PCSK9 INHIBITORS

Angela Pirillo[1,2], Alberico Luigi Catapano[2,3]

[1]Center for the Study of Atherosclerosis, E. Bassini Hospital, Cinisello Balsamo, Milan, Italy

[2]IRCCS MultiMedica, Sesto S. Giovanni, Milan, Italy

[3]Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy

Corresponding Author:

Prof. Alberico L. Catapano

Department of Pharmacological and Biomolecular Sciences, University of Milan and IRCCS Multimedica

Via Balzaretti, 9 - 20133 Milan ITALY

Phone number: +39250318302

Fax number: +39250318386

e-mail: alberico.catapano@unimi.it

Abstract

High levels of low density lipoprotein cholesterol (LDL-C) are directly associated with an increased risk of cardiovascular disease. Reducing LDL-C levels reduces the incidence of cardiovascular events, and the greater the reduction, the greater the clinical benefit. Several lipid-lowering approaches are available to achieve the LDL-C levels recommended by current guidelines based on individual cardiovascular risk; the first line therapy is represented by statins. However, many patients cannot achieve the recommended LDL-C levels even when treated with maximal tolerated dose of statins, or may experience statin-related muscle adverse events leading to therapy discontinuation. The discovery of the key role of proprotein convertase subtilisin kexin 9 (PCSK9) in the regulation of plasma LDL-C levels suggested it as a potential pharmacological target and led to the development of PCSK9 inhibitors for the management of LDL-C levels. Two of them are monoclonal antibodies (evolocumab and alirocumab) now approved for the treatment of hypercholesterolemia. New approaches to efficiently inhibit PCSK9 are currently under development.

Keywords: PCSK9, monoclonal antibodies, evolocumab, alirocumab, LDL-C, hypercholesterolemia, cardiovascular disease

Introduction

Low density lipoprotein (LDL) is a major risk factor for cardiovascular disease and a large number of epidemiological 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 events. In fact, 12% reduction in all-cause mortality, 19% reduction in coronary mortality and 17% reduction in any vascular cause of mortality per mmol/L 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 cardiovascular risk, as suggested by the comparison between more intensive and less intensive lipid-lowering therapies [2]. Thus, patients at high or very high cardiovascular 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 cardiovascular 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; this may occur for 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 cardiovascular 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 LDL-C levels below those achievable with the common cholesterol-lowering drugs.

PCSK9

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 (Figure 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]. This 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 plasmatic 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 was observed in subjects carrying loss-of-function mutations in *PCSK9* gene [13-17], while gain-of-function mutations are associated with an increased risk of premature cardiovascular disease [18-20]. These findings suggested that PCSK9 may be a useful pharmacological target for the control of hypercholesterolemia (Figure 2) and led to the development of two fully human monoclonal antibodies against circulating PCSK9, evolocumab and alirocumab, which are now approved for the treatment of hypercholesterolemia. The development of a third monoclonal antibody against PCSK9 (bococizumab) was recently halted due to a high titer of anti-drug antibodies which may significantly attenuate the LDL-C-lowering effect [21]. Another monoclonal antibody to PCSK9, named LY3015014, whose safety and efficacy has been so far tested in a phase 2 study but the development is still pending [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 pharmacological inhibition of PCSK9 may lead to extrahepatic effects of PCSK9 which may arise concerns about this approach. To date, there is no evidence indicating a direct association between lipid-lowering therapy with PCSK9 inhibitors with our 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 different groups of hypercholesterolemic patients with high cardiovascular risk; two meta-analyses have shown their safety and efficacy in reducing persistently LDL-C levels, [28-30], which translates into a lower incidence of cardiovascular 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≥2.6 mmol/L, <4.9 mmol/L) the administration of evolocumab every 2 weeks (Q2W) or every 4 weeks (Q4W) for 3 months produced a significant reduction of LDL-C levels either compared with ezetimibe or

placebo (Table 1); other lipids and lipoproteins, including VLDL-C and 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 placebo [35] (Table 1). All doses of evolocumab were more likely than placebo to reduce LDL-C levels below 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 Q2W or 420 mg Q4W), placebo or ezetimibe for 12 weeks: a significant additional reduction of LDL-C was observed (Table 1), and most patients (86-94%) achieved LDL-C levels <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 lipid-lowering therapy for 52 weeks (DESCARTES study): LDL-C were significantly reduced by 57% (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 observed in patients receiving placebo (-0.95% with evolocumab vs +0.05% with placebo) [39] (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 cardiovascular events after a median follow-up of 2.2 years, with a hazard ratio of 0.85 (95% CI, 0.79-0.92) for the primary endpoint (including cardiovascular death, myocardial infarction, stroke, hospitalization for unstable angina, or coronary revascularization) and 0.80 (95% CI, 0.73-0.88) for secondary endpoint (cardiovascular death, myocardial infarction, or stroke) [40] (Table 1). A pre-specified analysis of this trial showed that evolocumab significantly reduced the incidence of cardiovascular events with similar efficacy in patients with and without diabetes, but the absolute risk reduction was higher in patients with diabetes [41]; evolocumab did not increased the risk of new-onset diabetes and did not worsen glycaemia [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 a LDL-C level of 22 mg/dL [40]; this finding, together with the observations from the GLAGOV, suggests that a cardiovascular 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 adverse events following therapy, which may lead to the discontinuation of the statin therapy and the increase of cardiovascular 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] (Table 1). As monotherapy, evolocumab dose-dependently reduced LDL-C levels by 41% up to 63% compared with a 14.8% reduction achieved with ezetimibe [42]; 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 <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 (Table 1), and more than 75% of patients reached an LDL-C level <100 mg/dL, while the majority of the patients treated with ezetimibe were unable to achieve LDL target levels [43]. The incidence of myalgia among these patients was low [43]. These findings have been confirmed by the GAUSS-3 clinical trial (Table 1), which aimed at identifying patients with muscle symptoms confirmed by statin rechallenge and comparing the effect of evolocumab and ezetimibe [44].

Despite 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 do not achieve the recommended LDL-C level target even with maximally tolerated doses of drugs. These considerations pointed the attention on the need of new pharmacological approach for the treatment of these high cardiovascular 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 patients who do not respond well to conventional lipid-lowering therapies. Two specific studies aimed at evaluating the effect of evolocumab in HeFH patients [45, 46] (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 patient treated with evolocumab reached either LDL-C <100 mg/dL (70 and 89%) or <70 mg/dL (44 and 65%) [45]. The lipid profile was generally improved and a significant reduction of Lp(a), a cardiovascular 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 lipid-lowering therapy, confirmed the high ability of evolocumab to reduce significantly LDL-C levels (~60%) independently of the type of background lipid-lowering therapy [46]. Interestingly, some patients recruited as HeFH were then reclassified as homozyogotes; in these patients the reduction of LDL-C induced by evolocumab treatment were comparable to those observed in heterozygotes, and much greater than those reported in other studies on HoFH, probably due to 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 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%), while receptor negative patients did not respond to this therapy [47]; this suggests that the mechanism by which evolocumab reduce 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 to be investigated, as 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 cohort 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 (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 one or two defective LDLR mutations [48]. The ongoing TAUSSIG 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 (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 two 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 lipid-lowering therapy, and that patients on evolocumab had a significantly lower rate of cardiovascular events compared with patients on standard therapy (hazard ratio 0.47; 95CI, 0.28-0.78, P=0.003) [50] (Table 1), a finding that has been confirmed by the above discussed FOURIER trial [40].

Alirocumab

Alirocumab is a fully human monoclonal antibody 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 weeks [51] (Table 2). Almost all patients reached the LDL-C level <100 mg/dL and a great proportion reached the level <70 mg/dL [51]. Lp(a) levels significantly decreased in all tested conditions [51]; TC, non-HDL-C and apoB were also significantly reduced [51]. Similar observations were reported in other two 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]

(Table 2); all patients on alirocumab reached the LDL-C level target <100 mg/dL and >90% had a LDL-C level <70 mg/dL [52]. Alirocumab was effective in reducing LDL-C levels also in a population of young HeFH patients with a background therapy of low-to-moderate dose of atorvastatin: reductions ranged from 28.9% with alirocumab 150 mg Q4W up to 67.90% with 150 mg Q2W, compared with a reduction of 10.65% with placebo [53] (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 Q2W; 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 (Table 2). Most of these trials used a dosage of 75 mg Q2W uptitrated to 150 Q2W 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 (Table 2). The 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 <70 mg/dL [55]. The COMBO II study showed a greater efficacy of alirocumab in reducing LDL-C levels compared with ezetimibe in 104-weeks 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 controlled [57] (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 Q2W (switched to 150 mg Q2W 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), while 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 baseline [58] (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 patients [59]: LDL-C levels were reduced by -50.6% and -36.3%, respectively, while adding ezetimibe or doubling rosuvastatin dose was less effective(-14.4% and -11.0% with ezetimibe; -16.3% and-15.9% doubling rosuvastatin) [60] (Table 2). Altogether these results suggest that high CV risk patients with not controlled LDL-C levels despite on maximal tolerated lipid-lowering therapy may significantly benefit from the addition of alirocumab to their therapy.

As for evolocumab, a specific trial have 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<0.0001) (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) [61]. 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 [61]. The higher efficacy of alirocumab compared with ezetimibe has been reported also by the ODYSSEY MONO trial, conducted in patients on no lipid-lowering therapy [62] (Table 2).

To evaluate the log-term efficacy and safety of alirocumab, two studies have been designed to specifically address these question. 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 weeks [63] (Table 2); these reductions, which were maintained during the course of the study, did not differ between HeFH and non-HeFH patients [63]. 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 [63]. A post-hoc analysis reported a lower rate of major adverse cardiovascular events in alirocumab group than in placebo group (1.7% vs 3.3%, HR 0.52, nominal P=0.02), with cumulative probability of event curves tending to diverge over time[63]. The ongoing ODYSSEY OUTCOMES is evaluating the effect of adding alirocumab or placebo to the current lipid-lowering therapy in patients with a recent acute coronary syndrome; the primary outcome the time from randomization to first occurrence of a clinical cardiovascular event including CHD death, MI, fatal and non-fatal ischemic stroke and unstable angina requiring hospitalization [64]; 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 lipid-lowering therapy (Table 2). In the ODYSSEY FH I and FH II studies, patients (mean LDL-C levels 144.7 mg/dL) received alirocumab 75 mg Q2W 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 the majority of alirocumab receiving patients reached LDL-C levels <70 mg/dL [65]. Alirocumab also reduced apoB, non-HDL-C and Lp(a) [65]. The ODYSSEY JAPAN, which included 41 HeFH patients, reported similar results [66]. 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 Q2W or placebo for 78 weeks [67]. At week 24, in patients treated with alirocumab LDL-C were reduced by -45.7% compared with -6.6% with placebo (Table 3) [67]. 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 [63]. The ODYSSEY ESCAPE trial showed that, in HeFH patients undergoing regular lipoprotein apheresis, the addition of alirocumab to their lipid-lowering therapy led to the discontinuation of apheresis in 63.4% of patients, while in 29.3% of patients the standardized rate was reduced by at least 50% [68], which suggests that these specific subgroup 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) which 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 [69].

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 Q2W alirocumab dosing with 300 mg Q4W as a monotherapy or add-on to statin therapy in patients with moderate-to-very high CV risk, with a 48-week follow-up [70] (Table 2). The monthly dosing was as effective in reducing LDL-C levels as the Q2W dosing; the reductions were significant both

in patients on statin therapy and in patients not receiving statins [70]. The analysis of a subgroup of patients with atherosclerotic cardiovascular disease showed a significant reduction of LDL-C levels with alirocumab 300 mg Q4W both in patients on statin (-64.2%) and in the group not receiving statin (-56.8%) [71]; the reductions were similar to that observed in the whole population of the study [70]. 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 proportions of patients achieved the recommended LDL-C levels following the treatment with alirocumab. Alirocumab has been tested also as 150 mg dose Q4W in the ODYSSEY CHOICE II clinical trial, conducted in patients with inadequately controlled hypercholesterolemia and not on statin therapy due to muscle-related adverse events [72] (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% versus baseline with 75mg Q2W); some patients required a dose adjustment at week 12, which was associated with a higher LDL-C levels at baseline [72]. About 90% of the patients included in this trial had muscle-related adverse events with statin leading to therapy discontinuation, but during the treatment with alirocumab the rate of these events was low [72]. All these observation 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 positive effect on the costs of the therapy.

Other monoclonal antibodies to PCSK9

Bococizumab

Bococizumab is a humanized monoclonal antibody targeting PCSK9; as it contains a 3% murine sequence, this may result in the development of antidrug antibodies. 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 [73]. In SPIRE lipid-lowering program, which included several trials on bococizumab, high-titer antidrug antibodies developed in a high percentage of patients receiving bococizumab after week 12, which markedly reduced the magnitude and durability of LDL-C lowering (~50%) [21]. In addition to this, a high variability in the LDL-C lowering was observed among patients with no antidrug antibodies, a variation that was present as early as 12 weeks, and thus before the detection of antidrug 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 7-8 kDa following the activity of furin. This truncated form seems to be inactive at LDLR degradation [74, 75]. This observations can have a specific relevance when using monoclonal antibodies, as 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 [76]. 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 Q4W and -14.9% to -37.1% for Q8W 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 due to lower reductions of LDL-C induced compared with approved PCSK9 mAbs.

New approaches for the inhibition of PCSK9

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 [77]. BMS-962476 targets circulating PCSK9; in hypercholesterolemic mice overexpressing human PCSK9, it rapidly reduces cholesterol and free PCSK9 levels , and the treatment of cymolgous monkeys suppressed PCSK9 by more than 99% and LDL-C levels by ~55% [78]. BMS-962476 was well tolerated in healthy subjects on diet and LDL-C>130 mg/dL or statins and LDL-C>100 mg/dL; in these subjects it produced reductions of 48% reduction in LDL-C and >90%in PCSK9 levels at maximal dose [79]. Also the development of this molecule has been discontinued [80].

Inclisiran

A more recent approach for the inhibition of PCSK9 is through a biological process referred to as RNA interference (RNAi); 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 [81]. In healthy volunteers with serum LDL-C levels ≥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 [82].

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 in either a single ascending dose (25-800 mg) or multiple dose [83]; at day 84, in the single dose phase, 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% [83]. All multiple dose regimens massively reduced PCSK9 levels (up to 83.8%) and LDL-C levels (up to 59.7%) [83]. No serious adverse events were reported [83].

Inclisiran has been tested in a phase 2 trial (ORION-1) in patients at high cv risk with high LDL-C levels [84]. Patients were randomly assigned to receive a single dose of placebo or 200, 300, or 500 mg of inclisiran or two 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 two-dose 300 mg regimen of inclisiran (first injection at day 1, second injection at day 90) [84]. LDL-C levels were reduced in every patient enrolled in the trial [84]. 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 injecton-site reactions were uncommon and similar to those reported with monoclonal antibodies [84]. 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-PCSK9 vaccines

Active vaccination represents one of the recent approaches that are currently under investigation for cholesterol management and prevention of cardiovascular disease. The goal of this approach is to induce a therapeutic response similar to that induced by the administration of monoclonal antibodies, but with reduced interventions and avoiding the possibility of the formation of anti-drug antibodies, which may limit the pharmacological effect. To this end, a peptide-based anti-PCSK9 active vaccination has been evaluated in preclinical models [23]: vaccines induced generation of high-affine antibodies specific for PCSK9 [23]. This translated into a reduction of LDL-C up to 50% in treated animals [23]. The humoral immune response induced by vaccine persisted for one year, and LDL-C level reduction persisted for the entire period of the study [23]. Similar results were reported in another study which evaluated the effect of vaccination with various PCSK9 peptide 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, beside 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.

Conclusions

In summary, PCSK9 is a viable target for hypolipidaemic therapy. Tthe 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.

Legend to the Figures

Figure 1. Mechanism of action of PCSK9 and mAbs to PCSK9. In the absence of PCSK9, following the binding with LDL, LDLR is internalized then recycled to the cell surface to restart the cycle. PCSK9 binds to LDLR and target it to the degradation. MAbs to PCSK9 neutralize the circulating protein and block its binding to LDLR.

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

Conflict of interest

A.P. reports no disclosures. A.L.C. received research funding and/or honoraria for advisory boards, consultancy or speaker bureau from Aegerion, Amgen, AstraZeneca, Eli Lilly, Genzyme, Mediolanum, Merck or MSD, Pfizer, Recordati, Rottapharm, Sanofi-Regeneron, Sigma-Tau.

Table 1. Effect of evolocumab on LDL-C 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 weeks)	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 weeks)	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 weeks)	Fasting LDL-C≥85 mg/dL	Statin±ezetimibe	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 weeks)	Primary hypercholesterolemia and mixed dyslipidemia	Atorva 10/80 mg±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 weeks)	Fasting LDL-C≥75mg/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 weeks)	Fasting LDL-C≥80 mg/dL	Statin	1. 420 mg Q4W 2. Placebo	-61% vs placebo	Nominal change in PAV: -1.0%, p<0.001
FOURIER (5 years)	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 UA, stroke or coronary revascularization: HR 0.85 (0.79-0.92) p<0.001
GAUSS (12 weeks)	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 weeks)	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 we Part C: 2y)	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 ONGOING	

RUTHERFORD (12 weeks)	HeFH Fasting LDL-C≥100 mg/dL	Statin±ezetimibe	1. 350 mg Q4W 2. 420 mg Q4W 3. Placebo	1. -43.8% vs placebo 2. -56.4% vs placebo	
RUTHERFORD-2 (12 weeks)	HeFH Fasting LDL-C≥100 mg/dL	Statin±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 weeks)	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 weeks)	HoFH; LDL-C≥130 mg/dL	Stable LLT	1. 420 mg Q4W 2. Placebo	-30.9% vs placebo	
TAUSSIG (5 years)	Severe FH	Stable LLT	Q2W or Q4W	ONGOING (2020) Interim analysis: -20.6% at week 12	
OSLER-1 and OSLER-2 (1 year)	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=0.003

Table 2. Effect of alirocumab on LDL-C levels and cardiovascular outcomes in randomized clinical trials.

Clinical trial (duration)	**Characteristics of selected patients**	**Background LLT**	**Alirocumab dosing**	**LDL-C change**	**Cardiovascular outcomes**
DFI11565 (12 weeks)	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 weeks)	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 weeks)	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 weeks)	Established CHD or CHD equivalent, LDL-C ≥70 mg/dL	Max tolerated statin±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 weeks)	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 150mg Q2W if LDL-C ≥70 mg/dL at week 8) 2. Eze 10 mg	-29.8% vs eze	

ODYSSEY OPTIONS I (24 weeks)	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→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 weeks)	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 weeks)	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 weeks)	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 weeks)	HeFH or established CHD or CHD equivalent LDL-C ≥70 mg/dL	Max tolerated statin±other LLT	1.150 mg Q2W 2. Placebo	-61.9% vs placebo	
ODYSSEY OUTCOMES (64 months)	Recent ACS, LDL-C ≥70 mg/dL	Atorva 40/80 mg or rosuva 20/40 mg or the max tolerated dose	1. 75mg Q2W 2. 150 mg Q2W 3. Placebo	ONGOING (2017)	Time from randomization to first occurrence of CHD death, non-fatal MI, stroke or UA requiring hospitalization
ODYSSEY FH I and FH II (78 weeks)	HeFH; LDL-C≥100 mg/dL (for primary prevention) or LDL-C≥70 mg/dL (for secondary prevention)	Max tolerated statin±other LLT	1. 75mg 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 weeks)	HeFH or non-FH at high CV risk not at target	Stable statin therapy	1. 75mg Q2W (increased at 150 mg Q2W if LDL-C ≥70 mg/dL at week 8) 2. Placebo	-58.9% vs palcebo	
ODYSSEY HIGH FH (78 weeks)	HeFH LDL-C ≥160	Stable LLT	1. 150 mg Q2W 2. Placebo	-39.1% vs placebo	
ODYSSEY ESCAPE (18 weeks)	HeFH undergoing apheresis	Stable LLT	1. 150 mg Q2W 2. Placebo	-46.4% vs placebo	
ODYSSEY OLE (176 weeks)	HeFH who have completed one of the 4 parent studies			ONGOING (2017) Preliminary data at week48: -46.9%	
CHOICE I (48 weeks)	Hypercholesterolemic at moderate-to-very-high CV risk	±Statin ± other LLT	1. 300 mg Q4W 2. 75 mg Q2W	1. -52.4% vs placebo (no statin) -58.7 vs placebo (statin)	

			3. Placebo	2. -49.8% vs placebo (no statin) -51.4% vs placebo (statin)	
CHOICE II (24 weeks)	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	

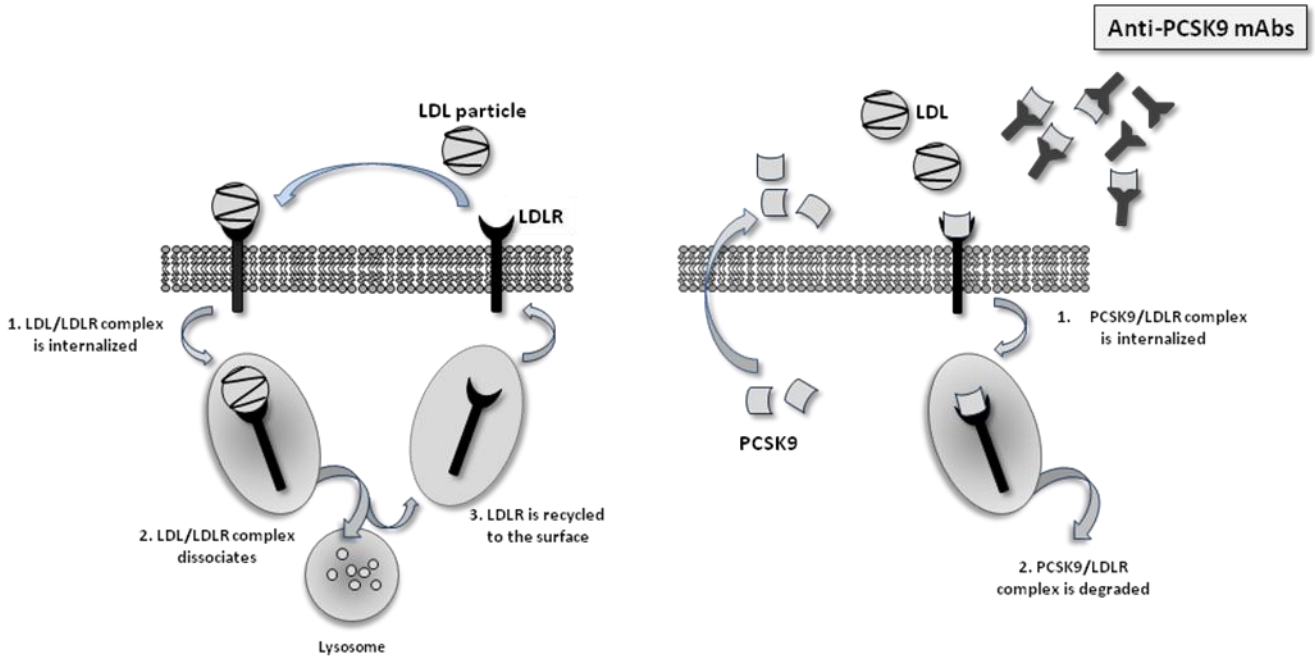
Anti-PCSK9 mAbs
LDL particle
LDLR
1. LDL/LDLR complex is internalized
2. LDL/LDLR complex dissociates
3. LDLR is recycled to the surface
Lysosome
LDL
PCSK9
1. PCSK9/LDLR complex is internalized
2. PCSK9/LDLR complex is degraded

Figure 1

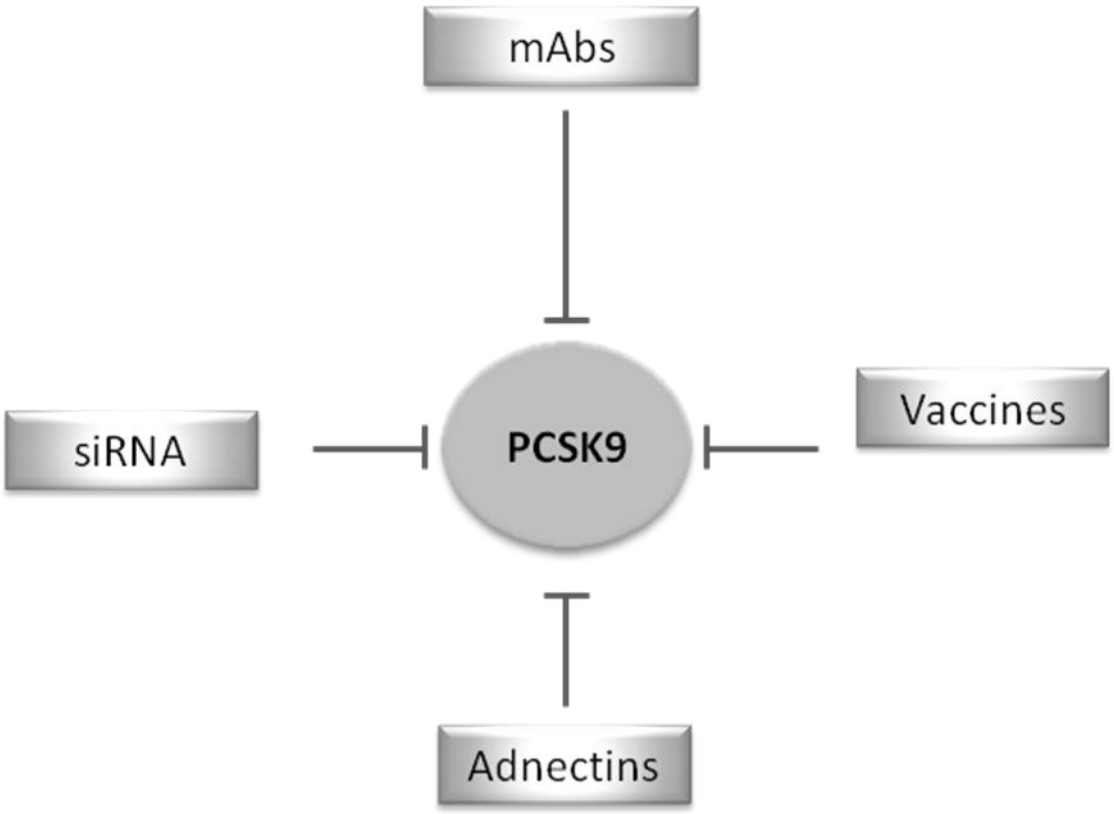

Figure 2

References

[1] Baigent, C.; Keech, A.; Kearney, P.M.; Blackwell, L.; Buck, G.; Pollicino, C.; Kirby, A.; Sourjina, T.; Peto, R.; Collins, R.; Simes, R.; Cholesterol Treatment Trialists, C., Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet,* **2005**, *366*, (9493), 1267-1278.

[2] Baigent, C.; Blackwell, L.; Emberson, J.; Holland, L.E.; Reith, C.; Bhala, N.; Peto, R.; Barnes, E.H.; Keech, A.; Simes, J.; Collins, R., Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. *Lancet,* **2010**, *376*, (9753), 1670-1681.

[3] Catapano, A.L.; Graham, I.; De Backer, G.; Wiklund, O.; Chapman, M.J.; Drexel, H.; Hoes, A.W.; Jennings, C.S.; Landmesser, U.; Pedersen, T.R.; Reiner, Z.; Riccardi, G.; Taskinen, M.R.; Tokgozoglu, L.; Verschuren, W.M.; Vlachopoulos, C.; Wood, D.A.; Zamorano, J.L., 2016 ESC/EAS Guidelines for the Management of Dyslipidaemias: The Task Force for the Management of Dyslipidaemias of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS) Developed with the special contribution of the European Assocciation for Cardiovascular Prevention & Rehabilitation (EACPR). *Atherosclerosis,* **2016**, *253*, 281-344.

[4] Naci, H.; Brugts, J.J.; Fleurence, R.; Tsoi, B.; Toor, H.; Ades, A.E., Comparative benefits of statins in the primary and secondary prevention of major coronary events and all-cause mortality: a network meta-analysis of placebo-controlled and active-comparator trials. *Eur J Prev Cardiol,* **2013**, *20*, (4), 641-657.

[5] Mills, E.J.; Wu, P.; Chong, G.; Ghement, I.; Singh, S.; Akl, E.A.; Eyawo, O.; Guyatt, G.; Berwanger, O.; Briel, M., Efficacy and safety of statin treatment for cardiovascular disease: a network meta-analysis of 170,255 patients from 76 randomized trials. *QJM,* **2011**, *104*, (2), 109-124.

[6] Tonelli, M.; Lloyd, A.; Clement, F.; Conly, J.; Husereau, D.; Hemmelgarn, B.; Klarenbach, S.; McAlister, F.A.; Wiebe, N.; Manns, B., Efficacy of statins for primary prevention in people at low cardiovascular risk: a meta-analysis. *CMAJ,* **2011**, *183*, (16), E1189-1202.

[7] Mills, E.J.; O'Regan, C.; Eyawo, O.; Wu, P.; Mills, F.; Berwanger, O.; Briel, M., Intensive statin therapy compared with moderate dosing for prevention of cardiovascular events: a meta-analysis of >40 000 patients. *Eur Heart J,* **2011**, *32*, (11), 1409-1415.

[8] Chan, D.K.; O'Rourke, F.; Shen, Q.; Mak, J.C.; Hung, W.T., Meta-analysis of the cardiovascular benefits of intensive lipid lowering with statins. *Acta Neurol Scand,* **2011**, *124*, (3), 188-195.

[9] Taylor, F.; Huffman, M.D.; Macedo, A.F.; Moore, T.H.; Burke, M.; Davey Smith, G.; Ward, K.; Ebrahim, S., Statins for the primary prevention of cardiovascular disease. *Cochrane Database Syst Rev,* **2013**, *1*, CD004816.

[10] Corrao, G.; Conti, V.; Merlino, L.; Catapano, A.L.; Mancia, G., Results of a retrospective database analysis of adherence to statin therapy and risk of nonfatal ischemic heart disease in daily clinical practice in Italy. *Clinical therapeutics,* **2010**, *32*, (2), 300-310.

[11] Leren, T.P., Sorting an LDL receptor with bound PCSK9 to intracellular degradation. *Atherosclerosis,* **2014**, *237*, (1), 76-81.

[12] Abifadel, M.; Varret, M.; Rabes, J.P.; Allard, D.; Ouguerram, K.; Devillers, M.; Cruaud, C.; Benjannet, S.; Wickham, L.; Erlich, D.; Derre, A.; Villeger, L.; Farnier, M.; Beucler, I.; Bruckert, E.; Chambaz, J.; Chanu, B.; Lecerf, J.M.; Luc, G.; Moulin, P.; Weissenbach, J.; Prat, A.; Krempf, M.; Junien, C.; Seidah, N.G.; Boileau, C., Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. *Nat Genet,* **2003**, *34*, (2), 154-156.

[13] Cohen, J.C.; Boerwinkle, E.; Mosley, T.H., Jr.; Hobbs, H.H., Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N Engl J Med,* **2006**, *354*, (12), 1264-1272.

[14] Cohen, J.; Pertsemlidis, A.; Kotowski, I.K.; Graham, R.; Garcia, C.K.; Hobbs, H.H., Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in PCSK9. *Nat Genet,* **2005**, *37*, (2), 161-165.

[15] Kathiresan, S., A PCSK9 missense variant associated with a reduced risk of early-onset myocardial infarction. *N Engl J Med,* **2008**, *358*, (21), 2299-2300.

[16] Kent, S.T.; Rosenson, R.S.; Avery, C.L.; Chen, Y.I.; Correa, A.; Cummings, S.R.; Cupples, L.A.; Cushman, M.; Evans, D.S.; Gudnason, V.; Harris, T.B.; Howard, G.; Irvin, M.R.; Judd, S.E.; Jukema, J.W.; Lange, L.; Levitan, E.B.; Li, X.; Liu, Y.; Post, W.S.; Postmus, I.; Psaty, B.M.; Rotter, J.I.; Safford, M.M.; Sitlani,

C.M.; Smith, A.V.; Stewart, J.D.; Trompet, S.; Sun, F.; Vasan, R.S.; Woolley, J.M.; Whitsel, E.A.; Wiggins, K.L.; Wilson, J.G.; Muntner, P., PCSK9 Loss-of-Function Variants, Low-Density Lipoprotein Cholesterol, and Risk of Coronary Heart Disease and Stroke: Data From 9 Studies of Blacks and Whites. *Circ Cardiovasc Genet,* **2017**, *10*, (4), e001632.
[17] Benn, M.; Nordestgaard, B.G.; Grande, P.; Schnohr, P.; Tybjaerg-Hansen, A., PCSK9 R46L, low-density lipoprotein cholesterol levels, and risk of ischemic heart disease: 3 independent studies and meta-analyses. *Journal of the American College of Cardiology,* **2010**, *55*, (25), 2833-2842.
[18] Hopkins, P.N.; Defesche, J.; Fouchier, S.W.; Bruckert, E.; Luc, G.; Cariou, B.; Sjouke, B.; Leren, T.P.; Harada-Shiba, M.; Mabuchi, H.; Rabes, J.P.; Carrie, A.; van Heyningen, C.; Carreau, V.; Farnier, M.; Teoh, Y.P.; Bourbon, M.; Kawashiri, M.A.; Nohara, A.; Soran, H.; Marais, A.D.; Tada, H.; Abifadel, M.; Boileau, C.; Chanu, B.; Katsuda, S.; Kishimoto, I.; Lambert, G.; Makino, H.; Miyamoto, Y.; Pichelin, M.; Yagi, K.; Yamagishi, M.; Zair, Y.; Mellis, S.; Yancopoulos, G.D.; Stahl, N.; Mendoza, J.; Du, Y.; Hamon, S.; Krempf, M.; Swergold, G.D., Characterization of Autosomal Dominant Hypercholesterolemia Caused by PCSK9 Gain of Function Mutations and Its Specific Treatment With Alirocumab, a PCSK9 Monoclonal Antibody. *Circ Cardiovasc Genet,* **2015**, *8*, (6), 823-831.
[19] Qiu, C.; Zeng, P.; Li, X.; Zhang, Z.; Pan, B.; Peng, Z.Y.F.; Li, Y.; Ma, Y.; Leng, Y.; Chen, R., What is the impact of PCSK9 rs505151 and rs11591147 polymorphisms on serum lipids level and cardiovascular risk: a meta-analysis. *Lipids Health Dis,* **2017**, *16*, (1), 111.
[20] Naoumova, R.P.; Tosi, I.; Patel, D.; Neuwirth, C.; Horswell, S.D.; Marais, A.D.; van Heyningen, C.; Soutar, A.K., Severe hypercholesterolemia in four British families with the D374Y mutation in the PCSK9 gene: long-term follow-up and treatment response. *Arterioscler Thromb Vasc Biol,* **2005**, *25*, (12), 2654-2660.
[21] Ridker, P.M.; Tardif, J.C.; Amarenco, P.; Duggan, W.; Glynn, R.J.; Jukema, J.W.; Kastelein, J.J.P.; Kim, A.M.; Koenig, W.; Nissen, S.; Revkin, J.; Rose, L.M.; Santos, R.D.; Schwartz, P.F.; Shear, C.L.; Yunis, C.; Investigators, S., Lipid-Reduction Variability and Antidrug-Antibody Formation with Bococizumab. *N Engl J Med,* **2017**, *376*, (16), 1517-1526.
[22] Kastelein, J.J.; Nissen, S.E.; Rader, D.J.; Hovingh, G.K.; Wang, M.D.; Shen, T.; Krueger, K.A., Safety and efficacy of LY3015014, a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 (PCSK9): a randomized, placebo-controlled Phase 2 study. *Eur Heart J,* **2016**, *37*, (17), 1360-1369.
[23] Galabova, G.; Brunner, S.; Winsauer, G.; Juno, C.; Wanko, B.; Mairhofer, A.; Luhrs, P.; Schneeberger, A.; von Bonin, A.; Mattner, F.; Schmidt, W.; Staffler, G., Peptide-based anti-PCSK9 vaccines - an approach for long-term LDLc management. *PLoS One,* **2014**, *9*, (12), e114469.
[24] Crossey, E.; Amar, M.J.; Sampson, M.; Peabody, J.; Schiller, J.T.; Chackerian, B.; Remaley, A.T., A cholesterol-lowering VLP vaccine that targets PCSK9. *Vaccine,* **2015**, *33*, (43), 5747-5755.
[25] Landlinger, C.; Pouwer, M.G.; Juno, C.; van der Hoorn, J.W.A.; Pieterman, E.J.; Jukema, J.W.; Staffler, G.; Princen, H.M.G.; Galabova, G., The AT04A vaccine against proprotein convertase subtilisin/kexin type 9 reduces total cholesterol, vascular inflammation, and atherosclerosis in APOE*3Leiden.CETP mice. *Eur Heart J,* **2017**, *38*, (32), 2499-2507.
[26] Norata, G.D.; Tavori, H.; Pirillo, A.; Fazio, S.; Catapano, A.L., Biology of proprotein convertase subtilisin kexin 9: beyond low-density lipoprotein cholesterol lowering. *Cardiovasc Res,* **2016**, *112*, (1), 429-442.
[27] Banach, M.; Rizzo, M.; Nikolic, D.; Howard, G.; Howard, V.; Mikhailidis, D., Intensive LDL-cholesterol lowering therapy and neurocognitive function. *Pharmacology & therapeutics,* **2017**, *170*, 181-191.
[28] Navarese, E.P.; Kolodziejczak, M.; Schulze, V.; Gurbel, P.A.; Tantry, U.; Lin, Y.; Brockmeyer, M.; Kandzari, D.E.; Kubica, J.M.; D'Agostino, R.B., Sr.; Kubica, J.; Volpe, M.; Agewall, S.; Kereiakes, D.J.; Kelm, M., Effects of proprotein convertase subtilisin/kexin type 9 antibodies in adults with hypercholesterolemia: a systematic review and meta-analysis. *Ann Intern Med,* **2015**, *163*, (1), 40-51.
[29] Li, C.; Lin, L.; Zhang, W.; Zhou, L.; Wang, H.; Luo, X.; Luo, H.; Cai, Y.; Zeng, C., Efficiency and safety of proprotein convertase subtilisin/kexin 9 monoclonal antibody on hypercholesterolemia: a meta-analysis of 20 randomized controlled trials. *J Am Heart Assoc,* **2015**, *4*, (6), e001937.
[30] Qian, L.J.; Gao, Y.; Zhang, Y.M.; Chu, M.; Yao, J.; Xu, D., Therapeutic efficacy and safety of PCSK9-monoclonal antibodies on familial hypercholesterolemia and statin-intolerant patients: A meta-analysis of 15 randomized controlled trials. *Scientific reports,* **2017**, *7*, (1), 238.

[31] Zhang, X.L.; Zhu, Q.Q.; Zhu, L.; Chen, J.Z.; Chen, Q.H.; Li, G.N.; Xie, J.; Kang, L.N.; Xu, B., Safety and efficacy of anti-PCSK9 antibodies: a meta-analysis of 25 randomized, controlled trials. *BMC Med,* **2015**, *13*, 123.
[32] Langslet, G.; Emery, M.; Wasserman, S.M., Evolocumab (AMG 145) for primary hypercholesterolemia. *Expert Rev Cardiovasc Ther,* **2015**, *13*, (5), 477-488.
[33] Koren, M.J.; Scott, R.; Kim, J.B.; Knusel, B.; Liu, T.; Lei, L.; Bolognese, M.; Wasserman, S.M., Efficacy, safety, and tolerability of a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 as monotherapy in patients with hypercholesterolaemia (MENDEL): a randomised, double-blind, placebo-controlled, phase 2 study. *Lancet,* **2012**, *380*, (9858), 1995-2006.
[34] Koren, M.J.; Lundqvist, P.; Bolognese, M.; Neutel, J.M.; Monsalvo, M.L.; Yang, J.; Kim, J.B.; Scott, R.; Wasserman, S.M.; Bays, H., Anti-PCSK9 monotherapy for hypercholesterolemia: the MENDEL-2 randomized, controlled phase III clinical trial of evolocumab. *Journal of the American College of Cardiology,* **2014**, *63*, (23), 2531-2540.
[35] Giugliano, R.P.; Desai, N.R.; Kohli, P.; Rogers, W.J.; Somaratne, R.; Huang, F.; Liu, T.; Mohanavelu, S.; Hoffman, E.B.; McDonald, S.T.; Abrahamsen, T.E.; Wasserman, S.M.; Scott, R.; Sabatine, M.S., Efficacy, safety, and tolerability of a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 in combination with a statin in patients with hypercholesterolaemia (LAPLACE-TIMI 57): a randomised, placebo-controlled, dose-ranging, phase 2 study. *Lancet,* **2012**, *380*, (9858), 2007-2017.
[36] Robinson, J.G.; Nedergaard, B.S.; Rogers, W.J.; Fialkow, J.; Neutel, J.M.; Ramstad, D.; Somaratne, R.; Legg, J.C.; Nelson, P.; Scott, R.; Wasserman, S.M.; Weiss, R., Effect of evolocumab or ezetimibe added to moderate- or high-intensity statin therapy on LDL-C lowering in patients with hypercholesterolemia: the LAPLACE-2 randomized clinical trial. *Jama,* **2014**, *311*, (18), 1870-1882.
[37] Blom, D.J.; Hala, T.; Bolognese, M.; Lillestol, M.J.; Toth, P.D.; Burgess, L.; Ceska, R.; Roth, E.; Koren, M.J.; Ballantyne, C.M.; Monsalvo, M.L.; Tsirtsonis, K.; Kim, J.B.; Scott, R.; Wasserman, S.M.; Stein, E.A., A 52-week placebo-controlled trial of evolocumab in hyperlipidemia. *N Engl J Med,* **2014**, *370*, (19), 1809-1819.
[38] Blom, T.; Back, N.; Mutka, A.L.; Bittman, R.; Li, Z.; de Lera, A.; Kovanen, P.T.; Diczfalusy, U.; Ikonen, E., FTY720 stimulates 27-hydroxycholesterol production and confers atheroprotective effects in human primary macrophages. *Circ Res,* **2010**, *106*, (4), 720-729.
[39] Nicholls, S.J.; Puri, R.; Anderson, T.; Ballantyne, C.M.; Cho, L.; Kastelein, J.J.; Koenig, W.; Somaratne, R.; Kassahun, H.; Yang, J.; Wasserman, S.M.; Scott, R.; Ungi, I.; Podolec, J.; Ophuis, A.O.; Cornel, J.H.; Borgman, M.; Brennan, D.M.; Nissen, S.E., Effect of Evolocumab on Progression of Coronary Disease in Statin-Treated Patients: The GLAGOV Randomized Clinical Trial. *Jama,* **2016**, *316*, (22), 2373-2384.
[40] Sabatine, M.S.; Giugliano, R.P.; Keech, A.C.; Honarpour, N.; Wiviott, S.D.; Murphy, S.A.; Kuder, J.F.; Wang, H.; Liu, T.; Wasserman, S.M.; Sever, P.S.; Pedersen, T.R., Evolocumab and Clinical Outcomes in Patients with Cardiovascular Disease. *N Engl J Med,* **2017**, *doi: 10.1056/NEJMoa1615664*.
[41] Sabatine, M.S.; Leiter, L.A.; Wiviott, S.D.; Giugliano, R.P.; Deedwania, P.; De Ferrari, G.M.; Murphy, S.A.; Kuder, J.F.; Gouni-Berthold, I.; Lewis, B.S.; Handelsman, Y.; Pineda, A.L.; Honarpour, N.; Keech, A.C.; Sever, P.S.; Pedersen, T.R., Cardiovascular safety and efficacy of the PCSK9 inhibitor evolocumab in patients with and without diabetes and the effect of evolocumab on glycaemia and risk of new-onset diabetes: a prespecified analysis of the FOURIER randomised controlled trial. *Lancet Diabetes Endocrinol,* **2017**.
[42] Sullivan, D.; Olsson, A.G.; Scott, R.; Kim, J.B.; Xue, A.; Gebski, V.; Wasserman, S.M.; Stein, E.A., Effect of a monoclonal antibody to PCSK9 on low-density lipoprotein cholesterol levels in statin-intolerant patients: the GAUSS randomized trial. *Jama,* **2012**, *308*, (23), 2497-2506.
[43] Stroes, E.; Colquhoun, D.; Sullivan, D.; Civeira, F.; Rosenson, R.S.; Watts, G.F.; Bruckert, E.; Cho, L.; Dent, R.; Knusel, B.; Xue, A.; Scott, R.; Wasserman, S.M.; Rocco, M., Anti-PCSK9 antibody effectively lowers cholesterol in patients with statin intolerance: the GAUSS-2 randomized, placebo-controlled phase 3 clinical trial of evolocumab. *Journal of the American College of Cardiology,* **2014**, *63*, (23), 2541-2548.
[44] Nissen, S.E.; Stroes, E.; Dent-Acosta, R.E.; Rosenson, R.S.; Lehman, S.J.; Sattar, N.; Preiss, D.; Bruckert, E.; Ceska, R.; Lepor, N.; Ballantyne, C.M.; Gouni-Berthold, I.; Elliott, M.; Brennan, D.M.; Wasserman, S.M.; Somaratne, R.; Scott, R.; Stein, E.A.; Investigators, G.-. Efficacy and Tolerability of Evolocumab vs Ezetimibe in Patients With Muscle-Related Statin Intolerance: The GAUSS-3 Randomized Clinical Trial. *Jama,* **2016**, *315*, (15), 1580-1590.

[45] Raal, F.; Scott, R.; Somaratne, R.; Bridges, I.; Li, G.; Wasserman, S.M.; Stein, E.A., Low-density lipoprotein cholesterol-lowering effects of AMG 145, a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 serine protease in patients with heterozygous familial hypercholesterolemia: the Reduction of LDL-C with PCSK9 Inhibition in Heterozygous Familial Hypercholesterolemia Disorder (RUTHERFORD) randomized trial. *Circulation,* **2012**, *126*, (20), 2408-2417.
[46] Raal, F.J.; Stein, E.A.; Dufour, R.; Turner, T.; Civeira, F.; Burgess, L.; Langslet, G.; Scott, R.; Olsson, A.G.; Sullivan, D.; Hovingh, G.K.; Cariou, B.; Gouni-Berthold, I.; Somaratne, R.; Bridges, I.; Wasserman, S.M.; Gaudet, D., PCSK9 inhibition with evolocumab (AMG 145) in heterozygous familial hypercholesterolaemia (RUTHERFORD-2): a randomised, double-blind, placebo-controlled trial. *Lancet,* **2015**, *385*, (9965), 331-340.
[47] Stein, E.A.; Honarpour, N.; Wasserman, S.M.; Xu, F.; Scott, R.; Raal, F.J., Effect of the proprotein convertase subtilisin/kexin 9 monoclonal antibody, AMG 145, in homozygous familial hypercholesterolemia. *Circulation,* **2013**, *128*, (19), 2113-2120.
[48] Raal, F.J.; Honarpour, N.; Blom, D.J.; Hovingh, G.K.; Xu, F.; Scott, R.; Wasserman, S.M.; Stein, E.A., Inhibition of PCSK9 with evolocumab in homozygous familial hypercholesterolaemia (TESLA Part B): a randomised, double-blind, placebo-controlled trial. *Lancet,* **2015**, *385*, (9965), 341-350.
[49] Raal, F.J.; Hovingh, G.K.; Blom, D.; Santos, R.D.; Harada-Shiba, M.; Bruckert, E.; Couture, P.; Soran, H.; Watts, G.F.; Kurtz, C.; Honarpour, N.; Tang, L.; Kasichayanula, S.; Wasserman, S.M.; Stein, E.A., Long-term treatment with evolocumab added to conventional drug therapy, with or without apheresis, in patients with homozygous familial hypercholesterolaemia: an interim subset analysis of the open-label TAUSSIG study. *Lancet Diabetes Endocrinol,* **2017**, *5*, (4), 280-290.
[50] Sabatine, M.S.; Giugliano, R.P.; Wiviott, S.D.; Raal, F.J.; Blom, D.J.; Robinson, J.; Ballantyne, C.M.; Somaratne, R.; Legg, J.; Wasserman, S.M.; Scott, R.; Koren, M.J.; Stein, E.A., Efficacy and safety of evolocumab in reducing lipids and cardiovascular events. *N Engl J Med,* **2015**, *372*, (16), 1500-1509.
[51] McKenney, J.M.; Koren, M.J.; Kereiakes, D.J.; Hanotin, C.; Ferrand, A.C.; Stein, E.A., Safety and efficacy of a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 serine protease, SAR236553/REGN727, in patients with primary hypercholesterolemia receiving ongoing stable atorvastatin therapy. *Journal of the American College of Cardiology,* **2012**, *59*, (25), 2344-2353.
[52] Roth, E.M.; McKenney, J.M.; Hanotin, C.; Asset, G.; Stein, E.A., Atorvastatin with or without an antibody to PCSK9 in primary hypercholesterolemia. *N Engl J Med,* **2012**, *367*, (20), 1891-1900.
[53] Stein, E.A.; Gipe, D.; Bergeron, J.; Gaudet, D.; Weiss, R.; Dufour, R.; Wu, R.; Pordy, R., Effect of a monoclonal antibody to PCSK9, REGN727/SAR236553, to reduce low-density lipoprotein cholesterol in patients with heterozygous familial hypercholesterolaemia on stable statin dose with or without ezetimibe therapy: a phase 2 randomised controlled trial. *Lancet,* **2012**, *380*, (9836), 29-36.
[54] Dufour, R.; Bergeron, J.; Gaudet, D.; Weiss, R.; Hovingh, G.K.; Qing, Z.; Yang, F.; Andisik, M.; Torri, A.; Pordy, R.; Gipe, D.A., Open-label therapy with alirocumab in patients with heterozygous familial hypercholesterolemia: Results from three years of treatment. *Int J Cardiol,* **2017**, *228*, 754-760.
[55] Kereiakes, D.J.; Robinson, J.G.; Cannon, C.P.; Lorenzato, C.; Pordy, R.; Chaudhari, U.; Colhoun, H.M., Efficacy and safety of the proprotein convertase subtilisin/kexin type 9 inhibitor alirocumab among high cardiovascular risk patients on maximally tolerated statin therapy: The ODYSSEY COMBO I study. *Am Heart J,* **2015**, *169*, (6), 906-915 e913.
[56] Cannon, C.P.; Cariou, B.; Blom, D.; McKenney, J.M.; Lorenzato, C.; Pordy, R.; Chaudhari, U.; Colhoun, H.M., Efficacy and safety of alirocumab in high cardiovascular risk patients with inadequately controlled hypercholesterolaemia on maximally tolerated doses of statins: the ODYSSEY COMBO II randomized controlled trial. *Eur Heart J,* **2015**, *36*, (19), 1186-1194.
[57] Robinson, J.G.; Colhoun, H.M.; Bays, H.E.; Jones, P.H.; Du, Y.; Hanotin, C.; Donahue, S., Efficacy and safety of alirocumab as add-on therapy in high-cardiovascular-risk patients with hypercholesterolemia not adequately controlled with atorvastatin (20 or 40 mg) or rosuvastatin (10 or 20 mg): design and rationale of the ODYSSEY OPTIONS Studies. *Clinical cardiology,* **2014**, *37*, (10), 597-604.
[58] Bays, H.; Gaudet, D.; Weiss, R.; Ruiz, J.L.; Watts, G.F.; Gouni-Berthold, I.; Robinson, J.; Zhao, J.; Hanotin, C.; Donahue, S., Alirocumab as add-on to atorvastatin versus other lipid treatment strategies: ODYSSEY OPTIONS I randomized trial. *The Journal of clinical endocrinology and metabolism,* **2015**, *100*, (8), 3140-3148.

[59] Farnier, M.; Jones, P.; Severance, R.; Averna, M.; Steinhagen-Thiessen, E.; Colhoun, H.M.; Du, Y.; Hanotin, C.; Donahue, S., Efficacy and safety of adding alirocumab to rosuvastatin versus adding ezetimibe or doubling the rosuvastatin dose in high cardiovascular-risk patients: The ODYSSEY OPTIONS II randomized trial. *Atherosclerosis,* **2016**, *244*, 138-146.
[60] Farnier, M.; Jones, P.; Severance, R.; Averna, M.; Steinhagen-Thiessen, E.; Colhoun, H.M.; Du, Y.; Hanotin, C.; Donahue, S., Efficacy and safety of adding alirocumab to rosuvastatin versus adding ezetimibe or doubling the rosuvastatin dose in high cardiovascular-risk patients: The ODYSSEY OPTIONS II randomized trial. *Atherosclerosis,* **2015**, *244*, 138-146.
[61] Moriarty, P.M.; Thompson, P.D.; Cannon, C.P.; Guyton, J.R.; Bergeron, J.; Zieve, F.; Bruckert, E.; Jacobson, T.A.; Kopecky, S.L.; Baccara-Dinet, M.T.; Du, L.; Pordy, R.; Gipe, D., Efficacy and safety of alirocumab vs ezetimibe in statin-intolerant patients, with a statin rechallenge arm: The ODYSSEY ALTERNATIVE randomized trial. *J Clin Lipidol,* **2015**, *9*, (6), 758-769.
[62] Roth, E.M.; McKenney, J.M., ODYSSEY MONO: effect of alirocumab 75 mg subcutaneously every 2 weeks as monotherapy versus ezetimibe over 24 weeks. *Future Cardiol,* **2015**, *11*, (1), 27-37.
[63] Robinson, J.G.; Farnier, M.; Krempf, M.; Bergeron, J.; Luc, G.; Averna, M.; Stroes, E.S.; Langslet, G.; Raal, F.J.; El Shahawy, M.; Koren, M.J.; Lepor, N.E.; Lorenzato, C.; Pordy, R.; Chaudhari, U.; Kastelein, J.J., Efficacy and safety of alirocumab in reducing lipids and cardiovascular events. *N Engl J Med,* **2015**, *372*, (16), 1489-1499.
[64] Schwartz, G.G.; Bessac, L.; Berdan, L.G.; Bhatt, D.L.; Bittner, V.; Diaz, R.; Goodman, S.G.; Hanotin, C.; Harrington, R.A.; Jukema, J.W.; Mahaffey, K.W.; Moryusef, A.; Pordy, R.; Roe, M.T.; Rorick, T.; Sasiela, W.J.; Shirodaria, C.; Szarek, M.; Tamby, J.F.; Tricoci, P.; White, H.; Zeiher, A.; Steg, P.G., Effect of alirocumab, a monoclonal antibody to PCSK9, on long-term cardiovascular outcomes following acute coronary syndromes: rationale and design of the ODYSSEY outcomes trial. *Am Heart J,* **2014**, *168*, (5), 682-689.
[65] Kastelein, J.J.; Ginsberg, H.N.; Langslet, G.; Hovingh, G.K.; Ceska, R.; Dufour, R.; Blom, D.; Civeira, F.; Krempf, M.; Lorenzato, C.; Zhao, J.; Pordy, R.; Baccara-Dinet, M.T.; Gipe, D.A.; Geiger, M.J.; Farnier, M., ODYSSEY FH I and FH II: 78 week results with alirocumab treatment in 735 patients with heterozygous familial hypercholesterolaemia. *Eur Heart J,* **2015**, *36*, (43), 2996-3003.
[66] Teramoto, T.; Kobayashi, M.; Tasaki, H.; Yagyu, H.; Higashikata, T.; Takagi, Y.; Uno, K.; Baccara-Dinet, M.T.; Nohara, A., Efficacy and Safety of Alirocumab in Japanese Patients With Heterozygous Familial Hypercholesterolemia or at High Cardiovascular Risk With Hypercholesterolemia Not Adequately Controlled With Statins- ODYSSEY JAPAN Randomized Controlled Trial. *Circ J,* **2016**, *80*, (9), 1980-1987.
[67] Kastelein, J.J.; Robinson, J.G.; Farnier, M.; Krempf, M.; Langslet, G.; Lorenzato, C.; Gipe, D.A.; Baccara-Dinet, M.T., Efficacy and safety of alirocumab in patients with heterozygous familial hypercholesterolemia not adequately controlled with current lipid-lowering therapy: design and rationale of the ODYSSEY FH studies. *Cardiovasc Drugs Ther,* **2014**, *28*, (3), 281-289.
[68] Moriarty, P.M.; Parhofer, K.G.; Babirak, S.P.; Cornier, M.A.; Duell, P.B.; Hohenstein, B.; Leebmann, J.; Ramlow, W.; Schettler, V.; Simha, V.; Steinhagen-Thiessen, E.; Thompson, P.D.; Vogt, A.; von Stritzky, B.; Du, Y.; Manvelian, G., Alirocumab in patients with heterozygous familial hypercholesterolaemia undergoing lipoprotein apheresis: the ODYSSEY ESCAPE trial. *Eur Heart J,* **2016**, *37*, (48), 3588-3595.
[69] Farnier, M.; Hovingh, G.K.; Langslet, G.; Dufour, R.; Baccara-Dinet, M.T.; Din-Bell, C.; Manvelian, G.; Guyton, J.R., Durability of Alirocumab Effect: Data From an Open-Label Extension to the ODYSSEY Program for Patients With Heterozygous Familial Hypercholesterolemia. *J Clin Lipidol,* **2017**, *11*, (3), 840.
[70] Roth, E.M.; Moriarty, P.M.; Bergeron, J.; Langslet, G.; Manvelian, G.; Zhao, J.; Baccara-Dinet, M.T.; Rader, D.J.; investigators, O.C.I., A phase III randomized trial evaluating alirocumab 300 mg every 4 weeks as monotherapy or add-on to statin: ODYSSEY CHOICE I. *Atherosclerosis,* **2016**, *254*, 254-262.
[71] Roth, E.; Moriarty, P.M.; Bergeron, J.; Langslet, G.; Manvelian, G.; Zhao, J.; Baccara-Dinet, M.T.; Rader, D.J., Efficacy and safety of the PCSK9 inhibitor alirocumab 300 mg every 4 weeks in patients with ASCVD. *Atherosclerosis,* **2017**, *263*, e102-e103.
[72] Stroes, E.; Guyton, J.R.; Lepor, N.; Civeira, F.; Gaudet, D.; Watts, G.F.; Baccara-Dinet, M.T.; Lecorps, G.; Manvelian, G.; Farnier, M.; Investigators, O.C.I., Efficacy and Safety of Alirocumab 150 mg Every 4 Weeks in Patients With Hypercholesterolemia Not on Statin Therapy: The ODYSSEY CHOICE II Study. *J Am Heart Assoc,* **2016**, *5*, (9).

[73] Ridker, P.M.; Revkin, J.; Amarenco, P.; Brunell, R.; Curto, M.; Civeira, F.; Flather, M.; Glynn, R.J.; Gregoire, J.; Jukema, J.W.; Karpov, Y.; Kastelein, J.J.P.; Koenig, W.; Lorenzatti, A.; Manga, P.; Masiukiewicz, U.; Miller, M.; Mosterd, A.; Murin, J.; Nicolau, J.C.; Nissen, S.; Ponikowski, P.; Santos, R.D.; Schwartz, P.F.; Soran, H.; White, H.; Wright, R.S.; Vrablik, M.; Yunis, C.; Shear, C.L.; Tardif, J.C., Cardiovascular Efficacy and Safety of Bococizumab in High-Risk Patients. *N Engl J Med,* **2017**, *376*, (16), 1527-1539.
[74] Essalmani, R.; Susan-Resiga, D.; Chamberland, A.; Abifadel, M.; Creemers, J.W.; Boileau, C.; Seidah, N.G.; Prat, A., In vivo evidence that furin from hepatocytes inactivates PCSK9. *J Biol Chem,* **2011**, *286*, (6), 4257-4263.
[75] Han, B.; Eacho, P.I.; Knierman, M.D.; Troutt, J.S.; Konrad, R.J.; Yu, X.; Schroeder, K.M., Isolation and characterization of the circulating truncated form of PCSK9. *J Lipid Res,* **2014**, *55*, (7), 1505-1514.
[76] Schroeder, K.M.; Beyer, T.P.; Hansen, R.J.; Han, B.; Pickard, R.T.; Wroblewski, V.J.; Kowala, M.C.; Eacho, P.I., Proteolytic cleavage of antigen extends the durability of an anti-PCSK9 monoclonal antibody. *J Lipid Res,* **2015**, *56*, (11), 2124-2132.
[77] Lipovsek, D., Adnectins: engineered target-binding protein therapeutics. *Protein engineering, design & selection : PEDS,* **2011**, *24*, (1-2), 3-9.
[78] Mitchell, T.; Chao, G.; Sitkoff, D.; Lo, F.; Monshizadegan, H.; Meyers, D.; Low, S.; Russo, K.; DiBella, R.; Denhez, F.; Gao, M.; Myers, J.; Duke, G.; Witmer, M.; Miao, B.; Ho, S.P.; Khan, J.; Parker, R.A., Pharmacologic profile of the Adnectin BMS-962476, a small protein biologic alternative to PCSK9 antibodies for low-density lipoprotein lowering. *J Pharmacol Exp Ther,* **2014**, *350*, (2), 412-424.
[79] Stein, E.A.; Kasichayanula, S.; Turner, T.; Kranz, T.; Arumugam, U.; Biernat, L.; Lee, J., LDL Cholesterol Reduction with BMS-962476, an Adnectin Inhibitor of Pcsk9: Results of a Single Ascending Dose Study. *Journal of the American College of Cardiology,* **2014**, *63*, (12), A1372.
[80] Mullard, A., Nine paths to PCSK9 inhibition. *Nature reviews. Drug discovery,* **2017**, *16*, (5), 299-301.
[81] Frank-Kamenetsky, M.; Grefhorst, A.; Anderson, N.N.; Racie, T.S.; Bramlage, B.; Akinc, A.; Butler, D.; Charisse, K.; Dorkin, R.; Fan, Y.; Gamba-Vitalo, C.; Hadwiger, P.; Jayaraman, M.; John, M.; Jayaprakash, K.N.; Maier, M.; Nechev, L.; Rajeev, K.G.; Read, T.; Rohl, I.; Soutschek, J.; Tan, P.; Wong, J.; Wang, G.; Zimmermann, T.; de Fougerolles, A.; Vornlocher, H.P.; Langer, R.; Anderson, D.G.; Manoharan, M.; Koteliansky, V.; Horton, J.D.; Fitzgerald, K., Therapeutic RNAi targeting PCSK9 acutely lowers plasma cholesterol in rodents and LDL cholesterol in nonhuman primates. *Proc Natl Acad Sci U S A,* **2008**, *105*, (33), 11915-11920.
[82] Fitzgerald, K.; Frank-Kamenetsky, M.; Shulga-Morskaya, S.; Liebow, A.; Bettencourt, B.R.; Sutherland, J.E.; Hutabarat, R.M.; Clausen, V.A.; Karsten, V.; Cehelsky, J.; Nochur, S.V.; Kotelianski, V.; Horton, J.; Mant, T.; Chiesa, J.; Ritter, J.; Munisamy, M.; Vaishnaw, A.K.; Gollob, J.A.; Simon, A., Effect of an RNA interference drug on the synthesis of proprotein convertase subtilisin/kexin type 9 (PCSK9) and the concentration of serum LDL cholesterol in healthy volunteers: a randomised, single-blind, placebo-controlled, phase 1 trial. *Lancet,* **2014**, *383*, (9911), 60-68.
[83] Fitzgerald, K.; White, S.; Borodovsky, A.; Bettencourt, B.R.; Strahs, A.; Clausen, V.; Wijngaard, P.; Horton, J.D.; Taubel, J.; Brooks, A.; Fernando, C.; Kauffman, R.S.; Kallend, D.; Vaishnaw, A.; Simon, A., A Highly Durable RNAi Therapeutic Inhibitor of PCSK9. *N Engl J Med,* **2017**, *376*, (1), 41-51.
[84] Ray, K.K.; Landmesser, U.; Leiter, L.A.; Kallend, D.; Dufour, R.; Karakas, M.; Hall, T.; Troquay, R.P.; Turner, T.; Visseren, F.L.; Wijngaard, P.; Wright, R.S.; Kastelein, J.J., Inclisiran in Patients at High Cardiovascular Risk with Elevated LDL Cholesterol. *N Engl J Med,* **2017**, *376*, (15), 1430-1440.