

Proprotein Convertase Subtilisin Kexin 9 Inhibitors



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KEYWORDS

- PCSK9 • Monoclonal antibodies • Evolocumab • Alirocumab • LDL-C • Hypercholesterolemia
- Cardiovascular disease • Proprotein convertase subtilisin kexin 9

KEY POINTS

- Low-density lipoprotein (LDL) is a major risk factor for cardiovascular disease.
- Patients at high or very high cardiovascular risk may benefit from large LDL-C reduction.
- Many patients often cannot achieve their LDL-C goals with the starting therapy.

INTRODUCTION

Low-density lipoprotein (LDL) is a major risk factor for cardiovascular disease (CVD), and a large number of epidemiologic studies have established the strong and direct relationship between high LDL-cholesterol (LDL-C) levels and coronary heart disease (CHD), and a wealth of clinical trials have shown that reducing LDL-C levels results in a reduced incidence of cardiovascular (CV) events. In fact, 12% reduction in all-cause mortality, 19% reduction in coronary mortality, and 17% reduction in any vascular cause of mortality per millimole per liter reduction in LDL-C have been observed,¹ and the higher the degree of reduction of LDL-C levels, the greater the benefit in terms of reduction of CV risk, as suggested by the comparison between more intensive and less intensive lipid-lowering therapies.² Thus, patients at high or very high CV risk may benefit from achieving the largest LDL-C reduction, as suggested by current guidelines,³ which need to be maintained over

time to gain a clinical benefit. However, many patients often cannot achieve their LDL-C goals with the starting therapy, which thus require adjustment based on the individual response to the lipid-lowering approach. Statins represent the first-line choice, and their efficacy in reducing CV morbidity and mortality in both primary and secondary prevention has been established in several clinical trials and meta-analyses.^{1,2,4–9}

However, despite the efficacy of statins, many patients do not reach their LDL-C level goals due to several reasons, including the occurrence of statin-related adverse events (mainly muscle-related disorders) leading to therapy discontinuation.¹⁰ In addition, a large proportion of patients with high or very high CV risk, including those with genetically determined forms of familial hypercholesterolemia (FH), does not achieve the recommended LDL-C level target even with maximally tolerated doses of drugs. Thus, there is the need of additional interventions that can efficiently reduce

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LDL-C levels below those achievable with the common cholesterol-lowering drugs.

PROPROTEIN CONVERTASE SUBTILISIN KEXIN 9

Proprotein convertase subtilisin kexin 9 (PCSK9) is a serine protease that plays a key role in the regulation of hepatic low-density lipoprotein receptor (LDLR) function, which represents the key regulator of cellular LDL uptake and plasma cholesterol levels (Fig. 1). Following the binding to LDL particles, the complex LDLR/LDL particle is internalized within the cell where it dissociates allowing receptor recycling and lysosomal degradation of LDL particle. When circulating PCSK9 binds to the epidermal growth factor-like repeat domain of LDLR, a conformational change of LDLR occurs, making it more vulnerable to degradation within lysosomes.¹¹ The binding PCSK9/LDLR thus results in a reduced LDLR surface expression, reduced LDL uptake, and increased plasma levels of LDL-C.

The relevance of PCSK9 as main regulator of plasmatc cholesterol levels derives from the observations that genetic variants of PCSK9 associated with loss or gain of function of this protein resulted in lower or higher levels of LDL-C, respectively.^{12,13} More importantly, an association with protection against cardiovascular disease (CVD) was observed in subjects carrying loss-of-function mutations in *PCSK9* gene,^{13–17} whereas gain-of-function mutations are associated with an increased risk of premature CVD.^{18–20} These findings suggested that PCSK9 may be a useful pharmacologic target for the control of hypercholesterolemia (Fig. 2) and led to the development

of 2 fully human monoclonal antibodies (mAbs) against circulating PCSK9, evolocumab and alirocumab, which are now approved for the treatment of hypercholesterolemia. The development of a third mAb against PCSK9 (bococizumab) was recently halted because of a high titer of antidrug antibodies, which may significantly attenuate the LDL-C-lowering effect.²¹ Another mAb to PCSK9, named LY3015014, the safety and efficacy of which have been so far tested in a phase 2 study, is still in development.²² Recently, other approaches are being developed, such as RNA interfering drugs; inclisiran is a long-acting RNA interference drug that produces a specific and sustained inhibition of hepatic PCSK9 synthesis. Finally, an approach for long-term LDL-C management through PCSK9-specific active vaccines has been evaluated in preclinical models.^{23–25}

It is worth noting that although PCSK9 targets mainly the LDLR in the liver, the protein is expressed also in extrahepatic tissues, including kidney, pancreas, and brain,²⁶ suggesting that pharmacologic inhibition of PCSK9 may lead to extrahepatic effects of PCSK9, which may raise concerns about this approach. To date, there is no evidence indicating a direct association between lipid-lowering therapy (LLT) with PCSK9 inhibitors with or without statins and the risk of cognitive disorders,²⁷ but specifically designed long-term clinical trials are awaited to clarify this aspect.

CLINICAL STUDIES ON EVOLOCUMAB AND ALIROCUMAB

Several clinical studies have evaluated the efficacy and safety of evolocumab and alirocumab in

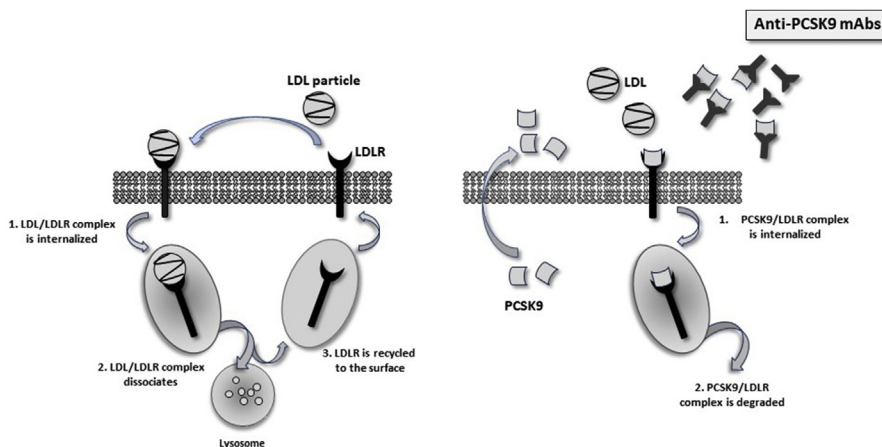


Fig. 1. Mechanism of action of PCSK9 and mAbs to PCSK9. In the absence of PCSK9, following the binding with LDL, LDLR is internalized and then recycled to the cell surface to restart the cycle. PCSK9 binds to LDLR and targets it to the degradation. MAbs to PCSK9 neutralize the circulating protein and block its binding to LDLR. (Modified from Pirillo A, Norata GD, Catapano AL. Advances in hypercholesterolemia. In: Chackalamannil S, editor. Comp Med Chem III. 3rd edition. Cambridge (MA): Elsevier; 2017. p. 669; with permission.)

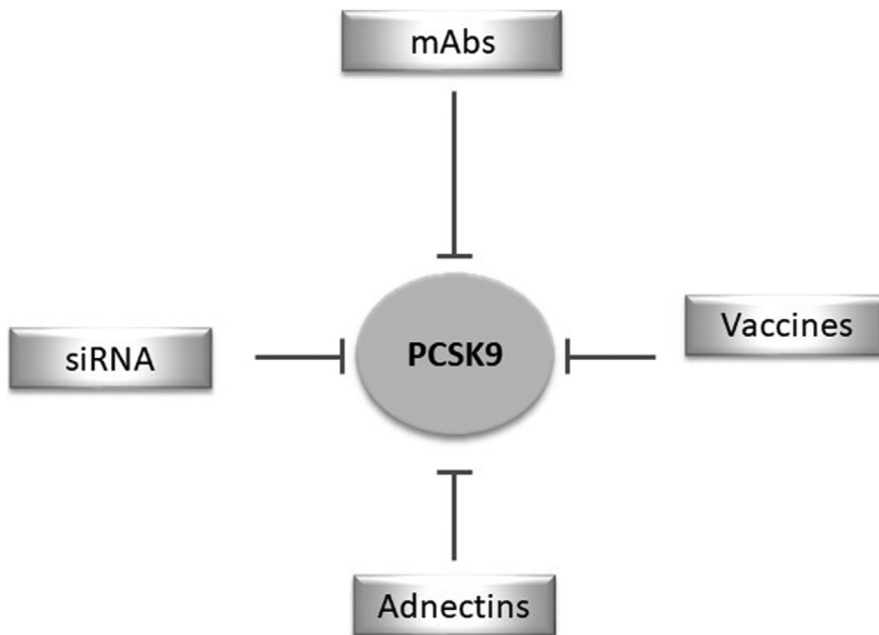


Fig. 2. Different approaches available or under current evaluation for the inhibition of PCSK9.

different groups of hypercholesterolemic patients with high CV risk; 2 meta-analyses have shown their safety and efficacy in reducing persistently LDL-C levels,^{28–30} which translates into a lower incidence of CV events.

Evolocumab

A large number of clinical trials have assessed the efficacy and safety of evolocumab in different groups of patients. Most of them were 12-week phase 2 and phase 3 trials in which participants showed significant reductions of LDL-C levels in the group treated with evolocumab compared with either placebo or ezetimibe^{31,32} (Table 1). Evolocumab was tested as monotherapy in the MENDEL studies, showing that in hypercholesterolemic patients (LDL-C ≥ 100 mg/dL [≥ 2.6 mmol/L], < 190 mg/dL [< 4.9 mmol/L]) the administration of evolocumab every 2 weeks or every 4 weeks for 3 months produced a significant reduction of LDL-C levels compared with either ezetimibe or placebo (see Table 1); other lipids and lipoproteins, including very low density lipoprotein cholesterol (VLDL-C) and lipoprotein(a) (Lp[a]), were also significantly reduced; adverse events were comparable among treatment groups.^{33,34}

Since then, evolocumab has been evaluated as add-on to background lipid-lowering therapies, mainly statins, in patients who did not reach their recommended LDL-C goal. The LAPLACE-TIMI 57 trial showed that in hypercholesterolemic patients taking a statin with or without ezetimibe

the administration of evolocumab at different doses for 12 weeks reduced LDL-C levels by up to 66% compared with placebo³⁵ (see Table 1). All doses of evolocumab were more likely than placebo to reduce LDL-C levels less than 70 mg/dL (1.8 mmol/L).³⁵ In the phase 3 LAPLACE-2 study, patients taking moderate- or high-intensity statin therapy were administered with evolocumab (140 mg every 2 weeks or 420 mg every 4 weeks), placebo, or ezetimibe for 12 weeks: a significant additional reduction of LDL-C was observed (see Table 1), and most patients (86%–94%) achieved LDL-C levels less than 70 mg/dL, compared with the group receiving ezetimibe (17%–62%).³⁶ Similar results were obtained when hyperlipidemic patients were treated with evolocumab added to a background LLT for 52 weeks (DESCARTES study): LDL-C levels were significantly reduced by 57% (see Table 1); no decrement in the efficacy of evolocumab was observed from week 12 to week 52 and was similar across all the background lipid-lowering therapies.³⁷ More patients in the evolocumab group experienced serious adverse events or events leading to drug discontinuation; however, the analysis of such events did not reveal a clear association with evolocumab use.³⁸ Recently, the GLAGOV study evaluated the effect of evolocumab in addition to statins in patients with angiographic coronary disease after 78 weeks.³⁹ Very low levels of LDL-C were observed in evolocumab-treated patients (mean value: 36.6 mg/dL), which associated with a decrease in percent atheroma volume (PAV) not

Table 1
Effect of evolocumab on low-density lipoprotein cholesterol levels and cardiovascular outcomes in randomized clinical trials

Clinical Trial (Duration)	Characteristics of Selected Patients	Background LLT	Evolocumab Dosing	LDL-C (% Change From Baseline)	Clinical Outcomes
MENDEL (12 wk) ³³	LDL-C ≥ 100 , <190 mg/dL	None	1. 70 mg, 105 mg, 140 mg q2w 2. 280 mg, 350 mg, 420 mg q4w 3. Eze 4. Placebo	1. -26.7% to -36.7% vs Eze; -37.3% to -47.2% vs placebo 2. -25.2% to -34.1% vs Eze; -43.6% to -52.5% vs placebo	
MENDEL-2 (12 wk) ³⁴	LDL-C ≥ 100 and <190 mg/dL	None	1. 140 mg q2w 2. 420 mg q4w 3. Eze 4. Placebo	1. -39.3% vs Eze; -57.4% vs placebo 2. -37.6% vs Eze; -54.8% vs placebo	
LAPLACE-TIMI 57 (12 wk) ³⁵	Fasting LDL-C ≥ 85 mg/dL	Statin $\pm$ Eze	1. 70, 105, or 140 mg q2w 2. 280, 350, or 420 mg q4w 3. Placebo	1. -41.8% to -66.1% vs placebo 2. -41.8% to -50.3% vs placebo	
LAPLACE-2 (12 wk) ³⁶	Primary hypercholesterolemia and mixed dyslipidemia	Atorva 10/80 mg $\pm$ Eze 10 mg; rosuva 5/40 mg; simva 40 mg	1. 140 mg q2w 2. 420 mg q4w 3. Eze 4. Placebo	1. -66% to -75% vs placebo; -39.6% to -47.2% vs Eze 2. -63% to -75% vs placebo; -38.9% to -41.1% vs Eze	
DESCARTES (52 wk) ³⁷	Fasting LDL-C ≥ 75 mg/dL	Diet; Diet + Atorva 10/80 mg; Diet + Atorva 80 mg + eze 10 mg	1. 420 mg q4w 2. Placebo	-57.0% vs placebo	
GLAGOV (78 wk) ³⁹	Fasting LDL-C ≥ 80 mg/dL	Statin	1. 420 mg q4w 2. Placebo	-61% vs placebo	Nominal change in PAV: -1.0%, $P < .001$
FOURIER (5 y) ⁴¹	CVD at high risk for a recurrent event LDL-C ≥ 70 mg/dL	Atorva 20 mg or equivalent	1. 140 mg q2w + statin 2. 420 mg q4w + statin 3. Placebo	-59% vs placebo	Time to CV death, MI, hospitalization for unstable angina, stroke, or coronary revascularization: HR 0.85 (0.79–0.92) $P < .001$

GAUSS (12 wk) ⁴²	Statin intolerant	Stable LLT therapy	1. 280, 350, or 420 mg q4w 2. 420 mg q4w + Eze 10 mg 3. Eze 10 mg	1. –26% to –35.9 vs Eze 2. –47.3% vs Eze	
GAUSS-2 (12 wk) ⁴³	Statin intolerant; subjects not at LDL-C goal	Not on statin or on low-dose statin	1. 140 mg q2w 2. 420 mg q4w 3. Eze 10	1. –38.1% vs Eze 2. –37.6% vs Eze	
GAUSS-3 (Part B: 24 wk; Part C: 2 y) ⁴⁴	Statin intolerant; subjects not at LDL-C goal	Stable LLT	Part A: atorva rechallenge Part B: evo q4w vs Eze Part C: evo q2w	Part B –37.8% vs Eze Part C <i>ONGOING</i>	
RUTHERFORD (12 wk) ⁴⁵	HeFH Fasting LDL-C ≥ 100 mg/dL	Statin $\pm$ ezetimibe	1. 350 mg q4w 2. 420 mg q4w 3. Placebo	1. –43.8% vs placebo 2. –56.4% vs placebo	
RUTHERFORD-2 (12 wk) ⁴⁶	HeFH Fasting LDL-C ≥ 100 mg/dL	Statin $\pm$ ezetimibe	1. 140 mg q2w 2. 420 mg q4w 3. Placebo	1. –59.2% vs placebo 2. –61.3% vs placebo	
TESLA part A (36 wk) ⁴⁷	HoFH; LDL-C ≥ 130 mg/dL	Stable LLT	1. 420 mg q4w for 24 we 2. +420 mg q2w for 12 we	1. –16.5% from baseline 2. –13.9% from baseline	
TESLA part B (12 wk) ⁴⁸	HoFH; LDL-C ≥ 130 mg/dL	Stable LLT	1. 420 mg q4w 2. Placebo	–30.9% vs placebo	
TAUSSIG (5 y) ⁴⁹	Severe FH	Stable LLT	q2w or q4w	<i>ONGOING (2020)</i> Interim analysis: –20.6% at week 12	
OSLER-1 and OSLER-2 (1 y) ⁵⁰	Subjects from phase 2 and 3 evolocumab trials		1. 420 mg q4w + standard therapy 2. Placebo	–61% vs placebo	HR: 0.47 (0.28–0.78), P = .003

Abbreviation: Eze, ezetimibe.

observed in patients receiving placebo (−0.95% with evolocumab vs +0.05% with placebo)³⁹ (see **Table 1**); a greater percentage of patients showed plaque regression with evolocumab than with placebo (64.3% and 47.3%, respectively).³⁹ This study showed for the first time that lowering LDL-C levels aggressively with a PCSK9 inhibitor as add-on to a background statin therapy may result in a reduction of atherosclerosis. The FOURIER trial confirmed and extended this finding, showing that adding evolocumab to a background lipid therapy (mainly statin) resulted in a reduction of CV events after a median follow-up of 2.2 years, with a hazard ratio (HR) of 0.85 (95% confidence interval [CI], 0.79–0.92) for the primary endpoint (including CV death, myocardial infarction [MI], stroke, hospitalization for unstable angina, or coronary revascularization) and 0.80 (95% CI, 0.73–0.88) for secondary endpoint (CV death, MI, or stroke)⁴⁰ (see **Table 1**). A prespecified analysis of this trial showed that evolocumab significantly reduced the incidence of CV events with similar efficacy in patients with and without diabetes, but the absolute risk reduction was higher in patients with diabetes⁴¹; evolocumab did not increase the risk of new-onset diabetes and did not worsen glycemia.⁴¹ A median of 30 mg/dL was reached following evolocumab treatment (median LDL-C at baseline: 92 mg/dL), and the reduction of LDL-C levels was maintained over time.⁴⁰ Interestingly, a 22% reduction of CV events was observed in the lowest quartile for baseline LDL-C level, in which patients reached an LDL-C level of 22 mg/dL⁴⁰; this finding, together with the observations from the GLAGOV, suggests that a CV benefit may be accrued even when LDL-C levels are reduced at levels well below those recommended by the current guidelines.³

Statin intolerance represents a major issue during the treatment of patients at high or very high CV risk; it is characterized by the occurrence of adverse events, mainly muscle-related events following therapy, which may lead to the discontinuation of the statin therapy and the increase of CV risk. The most effective drug used in alternative is ezetimibe, but given the relatively modest reduction achieved, it does not allow to reach the recommended LDL-C levels. Thus statin-intolerant patients, who need a very effective therapy to reduce their cholesterol levels, may significantly benefit from the therapy with anti-PCSK9. To address this question, evolocumab has been specifically tested in statin-intolerant patients in the GAUSS studies^{42–44} (see **Table 1**). As monotherapy, evolocumab dose dependently reduced LDL-C levels by 41% up to 63% compared with a 14.8% reduction achieved

with ezetimibe⁴²; more importantly, no signs of muscle-related adverse events were observed in evolocumab-treated patients.⁴² The association with ezetimibe further reduced LDL-C levels.⁴² A higher proportion of patients in the evolocumab group reached the LDL-C target less than 100 mg/dL; patients receiving the combination evolocumab + ezetimibe were more likely to reach the recommended LDL-C (90% for LDL-C <100 mg/dL and 62% for LDL-C <70 mg/dL).⁴² In the GAUSS-2 study, statin-intolerant patients were treated with evolocumab or ezetimibe for 12 weeks: evolocumab induced a significant reduction of LDL-C levels compared with ezetimibe (see **Table 1**), and more than 75% of patients reached an LDL-C level less than 100 mg/dL, whereas most of the patients treated with ezetimibe were unable to achieve LDL-C target levels.⁴³ The incidence of myalgia among these patients was low.⁴³ These findings have been confirmed by the GAUSS-3 clinical trial (see **Table 1**), which aimed at identifying patients with muscle symptoms confirmed by statin rechallenge and comparing the effect of evolocumab and ezetimibe.⁴⁴

Despite that commonly used cholesterol-lowering drugs have significantly improved the management of FH patients, particularly when used in combination, a large proportion of FH patients still does not achieve the recommended LDL-C level target even with maximally tolerated doses of drugs. These considerations directed the attention on the need of a new pharmacologic approach for the treatment of these high CV risk patients. Some drugs have been developed for these needs, including mipomersen and lomitapide, which reduce LDL-C levels by mechanisms that are independent of LDLR and thus are indicated specifically for homozygous FH (HoFH) patients who do not respond well to conventional lipid-lowering therapies. Two specific studies aimed at evaluating the effect of evolocumab in heterozygous FH (HeFH) patients^{45,46} (see **Table 1**). Evolocumab significantly reduced LDL-C levels in these patients at week 12 (43%–55%) when added to intensive statin therapy with or without ezetimibe.⁴⁵ A high percentage of patients treated with evolocumab reached either LDL-C less than 100 mg/dL (70% and 89%) or LDL-C less than 70 mg/dL (44% and 65%).⁴⁵ The lipid profile was generally improved, and a significant reduction of Lp(a), a CV risk factor whose levels are particularly elevated in FH patients, was observed.⁴⁵ The phase 3 RUTHERFORD-2 study, performed in HeFH patients on stable LLT, confirmed the high ability of evolocumab to reduce significantly LDL-C levels (~60%) independently

of the type of background LLT.⁴⁶ Interestingly, some patients recruited as HeFH were then reclassified as homozygotes; in these patients, the reduction of LDL-C induced by evolocumab treatment was comparable to those observed in heterozygotes, and much greater than those reported in other studies on HoFH, probably because of a residual receptor activity.⁴⁶ The pilot study TESLA part A, which recruited 8 HoFH patients (6 receptor-defective and 2 receptor negative) with a mean baseline LDL-C level of 440 mg/dL (11.4 mmol/L), showed that patients with defective LDLR activity had a significant reduction in their LDL-C levels following the treatment with evolocumab (~23%), whereas receptor-negative patients did not respond to this therapy.⁴⁷ This finding suggests that the mechanism by which evolocumab reduces LDL-C levels is through the upregulation of residual LDLR activity, but the large variation in the response of patients carrying the same mutation merits investigation, because it assumes that other factors may contribute to this observation. It is worth noting that the baseline PCSK9 levels of these patients was much higher than those reported in other cohorts of FH patients, which requires higher dose and more frequent administration of evolocumab to reduce PCSK9 levels at the same levels observed in other trials.⁴⁷ Another relevant finding of this study, that however needs to be verified in a larger population, is the similar reduction of Lp(a) levels observed in both receptor-defective and receptor-negative HoFH patients.⁴⁷ The TESLA part B trial reported similar results, with an overall 30.9% reduction of LDL-C levels compared with placebo (see [Table 1](#)); the intensity of the response was significantly correlated with the type of genetic defects, with receptor-negative patients not responding to the therapy and a maximal response (~40% reduction) in patients carrying 1 or 2 defective LDLR mutations.⁴⁸ The ongoing TAUSIG clinical trial will evaluate the long-term efficacy and safety of evolocumab in HoFH patients (completion date March, 2020). An interim subset analysis showed a 20.6% reduction of LDL-C levels, which is persistent at week 48 (see [Table 1](#)), and no differences were observed between patients with or without apheresis.⁴⁹

Patients who completed one of the described phase 2 or 3 studies were then enrolled in 2 open-label, randomized trials (OSLER-1 and OSLER-2); combined data from these 2 studies showed that evolocumab significantly reduced LDL-C levels by 61% compared with the standard LLT, and that patients on evolocumab had a significantly lower rate of CV events compared with patients on standard therapy (HR0.47; 95% CI, 0.28–0.78, $P = .003$)⁵⁰

(see [Table 1](#)), a finding that has been confirmed by the above discussed FOURIER trial.⁴⁰

Alirocumab

Alirocumab is a fully human mAb against PCSK9. Hypercholesterolemic patients on stable atorvastatin therapy administered with alirocumab showed a further reduction of LDL-C (40% up to 72%); the reductions were dose dependent and dose regimen dependent, with the highest efficacy observed when alirocumab was given every 2 weeks⁵¹ ([Table 2](#)). Almost all patients reached the LDL-C level less than 100 mg/dL, and a great proportion reached the level less than 70 mg/dL.⁵¹ Lp(a) levels significantly decreased in all tested conditions⁵¹; total cholesterol, non-high-density lipoprotein cholesterol (non-HDL-C), and apolipoprotein B (apoB) were also significantly reduced.⁵¹ Similar observations were reported in other 2 studies. A significant decrease of LDL-C levels was observed when alirocumab was added to atorvastatin 10 mg or 80 mg compared with atorvastatin 80 mg alone (–66.2%, –73.2%, and –17.3%, respectively)⁵² (see [Table 2](#)); all patients on alirocumab reached the LDL-C level target less than 100 mg/dL, and more than 90% had an LDL-C level less than 70 mg/dL.⁵² Alirocumab was effective in reducing LDL-C levels also in a population of young HeFH patients with a background treatment of low to moderate dose of atorvastatin: reductions ranged from 28.9% with alirocumab 150 mg every 4 weeks up to 67.90% with 150 mg every 2 weeks, compared with a reduction of 10.65% with placebo⁵³ (see [Table 2](#)). The patients who completed this study and were receiving stable statin + ezetimibe therapy entered the open-label extension during which they received alirocumab 150 mg every 2 weeks; after 3 years, sustained LDL-C reductions were observed (~60%), without specific safety signals, including in those patients who achieved very low levels of LDL-C (<25 mg/dL).⁵⁴

The ODYSSEY program, which included 14 phase 3 trials on alirocumab, aimed at evaluating the efficacy and safety of alirocumab alone or in combination with other lipid-lowering therapies in different groups of hypercholesterolemic patients (see [Table 2](#)). Most of these trials used a dosage of 75 mg every 2 weeks uptitrated to 150 every 2 weeks if LDL-C target is not reached after 8 weeks. The ODYSSEY COMBO studies (I and II) have evaluated the efficacy and safety of alirocumab in high CV risk patients with suboptimal levels of LDL-C at baseline despite on maximal tolerated dose of statin, with or without other lipid-lowering drugs (see [Table 2](#)). The

Table 2
Effect of alirocumab on low-density lipoprotein cholesterol levels and cardiovascular outcomes in randomized clinical trials

Clinical Trial (Duration)	Characteristics of Selected Patients	Background LLT	Alirocumab Dosing	LDL-C Change	CV Outcomes
DFI11565 (12 wk) ⁵¹	LDL-C ≥ 100 mg/dL	Atorva 10, 20, or 40 mg	1. 50, 100, 150 mg q2w 2. 200, 300 mg q4w 3. Placebo	1. -39.6% to -72.4% 2. -43.2%, -47.7% 3. -5.1%	
DFI11566 (8 wk) ⁵²	LDL-C ≥ 100 mg/dL	Atorva 10 mg	1. 150 mg q2w + atorva 10 mg 2. 150 mg q2w + atorva 80 mg 3. Atorva 80 mg	1. -66.2% 2. -73.2% 3. -17.3%	
R727-CL-1003 (12 wk) ⁵³	HeFH; LDL-C ≥ 100 mg/dL	Diet + statin	1. 150 mg q2w 2. 150 mg, 200 mg, 300 mg q4w 3. Placebo	1. -57.2% vs placebo 2. -18.2% to -31.9% vs placebo	
ODYSSEY COMBO I (52 wk) ⁵⁵	Established CHD or CHD equivalent, LDL-C ≥ 70 mg/dL	Maximum tolerated statin $\pm$ other LLT	1. 75 mg q2w (increased at 150 mg q2w if LDL-C ≥ 70 mg/dL at week 8) 2. Placebo	-45.9% vs placebo	
ODYSSEY COMBO II (52 wk) ⁵⁶	Established CHD or CHD equivalent, LDL-C ≥ 70 mg/dL with maximally tolerate dose of statin	High-intensity statins	1. 75 mg q2w (increased at 150 mg q2w if LDL-C ≥ 70 mg/dL at week 8) 2. Eze 10 mg	-29.8% vs Eze	
ODYSSEY OPTIONS I (24 wk) ⁵⁸	Very high CVD risk, LDL-C ≥ 70 mg/dL; high CVD risk and LDL-C ≥ 100 mg/dL	Atorva 20/40 mg	1. 75 mg q2w 2. Eze 10 mg 3. Doubling atorva dose 4. Atorva 40 mg $\rightarrow$ Rosuva 40 mg	Entry statin: Atorva 20 Atorva 40 1. -44.1% -54% 2. -20.5% -22.6% 3. -5% -4.8% 4. — -21.4%	
ODYSSEY OPTIONS II (24 wk) ⁵⁹	Very high CVD risk, LDL-C ≥ 70 mg/dL; high CVD risk and LDL-C ≥ 100 mg/dL	Rosuva 10/20 mg	1. 75 mg q2w 2. Eze 10 mg 3. Doubling rosuva dose	Entry statin: Rosuva 10 Rosuva 20 1. -50.6% -36.3% 2. -14.4% -11.0% 3. -16.3% -15.9%	

ODYSSEY ALTERNATIVE (24 wk) ⁶⁰	Moderate or high CV risk with statin intolerance, LDL-C ≥ 100 or ≥ 70 mg/dL	Non-statin LLT	1. 75 mg q2w 2. Eze 10 mg	–30.4% vs Eze	
ODYSSEY MONO (24 wk) ⁶¹	LDL-C ≥ 100 mg/dL and < 190 mg/dL	None	1. 75 mg q2w 2. Eze 10 mg	–31.6% vs Eze	
ODYSSEY LONG TERM (78 wk) ⁶²	HeFH or established CHD or CHD equivalent LDL-C ≥ 70 mg/dL	Maximum tolerated statin $\pm$ other LLT	1. 150 mg q2w 2. Placebo	–61.9% vs placebo	
ODYSSEY OUTCOMES (64 mo) ⁶³	Recent acute coronary syndrome, LDL-C ≥ 70 mg/dL	Atorva 40/80 mg or Rosuva 20/40 mg or the maximum tolerated dose	1. 75 mg q2w 2. 150 mg q2w 3. Placebo	ONGOING (2017)	Time from randomization to first occurrence of CHD death, nonfatal MI, stroke, or UA requiring hospitalization
ODYSSEY FH I and FH II (78 wk) ⁶⁴	HeFH; LDL-C ≥ 100 mg/dL (for primary prevention) or LDL-C ≥ 70 mg/dL (for secondary prevention)	Maximum tolerated statin $\pm$ other LLT	1. 75 mg q2w (increased at 150 mg q2w if LDL-C ≥ 70 mg/dL at week 8) 2. Placebo	FH I: –57.9% vs placebo FH II: –51.4% vs placebo	
ODYSSEY JAPAN (52 wk) ⁶⁵	HeFH or non-FH at high CV risk not at target	Stable statin therapy	1. 75 mg q2w (increased at 150 mg q2w if LDL-C ≥ 70 mg/dL at week 8) 2. Placebo	–58.9% vs placebo	
ODYSSEY HIGH FH (78 wk) ⁶⁶	HeFH LDL-C ≥ 160	Stable LLT	1. 150 mg q2w 2. Placebo	–39.1% vs placebo	
ODYSSEY ESCAPE (18 wk) ⁶⁷	HeFH undergoing apheresis	Stable LLT	1. 150 mg q2w 2. Placebo	–46.4% vs placebo	
ODYSSEY OLE (176 wk) ⁶⁸	HeFH who have completed one of the 4 parent studies			ONGOING (2017) Preliminary data at week 48: –46.9%	
CHOICE I (48 wk) ⁶⁹	Hypercholesterolemic at moderate to very high CV risk	$\pm$ Statin $\pm$ other LLT	1. 300 mg q4w 2. 75 mg q2w 3. Placebo	1. –52.4% vs placebo (no statin); –58.7 vs placebo (statin) 2. –49.8% vs placebo (no statin); –51.4% vs placebo (statin)	
CHOICE II (24 wk) ⁷¹	Inadequately controlled hypercholesterolemia	No statin Fenofibrate, ezetimibe, or diet	1. 150 mg q4w 2. 75 mg q2w 3. Placebo	1. –56.4% vs placebo 2. –58.2% vs placebo	

COMBO I study showed that alirocumab treatment induced a greater reduction of LDL-C levels (-48.2% at week 24, compared with -2.3% with placebo); in addition, a greater proportion of patients (77.5% on-treatment) reached the recommended LDL-C level less than 70 mg/dL.⁵⁵ The COMBO II study showed a greater efficacy of alirocumab in reducing LDL-C levels compared with ezetimibe in the 104-week treatment period, and many more patients achieved LDL-C goals.⁵⁶

The ODYSSEY OPTIONS studies were designed to evaluate the efficacy and safety of alirocumab in patients at high CV risk with LDL-C levels not adequately controlled⁵⁷ (see [Table 2](#)). The ODYSSEY OPTIONS I trial recruited patients with very high CVD risk and LDL-C ≥ 70 mg/dL (≥ 1.8 mmol/L) or high CVD risk and LDL-C ≥ 100 mg/dL (≥ 2.6 mmol/L); these patients were randomized to one of the following treatments: (1) alirocumab 75 mg every 2 weeks (switched to 150 mg every 2 weeks if LDL-C target was not achieved after 12 weeks) added to atorvastatin 20 or 40 mg; (2) ezetimibe 10 mg added to atorvastatin; (3) double atorvastatin dose; (4) switch from atorvastatin 40 mg to rosuvastatin 40 mg.⁵⁸ The greatest reductions of LDL-C levels were observed in patients treated with alirocumab as add-on (-44.1% and -54.0% with atorvastatin 20 or 40 mg), whereas the addition of ezetimibe reduced LDL-C levels by 20.5% and 22.6% , respectively, similarly to the reduction observed following the switch from atorvastatin to rosuvastatin; doubling the atorvastatin dose resulted in additional reduction of 5.0% and 4.8% compared with the baseline⁵⁸ (see [Table 2](#)). Patients treated with alirocumab were more likely to achieve the recommended LDL-C target; alirocumab also induced a higher reduction of apoB, non-HDL-C, and Lp(a) levels compared with all other treatments.⁵⁸ Similarly, the ODYSSEY OPTIONS II showed the higher efficacy of adding alirocumab to a background of rosuvastatin 10 or 20 mg in the same type of patients⁵⁹: LDL-C levels were reduced by -50.6% and -36.3% , respectively, whereas adding ezetimibe or doubling rosuvastatin dose was less effective (-14.4% and -11.0% with ezetimibe; -16.3% and -15.9% doubling rosuvastatin)⁵⁹ (see [Table 2](#)). Altogether, these results suggest that high CV risk patients with not controlled LDL-C levels despite on maximal tolerated LLT may significantly benefit from the addition of alirocumab to their therapy.

As for evolocumab, a specific trial has addressed the efficacy and safety of alirocumab in statin-intolerant patients, with a statin rechallenge arm. The ODYSSEY ALTERNATIVE trial showed that alirocumab was superior to ezetimibe

in reducing LDL-C levels in patients intolerant to statins, with reduction of -45% and -14.6% from baseline, respectively, at week 24 ($P < .0001$) (see [Table 2](#)); following the treatment with alirocumab, patients were more likely to achieve the recommended LDL-C levels (41.9% vs 4.4% with ezetimibe).⁶⁰ Myalgia was the most common adverse event reported in all groups, but alirocumab treatment was associated with the lowest rate of muscle-related adverse events.⁶⁰ The higher efficacy of alirocumab compared with ezetimibe has been reported also by the ODYSSEY MONO trial, conducted in patients on no LLT⁶¹ (see [Table 2](#)).

To evaluate the long-term efficacy and safety of alirocumab, 2 studies have been designed to specifically address these questions. The ODYSSEY LONG TERM, conducted in a population of high CV risk patients with LDL-C levels ≥ 70 mg/dL while receiving the maximal tolerated dose of statin, showed that addition of alirocumab produced a further -62% reduction of LDL-C levels after 24 weeks⁶² (see [Table 2](#)); these reductions, which were maintained during the course of the study, did not differ between HeFH and non-HeFH patients.⁶² Interestingly, a high percentage of patients (37.1%) reached very low levels of LDL-C (< 25 mg/dL), but the rate of adverse events in these patients was not increased.⁶² A post hoc analysis reported a lower rate of major adverse CV events in the alirocumab group than in the placebo group (1.7% vs 3.3% , HR 0.52, nominal $P = .02$), with cumulative probability of event curves tending to diverge over time.⁶² The ongoing ODYSSEY OUTCOMES is evaluating the effect of adding alirocumab or placebo to the current LLT in patients with a recent acute coronary syndrome; the primary outcome is the time from randomization to first occurrence of a clinical CV event, including CHD death, MI, fatal and nonfatal ischemic stroke, and unstable angina requiring hospitalization.⁶³ It is expected to be completed at the end of 2017.

The ODYSSEY program also included clinical trials evaluating the effect of alirocumab in HeFH patients with inadequate LDL-C level control despite maximal tolerated dose of LLT (see [Table 2](#)). In the ODYSSEY FH I and FH II studies, patients (mean LDL-C levels 144.7 mg/dL) received alirocumab 75 mg every 2 weeks uptitrated to 150 mg if LDL-C was ≥ 70 mg/dL (≥ 1.8 mmol/L) at week 8; LDL-C levels significantly decreased by 57.9% (FH I) and 51.4% (FH II) versus placebo. These reductions were maintained up to week 78, and most alirocumab-receiving patients reached LDL-C levels less than 70 mg/dL.⁶⁴ Alirocumab also reduced apoB, non-HDL-C, and Lp(a).⁶⁴ The ODYSSEY JAPAN

trial, which included 41 HeFH patients, reported similar results.⁶⁵ In the ODYSSEY HIGH FH trial, HeFH patients with LDL-C ≥ 160 mg/dL (≥ 4.1 mmol/L) despite maximally tolerated dose of statin with or without other lipid-lowering drugs were treated with alirocumab 150 mg every 2 weeks or placebo for 78 weeks.⁶⁶ At week 24, in patients treated with alirocumab, LDL-C levels were reduced by -45.7% compared with -6.6% with placebo.⁶⁶ This reduction was comparable with that observed in a subgroup of patients with HeFH with high baseline LDL-C levels in the ODYSSEY LONG TERM trial, which reported a -52.2% with alirocumab and -8.1% with placebo.⁶² The ODYSSEY ESCAPE trial showed that, in HeFH patients undergoing regular lipoprotein apheresis, the addition of alirocumab to their LLT led to the discontinuation of apheresis in 63.4% of patients, whereas in 29.3% of patients the standardized rate was reduced by at least 50%,⁶⁷ which suggests that these specific subgroups of HeFH patients may significantly benefit from an anti-PCSK9 therapy. The ODYSSEY OLE is an open-label extension study of 4 phase 3 studies (FH I, FH II, LONG TERM, HIGH FH) that is evaluating the long-term (176 weeks) efficacy and safety of alirocumab in patients with HeFH. Preliminary data show a mean reduction in LDL-C levels of 46.9% at week 48 compared with baseline; significant reductions are observed also in other parameters, including non-HDL-C, Lp(a), and apoB.⁶⁸

Recently, alirocumab has been approved as a once-monthly dosing option, based on the results of the ODYSSEY CHOICE I clinical trial, which compared the well-established 75 mg every 2 weeks alirocumab dosing with 300 mg every 4 weeks as a monotherapy or add-on to statin therapy in patients with moderate to very high CV risk, with a 48-week follow-up⁶⁹ (see [Table 2](#)). The monthly dosing was as effective in reducing LDL-C levels as the every 2 weeks dosing; the reductions were significant both in patients on statin therapy and in patients not receiving statins.⁶⁹ The analysis of a subgroup of patients with atherosclerotic CVD showed a significant reduction of LDL-C levels with alirocumab 300 mg every 4 weeks both in patients on statin (-64.2%) and in the group not receiving statin (-56.8%)⁷⁰; the reductions were similar to that observed in the whole population of the study.⁶⁹ The most relevant finding of this study is that the variations of LDL-C week by week are small, and at week 48, a large proportion of patients achieved the recommended LDL-C levels following the treatment with alirocumab. Alirocumab has been tested also as a 150-mg dose every 4 weeks in the ODYSSEY CHOICE II clinical

trial, conducted in patients with inadequately controlled hypercholesterolemia and not on statin therapy because of muscle-related adverse events⁷¹ (see [Table 2](#)). The LDL-C level reductions obtained with this dosing regimen did not differ from that observed with the reference dose (-51.7% vs -53.5% vs baseline with 75 mg every 2 weeks); some patients required a dose adjustment at week 12, which was associated with higher LDL-C levels at baseline.⁷¹ About 90% of the patients included in this trial had muscle-related adverse events with statins, leading to therapy discontinuation, but during the treatment with alirocumab, the rate of these events was low.⁷¹ All these observations represent a relevant step in the development of these drugs; in fact, reducing the timing of injection maintaining the effect on LDL-C levels may result in an increased adherence of the patients to the therapy, but can also have a positive effect on the costs of the therapy.

OTHER MONOCLONAL ANTIBODIES TO PROTEIN CONVERTASE SUBTILISIN KEXIN 9

Bococizumab

Bococizumab is a humanized mAb targeting PCSK9; because it contains a 3% murine sequence, the development of anti-drug antibodies may occur. Two randomized clinical trials have recently shown that bococizumab treatment did not reduce major adverse CV events in low-risk patients, whereas a benefit was reported for higher risk patients, despite similar LDL-C level reductions; the combined analysis of these 2 trials did not report benefit with respect to the primary end point.⁷² In the SPIRE lipid-lowering program, which included several trials on bococizumab, high-titer anti-drug antibodies developed in a high percentage of patients receiving bococizumab after week 12, which markedly reduced the magnitude and durability of LDL-C lowering ($\sim 50\%$).²¹ In addition, a high variability in the LDL-C lowering was observed among patients with no anti-drug antibodies, a variation that was present as early as 12 weeks, and thus before the detection of anti-drug antibodies.²¹ Bococizumab immunogenicity seems also to increase the rate of adverse events, such as injection-site reactions, that were higher than those previously reported with the other anti-PCSK9 mAbs.²¹ Following these observations, the development of bococizumab has been discontinued.

LY3015014

PCSK9 secreted from cells is formed by a 14-kDa prodomain associated noncovalently with a

60-kDa mature domain; in addition to this form, serum contains also a truncated form of PCSK9 (representing up to 40% of total circulating PCSK9) in which the N terminus of the catalytic domain is truncated by 7 to 8 kDa following the activity of furin. This truncated form seems to be inactive at LDLR degradation.^{73,74} This observation can have a specific relevance when using mAbs, because if the antibody binds to both intact and truncated forms, it may be consumed unproductively. LY3015014 antibody binds to intact but not to truncated form of PCSK9, thus blocking its interaction with LDLR but allowing the normal proteolytic cleavage of PCSK9 and limiting its accumulation.⁷⁵ LY3015014 has been tested in hypercholesterolemic patients as add-on to their background lipid-lowering therapy in a phase 2 randomized clinical trial.²² A dose-dependent significant and durable reduction of LDL-C levels was reported (−14.9% up to −50.5% for every 4 weeks and −14.9% to −37.1% for every 8 weeks dosing).²² LY3015014 also reduced significantly other lipid parameters, including non-HDL-C, apoB, and Lp(a).²² The development of LY3015014 has been discontinued because of lower reductions of LDL-C induced compared with approved PCSK9 mAbs.

NEW APPROACHES FOR THE INHIBITION OF PROPROTEIN CONVERTASE SUBTILISIN KEXIN 9

Adnectins

Adnectins are a new family of therapeutic proteins based on the 10th fibronectin type III domain, designed to bind with high affinity and specificity to therapeutic targets, which may translate into a higher pharmacologic activity and increased therapeutic efficacy.⁷⁶ BMS-962476 targets circulating PCSK9; in hypercholesterolemic mice overexpressing human PCSK9, it rapidly reduces cholesterol and free PCSK9 levels, and the treatment of cynomolgus monkeys suppressed PCSK9 by more than 99% and LDL-C levels by ~55%.⁷⁷ BMS-962476 was well tolerated in healthy subjects on diet and LDL-C greater than 130 mg/dL or statins and LDL-C greater than 100 mg/dL; in these subjects, it produced reductions of 48% in LDL-C and greater than 90% in PCSK9 levels at maximal dose.⁷⁸ Also, the development of this molecule has been discontinued.⁷⁹

Inclisiran

A more recent approach for the inhibition of PCSK9 is through a biological process referred to as RNA interference; this approach uses a small interfering

RNA (siRNA), which induces the degradation of specific mRNA, resulting in the suppression of the corresponding protein synthesis. Thus, preclinical studies have shown that the treatment with PCSK9-specific siRNA results in the reduction of PCSK9 and LDL-C plasma levels.⁸⁰ In healthy volunteers with serum LDL-C levels ≥ 116 mg/dL (≥ 3.00 mmol/L), a single intravenous dose of ALN-PCS, a siRNA that inhibits PCSK9 synthesis in a lipid nanoparticle formulation, resulted in a mean 70% reduction in circulating PCSK9 plasma levels and a 40% reduction of LDL-C.⁸¹

Inclisiran (ALN-PCSsc) is a long-acting synthetic siRNA against PCSK9 that is conjugated to triantennary N-acetylgalactosamine carbohydrates, which bind to the asialoglycoprotein receptors abundantly expressed in the liver, thus resulting in a specific uptake of inclisiran into hepatocytes. In a phase 1 trial, inclisiran has been tested in healthy volunteers with LDL-C ≥ 100 mg/dL (2.6 mmol/L) in either a single ascending dose (25–800 mg) or multiple dose⁸²; at day 84, in the single-dose phase, a dose of 300 mg or higher significantly reduced PCSK9 levels (up to a least-squares mean reduction of 74.5%), which corresponded to a reduction of LDL-C levels by ~50%.⁸² All multiple dose regimens massively reduced PCSK9 levels (up to 83.8%) and LDL-C levels (up to 59.7%).⁸² No serious adverse events were reported.⁸²

Inclisiran has been tested in a phase 2 trial (ORION-1) in patients at high CV risk with high LDL-C levels.⁸³ Patients were randomly assigned to receive a single dose of placebo or 200, 300, or 500 mg of inclisiran or 2 doses (at days 1 and 90) of placebo or 100, 200, or 300 mg of inclisiran; the greatest reduction in LDL-C levels (52.6%) was observed in patients who received the 2-dose 300-mg regimen of inclisiran (first injection at day 1, second injection at day 90).⁸³ LDL-C levels were reduced in every patient enrolled in the trial.⁸³ In terms of safety, during the 210 days of exposure to inclisiran, the rate of adverse events was similar in inclisiran and placebo group, and also injection-site reactions were uncommon and similar to those reported with mAbs.⁸³ So far, symptoms of immune activation after exposure to inclisiran were rare, but longer trials are warranted to evaluate the safety of a long-term exposure to this drug as well as to establish a long-term duration of the effect.

Anti-Proprotein Convertase Subtilisin Kexin 9 Vaccines

Active vaccination represents one of the recent approaches that are currently under investigation

for cholesterol management and prevention of CVD. The goal of this approach is to induce a therapeutic response similar to that induced by the administration of mAbs, but with reduced interventions and avoiding the possibility of the formation of anti-drug antibodies, which may limit the pharmacologic effect. To this end, a peptide-based anti-PCSK9 active vaccination has been evaluated in preclinical models²³: vaccines induced the generation of high-affine antibodies specific for PCSK9, resulting in the reduction of LDL-C up to 50% in treated animals.²³ The humoral immune response induced by vaccine persisted for 1 year, and LDL-C level reduction persisted for the entire period of the study.²³ Similar results were reported in another study that evaluated the effect of vaccination with various PCSK9 peptides in mice, and reported a consistent effect on LDL-C levels, that were even lower in vaccinated animals treated with statins.²⁴ The vaccine against PCSK9, besides the effect on LDL-C levels, also reduced plasma inflammatory markers and decreased significantly the atherosclerotic lesion area (–64%) and aortic inflammation in mice.²⁵ One main problem of this type of approach might be the high variability in the antibody response, thus suggesting the need of vaccine protocols able to induce a high-titer response; on the other hand, to be effective, it requires the maintenance of high levels of antibodies.

SUMMARY

In summary, PCSK9 is a viable target for hypolipidemic therapy. The current available drugs target the circulating protein, and clinical results are in line with the reduction of LDL-C induced by the drugs. Other therapies targeting PCSK9 albeit with different mechanisms are being developed and will certainly help to widen the range of possible choices for treatment. Whether PCSK9 inhibition will become directly second-line therapy is still pending, but data are accumulating especially from clinical trials, which will provide the wealth of evidence needed.

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