6)
	104 HeFH[b]	E 420 mg QM	−48.0 (−51.7 to −44.2)
TESLA, 12 weeks, Stein et al. (2013) [61]	8 HoFH	E 420 mg Q4W	−16.5±19.0[c]
	8 HoFH	E 420 mg Q2W	−13.9±27.2[c]
TESLA Part B, 12 weeks, Raal et al. (2015) [62]	16 HoFH	Placebo QM	7.9 (−2.7 to 18.5)
	33 HoFH	E 420 mg Q4W	−23.1 (30.7 to −15.4)
TAUSSIG, 12 weeks, Raal et al. (2017) [63]	106 HoFH	E 420 mg Q4W	−20.6±24.4[c]

LDL-C, low-density lipoprotein cholesterol; RUTHERFORD, Reduction of LDL-C with PCSK9 Inhibition in Heterozygous Familial Hypercholesterolemia Disorder; HeFH, heterozygous familial hypercholesterolemia; Q4W, every 4 weeks; E, evolocumab; Q2W, every 2 weeks; QM, every month; TESLA, The Trial Evaluating PCSK9 Antibody in Subjects With LDL Receptor Abnormalities; HoFH, homozygous familial hypercholesterolemia; TAUSSIG, Trial Assessing Long-Term Use of PCSK9 Inhibition in Subjects with Genetic LDL Disorders.
[a]Values are expressed as least-squares mean±standard error or (95% CI) unless indicated; [b]Pediatric heterozygous familial hypercholesterolemia; [c]Values are expressed as mean±standard deviation changes.

protein (LDL) Disorders (HAUSER-RCT), 157 pediatric patients aged 10 to 17 years with HeFH diagnosed by genetic testing or clinical diagnostic criteria with LDL-C ≥130 mg/dL (3.4 mmol/L) on stable lipid-lowering treatment for at least 4 weeks were treated with evolocumab 420 mg or placebo QM for 24 weeks [60]. The mean±SD age of the patients was 13.7±2.4 years and the placebo-corrected reduction in LDL-C at 24 weeks was 38.3%, or 68.6 mg/dL (1.8 mmol/L) in absolute terms. The effect was somewhat greater at 22 weeks than at 24 weeks.

Patients with HoFH with mutations in *LDLR* usually respond less well than those with HeFH to any treatment that involves the LDLR in the mechanism of action, such as statins and PCSK9 inhibitors. In the Trial Evaluating PCSK9 Antibody in Subjects With LDL Receptor Abnormalities (TESLA) pilot study, eight HoFH patients with *LDLR*-negative or *LDLR*-defective mutations on stable drug therapy were treated with open-label SC evolocumab 420 mg Q4W for at least 12 weeks, followed by 420 mg Q2W for an additional 12 weeks [61]. The two receptor-negative patients showed no reduction in LDL-C, whereas the receptor-defective patients showed mean±SD reductions in LDL-C of 19.3%±16% and 26.3%±20% with 4- and 2-week dosing, respectively, with a wide range of responses

(4% to 48% with 2-week dosing).

In the TESLA Part B phase 3 study, 50 eligible patients with HoFH who were on stable lipid-regulating therapy for at least 4 weeks and not receiving lipoprotein apheresis were randomized to treatment with SC evolocumab 420 mg or placebo Q4W for 12 weeks [62]. Of the 49 patients who received the study drug and completed the study, LDL-C was significantly reduced at 12 weeks with evolocumab by 30.9% (95% CI, 18.0% to 43.9%).

An interim subset analysis of the open-label phase 3 Trial Assessing Long-Term Use of PCSK9 Inhibition in Subjects with Genetic LDL Disorders (TAUSSIG) study involved 106 patients with HoFH, including 34 who were receiving apheresis at study entry, with a mean follow-up of 1.7 years [63]. The patients received evolocumab 420 mg SC QM, or 420 mg SC Q2W if on apheresis, and the dose could be increased for patients not on apheresis to Q2W after 12 weeks. The mean LDL-C level decreased from baseline at week 12 by 20.6%±24.4% (mean absolute decrease, 1.50±1.92 mmol/L) and at week 48 by 23.3%±30.8%. In patients not on apheresis, there was an additional mean reduction in LDL-C of 8.3%±13.0% (mean absolute decrease, 0.77±1.38 mmol/L) when dosing was increased from QM to Q2W.
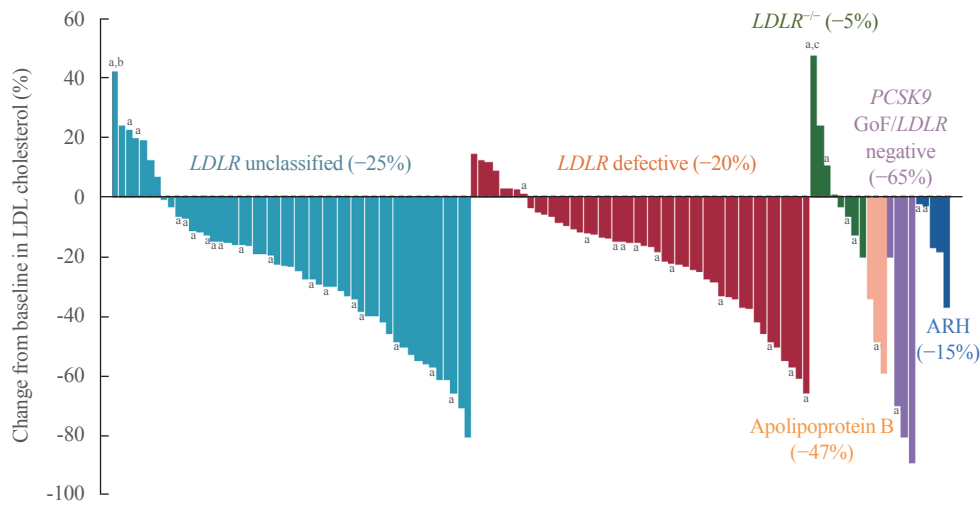
Fig. 2. Low-density lipoprotein cholesterol (LDL-C) change from baseline to week 12 by underlying genetic abnormality in the Trial Assessing Long-Term Use of PCSK9 Inhibition in Subjects with Genetic LDL Disorders (TAUSSIG) study with evolocumab added to conventional drug therapy in patients with homozygous familial hypercholesterolemia. Mean change in LDL-C is shown in parentheses after each genetic abnormality category. Adapted from Raal et al., with permission from Elsevier [63]. LDLR, low-density lipoprotein receptor; PCSK9, proprotein convertase subtilisin/kexin type 9; GoF, gain-of-function; ARH, autosomal recessive hypercholesterolemia. [a]Apheresis patient; [b]Patient missed apheresis before the week 12 blood draw due to a snowstorm; [c]Week 12 immediately after vacation, dietary indiscretion suspected.

There were considerable differences in responses between patients and according to mutation type and dosing regimen (Fig. 2). In the 47 receptor-defective patients not on apheresis, the mean±SD LDL-C reduction was 20.0%±19.6% at week 12 when they were being dosed QM, and 23.9%±21.9% at week 48, when they were up-titrated to dosing Q2W. The eight patients with *LDLR* negative mutations had a poor response, whereas the three patients with *APOB* mutations had a mean±SD 47.1%±12.2% reduction in LDL-C at week 12 and the four patients with a *PCSK9* GOF mutation combined with an *LDLR* mutation had a mean±SD 64.8%±31.0% reduction in LDL-C at week 12. The reductions in LDL-C did not significantly differ in patients on apheresis or not on apheresis, and some of those on apheresis were able to reduce the frequency of the procedure or even discontinue it.

In a final report from the TAUSSIG study, which included 106 patients with HoFH and 194 patients with severe HeFH on stable lipid-lowering therapy who received evolocumab for a median duration of 4.1 years at a dose of 420 mg QM or 420 mg Q2W, the mean±SD reduction in LDL-C from baseline to week 12 was 21.2%±25.0% in patients with HoFH and 54.9%± 17.4% in those with severe HeFH; this reduction was sustained over the period of the study [64]. In 48 patients with HoFH who were up-titrated from evolocumab 420 mg QM to 420 mg Q2W, the mean reduction in LDL-C increased from 19.6% at week 12

to 29.7% after 12 weeks of 420 mg Q2W, and 16 of the 61 patients receiving apheresis at enrollment were able to discontinue apheresis. Evolocumab was well tolerated and no patients developed neutralizing antibodies.

The Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk (FOURIER) trial examined cardiovascular outcomes with evolocumab in high-risk patients with established stable cardiovascular disease [65]. There was a significant reduction in the primary composite cardiovascular endpoint with a hazard ratio of 0.85 (95% CI, 0.79 to 0.92; $P<0.001$) for patients treated with evolocumab compared with placebo over a median duration of follow-up of 2.2 years.

In addition to reducing LDL-C, evolocumab decreases the levels of other atherogenic lipoproteins including Lp(a), which was reduced by median values of 24.7% and 21.7% with biweekly and QM doses of evolocumab, respectively, in an analysis of 10 clinical trials [66]. Changes in Lp(a) were quite variable, but were related to LDL-C reductions and appear to be mediated in part by increased LDLR uptake of Lp(a) particles.

Alirocumab

Alirocumab is a fully human mAb of the IgG1 isotype that binds with high affinity to PCSK9 [67,68]. It was approved by the FDA in 2015 as an adjunct to diet and maximally tolerated

Table 2. Changes in LDL-C in Studies with Alirocumab in Patients with Familial Hypercholesterolemia

Study, duration, and reference	Patients, number and type	Treatment	Change in LDL-C, %[a]
Phase 2 study, 12 weeks, Stein et al. (2012) [70]	15 HeFH	Placebo Q2W	−10.65±5.04
	15 HeFH	A 150 mg Q4W	−28.87±5.08
	16 HeFH	A 200 mg Q4W	−31.54±4.91
	15 HeFH	A 300 mg Q4W	−42.53±5.09
	16 HeFH	A 150 mg Q2W	−67.90±4.85
ODYSSEY FH I, 24 weeks; ODYSSEY FH II, 24 weeks, Kastelein et al. (2015) [72]	163 HeFH	Placebo Q2W	9.1±2.2
	322 HeFH	A 75/150 mg Q2W	−48.8±1.6
	55 HeFH	Placebo Q2W	2.8±2.8
	166 HeFH	A 75/150 mg Q2W	−48.7±1.9
ODYSSEY HIGH FH, 24 weeks, Ginsberg et al. (2016) [73]	35 HeFH	Placebo Q2W	−6.6±4.9
	71 HeFH	A 150 mg Q2W	−45.7±3.5
ODYSSEY KIDS, 8 weeks, Daniels et al. (2020) [79]	10 HeFH[b]	A 30/50 mg Q2W	−21.2±7.9
	10 HeFH[b]	A 40/75 mg Q2W	−46.1±8.3
	11 HeFH[b]	A 75/150 mg Q4W	−7.8±7.6
	11 HeFH[b]	A 150/300 mg Q4W	−44.5±7.6
ODYSSEY HoFH, 12 weeks, Blom et al. (2020) [80]	24 HoFH	Placebo Q2W	8.9±19.0
	45 HoFH	A 150 mg Q2W	−26.9±4.6

LDL-C, low-density lipoprotein cholesterol; HeFH, heterozygous familial hypercholesterolemia; Q2W, every 2 weeks; A, alirocumab; Q4W, every 4 weeks; ODYSSEY FH I, Efficacy and Safety of Alirocumab (SAR236553/REGN727) Versus Placebo on Top of Lipid-Modifying Therapy in Patients With Heterozygous Familial Hypercholesterolemia Not Adequately Controlled With Their Lipid-Modifying Therapy; ODYSSEY FH II, Study of Alirocumab (REGN727/SAR236553) in Patients With heFH (Heterozygous Familial Hypercholesterolemia) Who Are Not Adequately Controlled With Their LMT (Lipid-Modifying Therapy); ODYSSEY HIGH FH, Efficacy and Safety of Alirocumab (SAR236553/REGN727) Versus Placebo on Top of Lipid-Modifying Therapy in Patients With Heterozygous Familial Hypercholesterolemia; ODYSSEY KIDS, An 8-Week Dose-Finding Study to Evaluate the Efficacy and Safety of Alirocumab in Children and Adolescents With Heterozygous Familial Hypercholesterolemia; HoFH, homozygous familial hypercholesterolemia.

[a]Values are expressed as least-squares mean±standard error; [b]Pediatric heterozygous familial hypercholesterolemia.

statin therapy for the treatment of adults with HeFH or clinical ASCVD, who require additional lowering of LDL-C. The LDL-C responses to alirocumab in studies in patients with HeFH and HoFH are shown in Table 2.

A phase 1 study was conducted in patients with hypercholesterolemia, including some with HeFH [68]. The patients with HeFH and some of the subjects with non-FH were on atorvastatin. Doses of 50, 100, and 150 mg of alirocumab were given SC at day 1, 29, and 43. There was a dose-dependent maximum reduction in LDL-C seen at 8 to 15 days after the first dose, with mean reductions of 39.2%, 53.7%, and 61.0%, respectively, for the three doses. The response was similar in patients with and without concomitant atorvastatin treatment and in those with and without HeFH. The duration of LDL-C reduction was also dose-dependent and persisted longer in patients who were not taking atorvastatin. Those findings are consistent with statin treatment resulting in increased synthesis of PCSK9, which

would reduce the duration of the effect of alirocumab by increasing the target-mediated clearance of the antibody bound to PCSK9 [69].

In a phase 2 study in 77 patients with HeFH and LDL-C concentrations ≥2.6 mmol/L (100 mg/dL) who were on a stable statin dose, with or without additional ezetimibe, the patients were randomized to SC alirocumab 150, 200, or 300 mg Q4W alternating with placebo Q4W, or to alirocumab 150 mg or placebo Q2W, so they all received injections Q2W [70]. The mean reductions in LDL-C from baseline at week 12 for the different alirocumab doses were 28.9%, 31.5%, 42.5%, and 67.9%, respectively, compared with 10.7% with placebo. The reductions in LDL-C at 2 weeks after the doses given every 4 weeks were greater than those at 4 weeks, showing that the LDL-C reduction was not fully maintained for 4 weeks even with the 300 mg Q4W dose, whereas the 150 mg Q2W dose had a consistent effect that reached a maximum after 3 doses [70].

In a 3-year open-label treatment extension of the 12-week double-blind phase 2 study in HeFH patients, 58 patients received alirocumab 150 mg Q2W, and the mean±SD reduction in LDL-C was 65.4%±21.1% at week 24 and 56.0%±23.8% at week 148 [71]. Over the course of the study, 12 patients (20.7%) experienced a serious treatment-emergent adverse event and injection site reactions occurred in 21 (36.2%) patients; nonetheless, alirocumab was considered to be generally well-tolerated in the long term [71].

The ODYSSEY FH I and FH II trials were phase 3 trials in 486 and 249 patients with HeFH, respectively, who were not at the appropriate LDL-C target for primary or secondary prevention despite stable high-dose statin therapy with or without other lipid-lowering therapy [72]. As in the other ODYSSEY studies, the initial alirocumab dose was 75 mg Q2W and the dose was increased to 150 mg Q2W at week 12 if the LDL-C level was ≥ 1.8 mmol/L (70 mg/dL) at week 8. At week 24, LDL-C levels decreased relative to placebo by 57.9% and 51.4%, respectively, in the two studies (Fig. 3). These reductions were maintained through week 78. Adverse events that resulted in discontinuation of the study drug occurred with similar frequency with alirocumab and placebo. Injection site reactions were slightly more frequent in the alirocumab groups compared to placebo in FH I (12.4% vs. 11.0%) and FH II (11.4% vs. 7.4%), but these differences were not statistically significant.

The proportion of subjects with LDL-C <1.8 mmol/L at week 8 was 56.6% in FH I and 61.4% in FH II and the additional percentage reduction in LDL-C in subjects who had a dose increase was 15.1% in FH I and 16.9% in FH II. At week 24, an LDL-C level of <1.8 mmol/L was achieved with alirocumab by 59.8% of patients in FH I and 68.2% of patients in FH II [72].

In the ODYSSEY HIGH FH study, patients with HeFH and LDL-C ≥160 mg/dL despite the maximally tolerated statin dose with or without other lipid-lowering therapy were randomized to alirocumab 150 mg or placebo Q2W [73]. LDL-C was reduced by 39.1% compared to placebo at week 24 and 41% of patients treated with alirocumab were able to achieve predefined LDL-C goals, which were <70 mg/dL for very-high-risk patients and <100 mg/dL for high-risk patients. The mean±SD baseline LDL-C was 196.3±57.9 mg/dL in the alirocumab group and this was reduced to a mean±standard error (SE) level of 107.0±6.7 mg/dL at 24 weeks and the reductions were maintained to week 78. Injection-site reactions were more frequent in the alirocumab group (8.3%) than in the placebo group (5.7%). These were mostly mild in severity and did not result in study medication discontinuation.
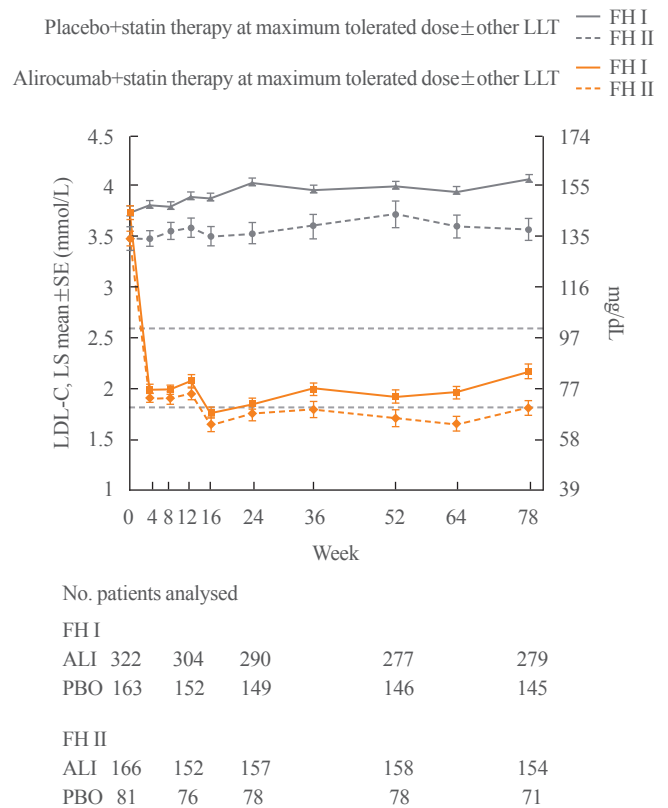
Fig. 3. Levels of calculated low-density lipoprotein cholesterol (LDL-C) over time (intention-to-treat analysis) in ODYSSEY FH I and FH II in patients with heterozygous familial hypercholesterolemia (FH) treated with alirocumab (ALI) or placebo (PBO). Adapted from Kastelein et al., with permission from Oxford University Press [72]. LLT, lipid-lowering therapy; LS, least squares; SE, standard error.

Some patients with HeFH were also included in the ODYSSEY LONG-TERM and ODYSSEY JAPAN phase 3 studies [74,75]. There were 27/144 and 14/72 patients with HeFH treated with alirocumab (75 mg Q2W) or with placebo, respectively, in the ODYSSEY JAPAN study [75]. The mean±SE reduction in LDL-C relative to placebo in the overall study was 64.1%± 2.2% at 24 weeks, and this reduction was maintained to week 52. The results for subjects with HeFH were not reported separately.

In the ODYSSEY LONG-TERM phase 3 study, 17.7% of patients had HeFH and they showed a similar response to alirocumab 150 mg Q2W as the overall trial patients, with a mean±SE reduction of 61.9%±1.3% in LDL-C at 24 weeks compared to placebo, and the effect remained consistent over a period of 78 weeks [74].

Of the patients with HeFH who completed the ODYSSEY LONG TERM study, 214 subsequently enrolled in the open-la-

bel extension (OLE) study, ODYSSEY OLE [76]. Following an 8-week washout period, patients were started on alirocumab 75 mg Q2W and the dose was increased to 150 mg Q2W from week 12 based on the physician's clinical judgment. The mean LDL-C reduction from the OLE baseline at week 96 was 46.8% for patients without an increase in the alirocumab dose and 55.4% for those with an increased dose. Consistent LDL-C reductions were maintained over a treatment period of up to 4 years including the 1.5 years of the original ODYSSEY LONG TERM trial.

In a *post hoc* analysis to examine whether age modified the efficacy and safety of alirocumab in patients with HeFH, pooled data from four phase 3 trials (ODYSSEY FH I, FH II, LONG TERM, and HIGH FH) were compared for the age subgroups of 18 to <45, 45 to <55, 55 to <65, and ≥65 years [77]. The mean LDL-C reductions were similar across age groups for the same alirocumab dose regimens, and treatment-emergent adverse events were similar in frequency in alirocumab- and placebo-treated patients in all age groups, with the exception that injection-site reactions were more common with alirocumab than with placebo, but declined in frequency with age.

In a multinational study that identified 164 HeFH patients with GOF mutations in PCSK9, there was a wide range of LDL-C levels and a high risk of premature ASCVD [78]. In 13 of these patients, a single dose of alirocumab 150 mg reduced LDL-C by a mean±SE of 53.7%±11.5% compared with placebo-treated patients at 2 weeks, and after all subjects received alirocumab 150 mg Q2W for 8 weeks, LDL-C was reduced by 73.3%±16.1% from baseline (*P*<0.0001).

The ODYSSEY KIDS study was a phase 2 dose-finding study in 42 pediatric HeFH patients aged 8 to 17 years with LDL-C ≥130 mg/dL (3.37 mmol/L) despite optimal statin treatment with or without other lipid-modifying therapies [79]. Alirocumab was given in various doses of 30 or 50 mg Q2W, 40 or 75 mg Q2W, 75 or 150 mg Q4W, and 150 or 300 mg Q4W chosen according to whether patients' body weight was <50 or ≥50 kg in four cohorts. The greatest reductions in LDL-C were mean±SE 46.1%±8.3% in the group receiving 40 or 75 mg Q2W and 44.5%±7.6% in the group receiving 150 or 300 mg Q4W.

Alirocumab 150 mg Q2W was tested in 69 adults with HoFH in the ODYSSEY HoFH trial [80]. Most patients were on high-intensity statin and ezetimibe, 10 patients were on lomitapide, and 10 patients were undergoing apheresis. The patients were randomized to alirocumab 150 mg or placebo Q2W for 12 weeks, and then all patients continued on open label alirocumab

150 mg Q2W for another 12 weeks. The mean baseline LDL-C level was 295.0 mg/dL in the alirocumab group and 259.6 mg/dL in the placebo group. The least squares mean difference in LDL-C from baseline after 12 weeks was -26.9% with alirocumab compared to 8.6% with placebo, resulting in a mean± SE reduction with alirocumab relative to placebo of 35.6%± 7.8%. The mean reduction in LDL-C from baseline to week 24, when all patients had received alirocumab was 27.3%, corresponding to an absolute reduction of 67.9 mg/dL. There was wide variation in LDL-C responses.

In a pooled analysis of eight ODYSSEY phase 3 clinical program trials in high cardiovascular risk populations with or without HeFH, alirocumab 150 mg Q2W reduced LDL-C by 60.4% compared with an increase of 0.5% with placebo [81]. There were also consistent reductions in apoB, non-high-density lipoprotein cholesterol, and Lp(a). Similar findings have been reported in other studies of alirocumab. In a pooled analysis of three double-blind phase 2 trials with alirocumab 150 mg Q2W, there was a 30% reduction in Lp(a) compared to placebo (*P*<0.0001) [82].

The Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome with Alirocumab (ODYSSEY Outcomes) trial randomized patients to placebo or to alirocumab at 1 to 12 months after hospitalization for acute coronary syndrome [83]. The 15% reduction in the primary composite CV endpoint was significant (*P*<0.001) and all-cause mortality was also reduced after a median follow-up of 2.8 years. Patients with baseline LDL-C >100 mg/dL (2.6 mmol/L) had a greater absolute benefit with alirocumab than those with lower values for the primary composite endpoint.

INCLISIRAN

Inclisiran is a long-acting small interfering RNA (siRNA) agent that prevents translation of *PCSK9* messenger RNA (mRNA), leading to decreased PCSK9 protein concentrations in hepatocytes and plasma. It was previously known as ALN-PCSsc, which was developed from ALN-PCS. It consists of two synthetic RNA strands conjugated to triantennary N-acetylgalactosamine (GalNAc), which is a ligand for the asialoglycoprotein receptor (ASGPR) expressed on hepatocytes, providing high-affinity binding with rapid and selective uptake of the compound into the liver. In the liver, it forms the RNA-inducing silencing complex (RISC), which prevents intracellular translation of *PCSK9* mRNA to protein (Fig. 1) [84].

Inclisiran is undergoing development in the ORION clinical

Table 3. Changes in LDL-C in Studies with Inclisiran in Patients with Familial Hypercholesterolemia

Study, duration, and reference	Patients, number and type	Treatment	Change in LDL-C, %
ORION-2, 180 days, Hovingh et al. (2020) [89]	4 HoFH	I 300 mg on days 1 and 90/104	−37.0 to 3.3
ORION-5, 24 months, (NCT03851705) [90]	56 HoFH randomized 2:1 to I or placebo	Placebo on days 1, 90 / I 300 mg on days 1, 90, 270, 450, 630	Awaited
ORION-9, 18 months, Raal et al. (2020) [73]	240 HeFH / 242 HeFH	Placebo on days 1, 90, 270, 450 / I 300 mg on days 1, 90, 270, 450	8.2 (4.3 to 12.2) / −39.7 (−43.7 to −35.7)

Values are expressed as range or mean (95% confidence interval).
LDL-C, low-density lipoprotein cholesterol; HoFH, homozygous familial hypercholesterolemia; I, inclisiran; HeFH, heterozygous familial hypercholesterolemia.

development program [85]. A phase 1 dose-finding study showed that SC doses of 300 mg or more can reduce LDL-C by more than 50% for at least 6 months [86]. The ORION-1 trial was a phase 2 study that examined the effects of different doses of inclisiran in subjects with high cardiovascular risk and elevated LDL-C despite maximally tolerated statin therapy, including 6% of subjects with FH [87]. Inclisiran produced dose-dependent reductions in plasma PCSK9 and LDL-C levels, and the 2-dose 300 mg regimen gave the greatest reduction in LDL-C. Injection-site reactions occurred in 5% of the subjects given inclisiran. A 1-year follow-up study of the ORION-1 trial showed that inclisiran produced durable reductions in LDL-C over 1 year [88]. The effects of inclisiran in patients with FH are shown in Table 3.

In the phase 2 ORION-2 Pilot study in four patients with HoFH and LDL-C >500 mg/dL, inclisiran 300 mg was given on day 1 and day 90 or 104 and the change in LDL-C at day 180 varied between patients from −37.0% to 3.3% [89]. The ORION-5 study (NCT03851705) is an ongoing phase 3 trial in 56 patients with HoFH who are being treated with inclisiran 300 mg or placebo on days 1 and 90 in Part 1, while in Part 2 all the study subjects will receive a dose of 300 mg inclisiran SC on day 270, day 450, and day 630 [90].

In ORION-9, 482 patients with HeFH received placebo or inclisiran 300 mg SC at baseline, 3 months later, and then every 6 months for a total of four doses [91]. At day 510, the mean reduction in LDL-C with inclisiran was 39.7% compared with an increase of 8.2% in the placebo group, giving a mean placebo-adjusted LDL-C reduction of 47.9% at day 510 and a time-averaged LDL-C reduction of 44.3% over the 18-month trial. In a single patient with a PCSK9 GOF variant, the placebo-adjusted LDL-C reduction was 89.7%.

The ORION-4 trial (NCT03705234) is an ongoing cardiovascular outcome trial to examine the effect of inclisiran on cardiovascular events in about 15,000 patients at very high cardiovascular risk over 5 years [92].

Overall, inclisiran and the mAbs that inhibit PCSK9 are very well-tolerated apart from a small number of injection-site reactions, which are generally mild. These treatments reduce LDL-C by more than 50% in HeFH patients when given in addition to treatment with statins and other lipid-lowering drugs and have a similar effect to that seen in subjects without FH. The reductions in LDL-C in HoFH patients are smaller and appear to depend on the type of mutation in the *LDLR* gene. PCSK9 inhibitors therefore represent a very useful therapy for the majority of patients with FH. Mendelian randomization studies suggested that genetic variants in PCSK9 associated with lower LDL-C levels were also associated with an increased risk of new-onset type 2 diabetes [93,94]. This has not been seen in clinical studies with the PCSK9 inhibitors, but is known to be a risk with statin therapy [95,96].

ADDITIONAL TREATMENTS

The PCSK9 inhibitors can bring a substantial number of patients with HeFH to goal in combination with high-intensity statin therapy and ezetimibe. However, very few patients with HoFH will reach the LDL-C target with the addition of PCSK9 inhibitors and other treatments are usually required. Currently, lomitapide and apheresis are the main options. Lomitapide is a microsomal triglyceride transfer protein inhibitor given orally [97]. It prevents the formation of apoB-containing lipoproteins in the intestines and liver. There are concerns over adverse effects, which were mainly gastrointestinal problems and hepatic steatosis, but recent reports show that in clincal practice it has been used in lower doses than in the phase 3 study, with reduc-

tions in LDL-C of more than 50% in many patients and less severe adverse events [98]. Data from the Lomitapide Observational Worldwide Evaluation Registry (LOWER) show that the median dose was 10 mg and the mean reduction in LDL-C was 33% overall, or 45% in patients remaining on lomitapide treatment [99].

Bempedoic acid, previously known as ETC-1002, is an inhibitor of ATP citrate lyase (ACL), which is an enzyme in the cholesterol synthesis pathway upstream of the statin target, 3-hydroxy-3-methylglutaryl-CoA reductase [100]. It was approved by the FDA for the treatment of adults with HeFH or established ASCVD who require additional lowering of LDL-C in February 2020 [101]. It is given in the form of an inactive prodrug that is activated selectively in the liver, so it is less likely to cause the skeletal muscle problems that may occur with statins [102].

Bempedoic acid produces additional reductions in LDL-C of about 18% to 20% when added to statins and has an additive effect when given with ezetimibe [103]. It may prove to be particularly useful in FH patients with statin intolerance. A cardiovascular outcome study with bempedoic acid, Cholesterol Lowering via BEmpedoic Acid, an ACL-inhibiting Regimen (CLEAR) Outcomes (NCT02993406), is ongoing [104].

Other promising treatments, which were origally developed to reduce triglycerides but may be particularly relavant for patients with HoFH, are those that target angiopoietin-like protein 3 (ANGPTL3), which inhibits hepatic and endothelial lipases. Evinacumab is a mAb directed against ANGPTL3. In a study in 65 patients with HoFH receiving stable lipid-lowering therapy, intravenous evinacumab 15 mg/kg Q4W for 24 weeks reduced LDL-C relative to placebo by 49.0% (95% CI, 33.1% to 65.0%; $P < 0.001$) with no major adverse effects [105].

Vupanorsen, or AKCEA-ANGPTL3-LRx, is a GalNAc-modified antisense oligonucleotide (ASO) that is more potent than an earlier non-GalNAc-modified ANGPTL3 ASO. An early study showed it reduced triglycerides in healthy subjects with and without elevated triglyceride levels when given SC once weekly [106]. Vupanorsen at a dose of 20 mg QW or higher doses Q4W was effective in reducing triglycerides and other atherogenic lipoproteins in patients with diabetes and hypertriglyceridemia without any major adverse effects [107]. The reduction in LDL-C was only about 7%, and HDL-C was also reduced.

ARO-ANG3 is a siRNA with a ligand for hepatic ASGPRs that is in development. Preliminary results showed that it reduced LDL-C by 23% to 37% in 17 patients with HeFH and was well tolerated [108]. Like inclisiran, ARO-ANG3 is likely

to have a longer duration of action than the ASOs and may therefore prove to be a useful addition in patients with HeFH and HoFH.

CONCLUSIONS

Patients with FH are at high or very high risk for ASCVD. Many patients with HeFH do not reach LDL-C targets with statins and ezetimibe, and most patients with HoFH do not reach LDL-C targets with currently available treatments including apheresis. Inhibition of PCSK9 with mAbs or siRNA can reduce LDL-C in patients with HeFH to a similar extent as in those without FH, by about 50% to 60%, and the effect has been durable over the period of the studies. The reduction in LDL-C is less in patients with HoFH (20% to 35% on average) and varies according to the type of mutation. PCSK9 inhibitors also reduce Lp(a), which may be an advantage in many patients with FH, and they have a good safety profile apart from a relatively low rate of mild injection-site reactions. Other treatments that can reduce LDL-C without involving the LDLR are needed for patients with HoFH, and ANGPTL3 inhibitors may prove to have an important role.

CONFLICTS OF INTEREST

Brian Tomlinson has acted as consultant or speaker for Amgen Inc., Kowa, and Merck Serono, for which he received honoraria. The other authors have no conflicts to disclose.

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