Dyslipidaemias and cardiovascular disease: focus on the role of PCSK9 inhibitors

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Abstract

Genetic, experimental and clinical studies have consistently confirmed that inhibition of proprotein convertase subtilisin/kexin type 9 (PCSK9) can result in significant LDL-C lowering, and two fully human PCSK9 mononclonal antibodies have received regulatory approval for use in high-risk patients. Co-administration of PCSK9 with statins has resulted in extremely low LDL-C levels with excellent short-term safety profiles. While results from Phase III clinical trials provided exciting evidence about the role of PCSK9 inhibitors in reducing cardiovascular event rates,their impact on mortality remains less clear. PCSK9 inhibitor therapy can be considered for high risk patients who are likely to experience the largest cardiovascular risk reduction benefit.

Keywords

PCSK9, Proprotein Convertase Subtilisin/Kexin type 9, Alirocumab, Evolocumab, LDL-cholesterol, monoclonal antibodies

Introduction

Genetic and epidemiological studies have consistently confirmed the causal role of low density lipoprotein (LDL-C) in the pathogenesis of atherosclerosis, and lowering of LDL-C has been the main target for both primary and secondary prevention strategies [1-4]. Among the different antilipidemic medications, the 3-hydroxy-methylglytaryl coenzyme A reductase inhibitors (HMG-CoA; statins) have been the most widely prescribed drugs [5-6]. LDL-C lowering up to 30-50% has been reported depending on the potency of statin used and this reduction translated in improved cardiovascular (CV) outcomes [7-8]. In 2005, the meta-analysis of Cholesterol Treatment Trialists' (CTT) collaboration documented that lowering of LDL-C by 1 mmol/L (38.7 mg/dl) corresponds to a 20% reduction in major vascular events [9]. Five years later, the same team revealed that an additional 0.5 mmol/L reduction in LDL-C using intensive statin therapy was associated with a 28% reduction in the incidence of vascular events [10]. Following these data, the treatment targets for LDL-C were updated in prevention guidelines to reflect the common knowledge that the lower LDL-C, the better the CV benefit [6, 8, 11-14] (Table 1). Further analysis of statin trials, however, has revealed that more than 40% of high risk patients fail to reach the LDL-C recommended targets of <1.8mmol/L (70mg/dL) with statins, and this has opened the pipeline for the use and development of additional LDL-C lowering compounds [15-17].

Over the last few years, pharmacological developments have been remarkable to meet primarily the needs for patients who are intolerant to statins [18-22]. The cholesterol absorption inhibitor ezetimibe was approved for clinical use in 2003, but it was only in 2015 that the results of the IMPROVE-IT trial confirmed that administration of ezetimibe to simvastatin 40mg/d in patients with a recent acute coronary syndrome resulted in a 24% additional lowering of LDL-C and a 13% relative reduction in myocardial infarction [23-24]. Since then, new LDL-C lowering therapies have been approved as adjunctive therapy to statins. Lomitapide and mipomersen are approved for use in patients with homozygous familial hypercholesterolaemia (FH), when standard therapy has been ineffective [25], and the proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors evolocumab and alirocumab have been approved for use in patients with FH and those with clinically evident CV disease with increased LDL-C levels

despite antilipidemic therapy [26-27]. In this review, we discuss the biology of PCSK9 receptors, summarize recent developments about their inhibition and document results from recent clinical trials.

The role of PCSK9 in modulating LDL-C metabolism (Figure 1a)

PCSK9 was unknown in the scientific community until 2003 when Abifadel et al linked new mutations found in PCSK9 gene in two French families with FH [28-29]. PCSK9 is expressed from a single gene on chromosome 1p32 as an inactive 692 amino acid (pro-PCSK9) [30]. The protein is translocated to the endoplasmic reticulum where it undergoes autocleavage and transformation to a mature PCSK9 protein. If the PCSK9 does not undergo autocleavage, it is not secreted in the circulation and has no function [31-33]. Circulating PCSK9 binds to the epidermal growth factor-like repeat A (EGF-A) domain on the LDL receptor (LDLR) [34-36]. When the LDLR is bound by PCSK9 in the extracellular space, the LDLR does not recycle to the cell surface and is targeted for lysosomal degradation thus reducing the number of LDLRs and the uptake of cholesterol from the circulation [37-39]. There are also data to suggest that apart from this extracellular pathway there might be an intracellular pathway that PCSK9 is acting on to enhance LDLR degradation [40-41].

The interaction of PCSK9 with LDLR is a dynamic process. PCSK9 is expressed in the hepatocyte and to a lesser extent in the brain, intestine and kidney [42]. Its expression is modulated by the transcription factor, sterol-responsive element binding protein (SREBP2) and by the hepatocyte nuclear factor 1a [43-47], [48-49]. These factors are responsive to the cholesterol concentration in the hepatocyte. When the cholesterol concentration falls below a certain level, the transcription factors upregulate HMG-CoA reductase and LDLR to increase the cholesterol level in the hepatocyte. At the same time, newly synthesized PCSK9s are secreted to limit this process. Statins promote the upgregulation of both PCSK9 and LDLR and clinical data suggests that co-administration of statins and PCSK9 inhibitors result in greater LDL-C lowering [50-51]. The interaction between PCSK9 and LDLR at the cell surface does not take place in the adrenals [52] and in some areas of the brain [53-54], thus these cells will not be deprived of cholesterol following administration of drugs that alter the activity of PCSK9.

In population studies, PCSK9 plasma levels vary during the course of the day with higher levels reported early hours in the morning and lower levels in the afternoon [55]. Females have higher PCSK9 levels compared to males and

this difference is amplified with increasing age [56-57]. PCSK9 plasma levels are also influenced by the expression of another receptor sortilin, which is involved in the intracellular trafficking of PCSK9 [57-58]. Decrease in sortilin expression leads to low circulating PCSK9 levels and reduced LDL-C levels [59]. Increased body mass index, diet and exercise are major modulators of sortilin receptor [60].

From PCSK9 discovery to PCSK9 inhibition as a novel lipid lowering therapy (Figure 1b)

Following the discovery of PCSK9 gene, a number of PCSK9 mutations have been identified [61-63]. Gain of function mutations result in tighter binding affinity between PCSK9 and LDLR or increased PCSK9 levels and increased LDL-C blood levels [32, 64-65]. Other "loss of function" mutations result in lower affinity binding between PCSK9 and LDLR or lower levels of functional PCSK9 and have been associated with reduced blood cholesterol levels [66-67]. In 2006, Cohen et al confirmed this principle by demonstrating that carriers of "loss of function" mutations had lower LDL-C levels (mean levels 53-88 mg/dl) compared to those without the mutations and their CV risk was reduced up to 88% [68]. Rare cases of healthy individuals who had two "loss of function" mutations in PCSK9 gene and extremely low plasma PCSK9 and LDL-C levels (mean levels 14 to 16mg/dl) have also been identified and provided the proof of concept about the safety of extremely low LDL-C levels [69]. These reports were reassuring and have prompted the extensive research in the development of PCSK9 inhibitors.

Both intracellular and extracellular approaches of PCSK9 inhibition have been reported in animal and clinical studies [70-78] and two categories are under clinical development; monoclonal antibodies (mAbs) for PCSK9 and small interfering RNAs (siRNAs) [79-81], [82-83](Table 2). Monoclonal antibodies bind selectively to extracellular PCSK9 and prevent its interaction with LDLR, thus allowing - for a period of time - the hepatic uptake of circulating LDL-C particles [83-84]. Two human PCSK9 mAbs (alirocumab, evolocumab) have been developed and already approved for use in USA and EU [26-27]. These share different pharmacokinetic and pharmacodynamic characteristics, and a number of clinical trials on different populations will report on their long-term efficacy and safety [85-86], [87-88] (Table 3). Another humanized mAb, bococizumab has also been composed and studied, but its use was halted during phase III clinical trials due to high immunogenicity, injection site reactions and attenuation of LDL-C lowering effect over time [89].

The siRNAs can enhance the degradation of the messenger RNA and suppress the synthesis of proteins [90]. Inhibition of PCSK9 by long-acting RNA interference occurs solely in the liver and has been associated with significant reductions in PCSK9 and LDL-C levels, and this effect appears to be dose related [81, 91-92]. Data also suggests that a single siRNA-bound RNA-induced silencing complex is catalytic and cleaves many transcripts. These compounds, when administered together with statins, are likely to have a greater effect considering that the latter upregulates PCSK9 levels [79, 91]. Inclisiran is one of those long-acting siRNAs, which is delivered subcutaneously (SC) and is directed against PCSK9. Initial clinical studies (ORION-1) suggested that inclisiran administration is safe with only mild to moderate reported side effects [91]. In patients who had high risk of CV disease and high LDL-C levels despite receiving the maximum possible dose of statin therapy, inclisiran therapy resulted in greater reduction in LDL-C cholesterol levels at 180 days than did placebo. The greatest reduction (52.6%) was observed in association with the two-dose 300mg SC administration [93]. Further developments in the field of PCSK9 inhibition are expected in the form of vaccines, small molecule inhibitors and antibodies with longer effectiveness [91, 94].

Efficacy of PCSK9 mAb on LDL-C lowering in different populations: Evidence from clinical trials (Table 4)

A large number of clinical trials with PCSK9 mAbs have now been completed and have demonstrated that these agents lead to substantial reductions in LDL-C when administered as monotherapy or in combination with statins and/or ezetimibe to patients with non FH and FH. Phase I and II trials on efficacy of evolocumab demonstrated that dosing patterns of 140 mg every two weeks (Q2W) or 420 mg once a month (QM) caused LDL-C reduction up to 66% and 50% respectively [95-104].

Following these encouraging results, PROFICIO constitutes the completed phase III programme evaluating the efficacy of evolocumab. The Phase III data demonstrated that evolocumab monotherapy led to mean LDL-C reductions of 69% compared with placebo on two dose regimens (140 mg Q2W and 420 mg QM) [105-106]. Adding evolocumab to statin background therapy with or without other lipid lowering agent (ezetimibe) reduced LDL-C by up to 57% more than placebo and 40% more than ezetimibe at week 12 [107]. The extended therapy to 52 weeks (DESCARTES) with the co-administration of statins resulted in up to 62% LDL-C reduction [108, 109]. Further studies (FOURIER, OSLER 2, Tesla part B and TAUSSING) will provide long-term results about LDL-C levels with the use of evolocumab [110-112]. The RUTHERFORD-2 study, which involved participants with heterozygous FH on statins and/or ezetimibe, reported that evolocumab co-administration (140mg Q2W or 420mg QM) was associated with 60% reduction in LDL-C levels at 12 weeks regardless of the nature of the underlying LDLR mutation [113]. A recent meta-analysis of 8 randomized controlled trials (RCT) confirmed that evolocumab therapy

at 140 mg Q2W has been more efficacious in modulating LDL-C levels and other lipid parameters compared to 420 mg QM [114]. However, differences in the curative effects using the two evolocumab treatment doses remains to be determined.

Similar data were shared from Phase I and II clinical trials with Alirocumab in FH and non FH patients [86, 96, 115-116]. In most patients, the initial administration of 75mg alirocumab Q2W SC followed by uptitration to 150 mg Q2W in patients who could not achieve their LDL-C lowering goal, was regarded as an effective dosage regime [115-117]. Using alirocumab as monotherapy, a 50% reduction in LDL-C has been reported and when used as combined therapy with statins, LDL-C was reduced up to 65% [96]. ODYSSEY is a clinical Phase III trial program, which includes 18 clinical trials focusing on long-term efficacy and safety of alirocumab [118-129]. Monotherapy with 75/150 mg alirocumab resulted in reduction of LDL levels by 56.4 - 58.2% compared to placebo and by 30.5 - 31.6% compared to ezetimibe. These LDL-C lowering effects improved from 45.9% to 64.1% following co-administration with statin therapy compared to placebo, but no change was shown compared to ezetimibe [119-120, 130]. Consequently, alirocumab, in combination with different lipid lowering treatments, reduces significantly LDL-C concentration and resulted in about 82% of patients to achieve the European LDL-C-targets [122-123, 125-126, 131-132]. The ODYSSEY DIABETES trial addresses the impact of alirocumab along with high intensity statin therapy in patients at increased CV risk with Type 1 and Type 2 diabetes (T2D). In the T2D group a 75 mg dose of alirocumab Q2W resulted in up to 50% LDL-C reduction at week 24 [133] and with this dosage pattern 80% of patients achieved the recommended LDL-C target [134]. In ODYSSEY FH I and FH II, treatment with alirocumab 75mg Q2W in addition to a maximally tolerated statin dose, with or without other lipid lowering agent, was associated with significant reduction (up to 49%) in LDL-C at week 24, and these low LDL-C levels were maintained over a period of 52 weeks [127]. The results of the ODYSSEY ESCAPE trial suggested a potential role for alirocumab in patients with heterozygous FH who are refractory to treatment and receive regular lipoprotein apheresis therapy. In those patients, alirocumab not only reduced plasma LDL-C levels but also delayed the need for apheresis [129].

Pleiotropic effects of PCSK9 mAbs

In addition to lowering LDL-C levels, off-target actions with the use of PCSK9 inhibitors have been identified. With alirocumab therapy, levels of apo B were reduced by 37-54% whilst non– high-density lipoprotein cholesterol (non-HDL-C) decreased by 41-52% in ODYSSEY LONG-TERM and ODYSSEY MONO trials respectively [125, 135]. In the LAPLACE trial, evolocumab was associated with a 12% reduction in triglycerides in the Q2W regime and

with 6–16% reduction in the QM dosage regime. High-density lipoprotein cholesterol (HDL-C) levels were modestly increased (5–10%) in both groups [106]. A decrease in cholesterol ester transfer protein (CETP) activity as well as decreases in the concentrations of triglyceride rich lipoproteins and LDL-C particles with the use of PCSK9 mAbs may all contribute to the reported increases in HDL-C levels [136-137]. Interestingly, the results of a recent meta-analysis, suggested that 140mg evolocumab therapy administered Q2W had a better effect on the levels of non-HDL-C and ratio of apolipoprotein B to apolipoproteinA1 (apoB/ApoA1) compared to the 420mg QM at 12 weeks of follow up [114]. Significant dose-dependent reductions in lipoprotein a [Lp(a)] up to 30% were also noted in clinical trials of alirocumab and evolocumab [138-139]. Increased clearance of Lp(a) via the LDLR [140] and direct actions on Lp(a) hepatic synthesis, secretion and/or assembly [141-143] have been proposed as possible mechanisms which can explain the noted associations. These data are exciting considering that Lp(a) is an independent risk factor for CV disease and as yet there is no effective Lp(a) lowering therapy available.

Apart from lipid lowering actions, PCSK9 inhibitors have also been shown to modulate inflammatory pathways in experimental studies. In a randomized controlled trial of a murine peritonitis model, PCSK9 inhibitors decreased pro-inflammatory cytokines [144]. Consistent with this finding, increases in LDL-C related protein 1, which facilitates degradation of bacterial toxins and apoptotic cell debris, with PCSK9 inhibition, have been reported in experimental studies confirming a potential anti-inflammatory action for these drugs [144-145]. PCSK9 is a critical regulator of the expression of proinflammatory cytokines in the atheromatous plaques [146] and its overexpression is associated with increased aortic plaque infiltration by macrophages in mice [147]. Serum PCSK9 levels are linearly associated with the fraction and amount of necrotic core tissue in coronary atherosclerosis [148] which suggests that targeting PCSK9 might be beneficial for the treatment of atherosclerotic disease. However, despite the wealth of experimental data which supports the link between inflammatory pathways and PCSK9 inhibition, there is no clinical data as yet to support this association [149-150].

PCSK9 and atherosclerotic disease

In preclinical studies, Chan et al demonstrated in asymptomatic individuals that PCSK9 levels are associated with carotid intima media thickness [151]. In another study, in asymptomatic patients with FH, PCSK9 levels were independently predictive of coronary artery calcium score [152]. In the Chinese Multi-provincial cohort study, serum PCSK9 levels in asymptomatic people were associated with progression of atherosclerosis as reflected by total plaque area [153]. Using intravascular ultrasound (IVUS) serially, reduction of atherosclerotic burden was

documented when LDL-C levels of <1.8mmol/L were achieved in statin trials [154-155]. In the IVUS GLAGOV trial, it was demonstrated that further LDL-C lowering by evolocumab on top of background optimized statin therapy for a period of 18 months led to additional 1% atheroma regression in 846 patients [156]. In more advanced stages of atherosclerosis, PCSK9 levels were associated with necrotic core fraction in coronary plaque [151].

Given the results from experimental and clinical studies, which demonstrate a link between PCSK9 and atherosclerosis [157], one can hypothesize that PCSK9 serum levels could be a useful biomarker for atherosclerotic disease risk stratification [158]. Results, however from both primary and secondary prevention clinical trials have provided inconsistent results about the utility of PCSK9 as a routine clinical biomarker [149, 158-162]. To shed some light on this discrepancy, Vlachopoulos et al conducted a meta-analysis of 9 longitudinal studies [150]. Although the results of this meta-analysis demonstrated a modest but yet significant positive association between PCSK9 plasma levels and risk of total CV events, routine measurement of PCSK9 as a means to monitor atherosclerotic disease progression cannot be recommended.

PCSK9 inhibitors and Cardiovascular outcomes

The benefit of PCSK9 inhibition on CV outcome has been demonstrated in different populations. In the FOURIER trial, evolocumab treatment reduced significantly both LDL-C levels and the risk for major CV events [110]. The magnitude of this effect was independent of the presence or absence of diabetes at baseline [163]. More recently, as part of the FOURIER study, in patients with peripheral arterial disease, evolocumab therapy added to moderate to high intensity statin therapy, reduced the primary end point by 21% and the composite of CV death, myocardial infarction or stroke by 27% [108, 164]. It is interesting also to note that patients from the FOURIER trial had significant reductions in CV events even with a short-term follow up, thus it is likely that a greater clinical benefit with longer-term follow up can be anticipated [156, 165]. This hypothesis, however, will have to be confirmed.

As far as alirocumab therapy is concerned, a post hoc analysis of 10 ODYSSEY trials, which included patients randomized to alirocumab 75/150mg or control, demonstrated that the greater % reduction in LDL-C and the lower the achieved LDL-C levels, the lower the incidence of major adverse events (MACE) [166]. Similar results have been presented in another post hoc analysis which showed a reduction of MACE incidence by 48% in hypercholesterolemic patients who were receiving alirocumab therapy compared with those on placebo [125]. In the ODYSSEY OUTCOME trial, the risk of recurrent ischemic CV events was lower among those who received

alirocumab therapy than among those who received placebo[167]. In this trial, in comparison to FOURIER trial, a greater percentage of patients were on high intensity statin therapy and follow up was for 2.8 years.

Results from the SPIRE CV outcome studies were also reported recently. In these studies, 27438 patients at increased CV risk were assigned to receive bococizumab therapy (150mg SC every 2 weeks) or placebo. The primary endpoints were non-fatal myocardial infarction, stroke, hospitalization from angina requiring revascularization and CV death. In SPIRE 1 study, patients had baseline LDL-C >1.8mmol/L whereas in SPIRE 2 study, baseline LDL-C levels were >2.6mmol/L [168-169]. Although the SPIRE programme was stopped prematurely due to anti-drug antibody formation, it is worth reporting that a 21% reduction in the incidence of MACE was reported over a 12 month period in SPIRE 2 trial [168]. Regardless of discontinuation of bococizumab development, these results are in agreement with the results of the FOURIER trial and support the beneficial role of PCSK9 inhibitors on CV outcome.

Finally, analysis of data from 14 studies with genetic information showed that every unit reduction in LDL-C was associated with similar reduction in the incidence of CV events and diabetes risk in patients with PCSK9 or HMGCR variants [170]. These results emphasize the need for lower LDL-C levels to further reduce CV events.

Safety and tolerability

The short-term safety and tolerability profile of the PCSK9 mAbs is promising as no significant adverse events have been reported in Phase II and III clinical trials. The safety analysis for evolocumab was integrated in the PROFICIO study [171]. Evolocumab therapy was not associated with significant risk for hepatotoxicity, muscle-related adverse events, or neurocognitive problems. Regarding cognitive function assessment, EBBINGHAUS, a sub-study of the FOURIER study is the first prospective study designed to evaluate any potential relationship between PCSK9 inhibitors and changes in memory, attention, and reaction time by using a standardized Cambridge Neuropsychological Test rather than relying on self-reported symptoms [172]. The recently presented results are reassuring as they show no significant differences in neurocognitive measures between the evolocumab and placebo treated groups [172]. Evolocumab administration, in the home-use setting, was associated with similar rates of injection-site reactions and adverse device effects as those seen in treatment groups from PROFICIO studies [173]. These results further confirm the safety of its use. Patients with liver impairment can be safely treated with evolocumab without the need for dose adjustment relative to their liver function and they demonstrate similar reductions in LDL-C to those seen in other patient groups [174]. Finally, pooled safety analysis of evolocumab

therapy in over 6000 patients from double blind and open label extension studies demonstrated that the incidence of non-neutralizing antibody binding, was between 0.1% -0.4% and the prolonged evolocumab therapy up to 48 weeks did not seem to enhance the development of this binding [171].

The safety profile of alirocumab therapy was confirmed in the analysis of 14 randomized controlled trials [175]. No significant difference in the incidence of musculoskeletal and neurologic and neurocognitive events was found between alirocumab treated groups and control groups. The incidence of abnormalities in liver function tests was also similar between groups whereas local injection site reactions were higher in alirocumab group (7.4%) compared to placebo (5.3%). A substantial difference in the occurrence of blurred vision linked to cataract was noticed in alirocumab-treated patients with baseline LDL-C < 25 mg/dl compared to those with LDL-C ≥ 25mg/dl [175-176]. Considering that lens have high cholesterol content, this finding was of concern. However, the results of a recent meta-analysis in patients > 18 years do not support a link between low LDL-C and cataract incidence [177]. The development of antibodies against alirocumab was also observed in a small number of patients but this was not related to the presence of any other clinical signs or symptoms [175].

The risk of diabetes development has also been a concern with PCSK9 inhibition. In patients with PCSK9 variants, LDL-C reduction of 1 mmol/l was associated with a 15-20% increased incidence of diabetes [178]. This association, however, was not confirmed in the published clinical trials of evolocumab and alirocumab [179]. In FOURIER study, evolocumab did not increase the risk of new-onset diabetes or worsened glycemia over a median of 2.2 years of follow-up in patients without diabetes or pre-diabetes at baseline [163]. Similarly, no excess of new-onset diabetes associated with evolocumab use was documented in smaller, open label extension studies up to 4 years [180]. Further safety and tolerability data are awaited from the large long-term ongoing phase III trials.

Future clinical perspectives of the PCSK9 inhibitors

PCSK9 inhibition using mAbs is currently the most advanced and effective approach in achieving low LDL-C levels in conjunction with statin therapy and two PCSK9 mAbs (evolocumab and alirocumab) have received regulatory approval in many countries for treatment of severe hypercholesterolaemia. The high cost related to the use of PCSK9 antibodies and the requirement for multiple injections, however, presents a challenge for the widespread clinical use of these medications. From the available clinical outcomes trials, PCSK9 mAbs are justified for use only in patients with FH and individuals with a history of adverse cardiovascular event and high LDL-C due to statin intolerance or

resistance. Assuming that the results of the long-term large outcome trials will confirm the positive effects of PCSK9 inhibition on CV outcomes, a broader use of PCSK9 inhibitors is anticipated. Appropriate education and close monitoring of patients as well as further developments for alternative regimens with less frequent doses or other route of administration will be needed. The development of the novel agent, inclisiran is a step forward to this direction. In a phase I trial (ORION-1 study), the two-dose starting regime followed by a 6-monthly regimen led to robust sustained LDL-C reduction without the injection burden and without worsening glycaemia [93, 181]. The inclisiran-treated groups also had lower apoB, non-HDL-C, and Lp(a) but higher HDL-C [182]. The results of the ORION-4 outcomes study, however will determine whether these initial results can translate into significant CV benefits. Finally, although the scientific community is accepting that the lower LDL-C the better, further studies are needed to confirm the results of the genetic studies, which suggest the longer exposure to lower LDL-C the better the CV outcome, and these will provide the foundation for the widespread use of PCSK9 inhibitors in primary prevention.

Conclusions

The discovery of PCSK9 inhibitors has been an exciting advance in anti-lipidemic therapy. Fully human PCSK9-mAbs offer a stable 60% mean LDL-C reduction either as an adjunct to statin therapy or as monotherapy and have been associated with reduction in the incidence of CV events in high risk populations. Although the results of long-term large ongoing studies of PCSK9 mAbs are pending, the available data on efficacy and safety are reassuring and suggest that treatment with PCSK9 inhibitors has the potential to transform the prognosis of patients with hypercholesterolemia in the presence or absence of established CV disease.

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Figure legend

Figure 1:

A. Low density lipoprotein-cholesterol (LDL-C) metabolism in the presence of proprotein convertase subtilisin/kexin type 9 (PCSK9). PCSK9 is synthesized in the liver and then is secreted and circulates in the plasma where it binds to LDL receptors (LDLR). The PCSK9 & LDLR complex is internalized and undergoes degradation in lysosome. A decrease in LDLR results in an increase of serum LDL-C. Following statin therapy, decreased hepatic cholesterol as a result of 3-hydroxy-3-methyl-glutaryl-coenzyme (HMG-CoA) reductase inhibition, leads to activation of sterol-responsive element binding protein (SREBP) and enhanced PCSK9 expression.

B. Intrahepatic and extrahepatic targets of PCSK9 inhibition: This figure demonstrates different areas of PCSK9 inhibition []

a. anti-sense oligonucleotides (ASO) bind to PCSK9 messenger ribonucleic acids (mRNA). As a result of this binding mRNA is degraded or translation of mRNA to protein is prohibited.

b. Small interfering ribonucleic acids (siRNA) can block transcription of PCSK9 mRNA.

c. Human monoclonal antibodies, adnectins & mimetic peptides can bind to PCSK9, preventing PCSK9 from binding to LDLR. After endocytosis, few receptors are degraded in the lysosome and more LDLR is recycled back to the surface of the cell.

Table 1. Differences among major guidelines and recommendations

Organisation	Cardiovascular risk scoring	Recommended treatment	LDL target	Other lipid parameter target (for treatment of Follow-Up)	PCSK9 inhibitors
ACC/AHA 2016 [13]	Pooled Cohort Equations	High or moderate intensity statin according to the level of ASCVD	LDL-C reduction by 30-50% or ≥ 50%	Non-HDL	Recommended as a second non statin agent
NLA consensus 2015/2017 [8,12]	Framingham Risk Score	Statins: mainstay treatment	Treatment goal <100 mg/dL and < 70 mg/dL for LDL-C in individuals at low, moderate or high, and at very high risk ASCVD risk, respectively	Co-primary therapeutic targets for non-HDLc: <130 and <100 mg/dL in individuals at low, moderate or high and at very high ASCVD risk, respectively	Recommended as a second non statin agent
NICE 2014 [14]	QRISK2	Atorvastatin 20 mg for primary and 80 mg for secondary prevention	Use of specific statin and dose in primary and secondary prevention*	Non HDL as a marker in follow-Up	Recommended as a second non statin agent
ESC/EAS 2016 [6]	SCORE charts	Statins	The target for LDL-C levels was 2.6 mmol/l in high risk population and 1.8 mmol/L in very high risk population	Non-HDL-C and ApoB should be considered as a secondary target treatment	Recommended as a second non statin agent
CCS 2016 [11]	Modified Framingham	Statins: first-line therapy	LDL-C <2 mmol/l or 50% LDL-C reduction	Non HDL and apoB: alternate agents	Recommended as a second non statin agent
			* 20%–30%: low intensity 31%–40%: medium intensity > 40%: high intensity		

ACC/AHA, American College of Cardiology/American Heart Association; Apo-B: Apolipoprotein B; ASCVD: atherosclerotic cardiovascular disease; CCS, Canadian Cardiovascular Society; ESC/EAS, European Society of Cardiology/European Atherosclerosis Society; HDL-C: high-density lipoprotein cholesterol LDL-C: low-density lipoprotein cholesterol; NICE, National Institute for Health and Care and Excellence; NLA, National Lipid Association; PCSK9, Proprotein convertase subtilisin/kexin 9; SCORE, Systematic Coronary Risk Evaluation

Table 2. Pathways of PCSK9 inhibition

Mechanisms	Agents

Binding and blocking of the complex PCSK9-LDLR	**Small mimetic peptides** [70-72] - short amino acid sequences mimicking the EGF-A, catalytic domain, prodomain, or C-terminal domain of PCSK9 - SX-PCSK9: Peptide-mimetic inhibitor binding to the EGF-A domain of PCSK9 (preclinical phase) **Adnectins** [73] - BMS-962476: In first human trial, 60/64 subjects completed the 46 day study; LDL-C reduction up to 48% occurred between days 4 and 14. **Monoclonal antibodies (mAbs)** - fully humanized and humanized mAbs [26,27] - other mAbs: LY3015014 [82]
Reduction of hepatic PCSK9 synthesis/ Inhibition of PCSK9 expression	**Small molecules** [74,75]: berberine **Antisense oligonucleotides** (ASO) [76]: - In mice, administration of ASO:ISIS 394814 reduced LDL-C up to 32%. - A phase I clinical trial was terminated due to safety concerns. **Small interfering RNAs** (siRNA)=> ALN-PCS02/inclisiran [79-81], [91]: - phase 1 human trial: a mean 40% reduction of LDL-C over 84 days - results of ORION-1: time-averaged reduction in LDL-C was 39% and 46% after one and two injections of inclisiran 300 mg respectively - ORION- 4: an ongoing clinical trial of inclisiran to evaluate cardiac outcomes in high risk patients **Clustered Regularly Interspaced Short Palindromic Repeats (**CRISPR**)/CRISPR associated protein 9** (Cas9) [78] - gene-editing strategy to correct aberrant PCSK9 gene sequence; - In mice, plasma cholesterol levels were reduced by 35%–40%.
Interference on PCSK9 secretion	Two specific mediators targets: **Sortilin, Sec2a** [58,59]

EGF-A: epidermal growth factor precursor homology domain A; mAbs: monoclonal antibodies; LDL-C: low-density lipoprotein cholesterol PCSK9: Proprotein convertase subtilisin kexin-like 9

Table 3. Pharmacokinetic and pharmacodynamics of available human PCSK9 monoclonal antibodies.

	Alirocumab [85, 86]	**Evolocumab** [87,88]
Chemistry	Human G1 isotype immunoglobulin	Human G2 isotype immunoglobulin

Dosage	starting dose 75 mg Q2W, if response inadequate up to 150mg Q2W	140 mg Q2W or 420 mg QM
Administration	SC	SC
Absorption	Median Tmax:3-7 days; absolute bioavailability: 85%;	Median Tmax: 3-4 days; absolute bioavailability: 72%;
Distribution	0.04-0.05 L/kg	3.3 L/kg
Metabolism	Effective half life: 17-20 days	Effective half life: 11-17 days
Pharmacodynamics	After a single SC dose of 75 or 150 mg, max PCSK9 suppression within 4-8 hrs	After a single SC dose of 140 or 420 mg, max PCSK9 suppression within 4 hrs
		Mean nadir in LDL-C lowering effect by 14 and 21 days
		Both dosage regimens QM => same average LDL-C lowering level
	Free PCSK9 concentrations back to baseline when antibodies levels were below the quantitation limit	Free PCSK9 concentrations back to baseline when antibodies levels were below the quantitation limit

LDL-C: low-density lipoprotein cholesterol; PCSK9: Proprotein Convertase Subtilisin/Kexin type 9; Q2W: twice a week; QM: once a month; SC: subcutaneously; T max: the time taken to reach the maximum concentration

Table 4. Clinical trials of PCSK9

PHASE I							
Evolocumab (EVO)	**Study name/ Reference**	**Population type & background therapy**	**Medical treatment**	**Duration of treatment**	**% LDL-C change from baseline to study end**	**Difference between % LDL-C change (Evol vs PL)**	**Impact on other lipid parameters: increase (+) or reduction (-)[§]**
	Dia et al. [95]	one arm: healthy adults (N=53)	EVO: 7 mg SC 21 mg SC 70 mg SC 210 mg SC 420 mg SC 21mg IV 420 mg IV PL	12 to 16 weeks	N/A	EVO > 21 mg: -64%*	EVO >21mg TC: (-) ApoB: (-)
		second arm: hyper-cholesterolemic adults on low to moderate-dose statins; 1 subject with HeFH (N=57)	EVO SC: 14mg QW 35mg QW, 140 mg Q2W 280 mg Q2W 420 mg QM PL	6 or 8 weeks	mean LDLc levels - 60%[I]	mean LDLc levels -75%[I]	TC: (-) ApoB: (-) Lpa: (-)
Alirocumab (ALI)	**Study name/ Reference**	**N & population type**	**Medical treatment**	**Duration of treatment**	**% LDL-C change from baseline to study end**	**Difference between % LDL-C change (ALI vs PL or ALI vs atorvastatin or diet)**	**Impact on other lipid parameter: increase (+) or reduction (-)[§]**

	Stein et al [96] NCT01026597	40 healthy individuals without CVRF	0.3 mg/kg IV 1 mg/kg IV 3 mg/kg IV 6 mg/kg IV 12 mg/kg IV PL	single dose	─ 34.2% ─ 48.3% ─ 63.5% ─ 62.8% ─ 71.5% + 6.1%		─ 28.1%[I] ─ 42.2%[I] ─ 57.4%[I] ─ 56.5%[I] ─ 65.4%[I]		N/A
	Stein et al [96] NCT01074372	32 healthy individuals without CVRF	0.3 mg/kg SC 1 mg/kg SC 3 mg/kg SC 6 mg/kg SC 12 mg/kg SC PL	single dose	─ 45.8% ─ 53.2% ─ 51.8% ─ 59% + 13.3%		─ 32.5%[¥] ─ 39.9%[¥] ─ 38.5%[¥] ─ 45.7%[¥]		N/A
	Stein et al [96] NCT01161082	21 individuals with HeFH on Atrovastatin (A) therapy & 40 non HeFH individuals on **Atorvastatin** Therapy (**A**) or **Diet** Only (**D**)	 50 mg SC 100 mg SC 150 mg SC PL	dose on day 1, day29 & day 43	Pts on: **A** N/A N/A ─ 56.1% + 4%	**D** N/A N/A ─ 54.7% + 3.3%	**HeFH/nonHeFH** on: **A** ─ 39.2%[I] ─ 53.7%[I] ─ 61%[I]	**D** N/A N/A ─ 57% (p=0.0023)	TC: (─) ApoB: (─) Lpa: (─)
PHASE II									

Evolocumab (EVO)	Study name/ Reference	N & population type	Medical treatment	Duration of treatment	% LDL-C change from baseline to study end	Difference between % LDL-C change (Evol vs EZE/PL/Statin)	Impact on other lipid parameters: increase (+) or reduction (-)[§]
	GAUSS-2 [97]	157 individuals with statin intolerance (FH and non FH)	EVO SC: 280 mg QM 350 mg QM 420 mg QM EVO 420 mg QM SC plus EZE EZE plus PL	12 weeks	 – 40.8% – 42.6% – 50.7% – 63% – 14.8%	 – 26%[I] – 27.8%[I] – 35.9%[I] – 47.3%[I]	 TC: (-) HDL: (+) ApoB: (–) ApoA1: (+) Lpa: (–)
	LAPLACE-TIMI 57 [98]	629 individuals (FH and non-FH) on statin combination therapy (± EZE)	EVO Q2W SC: 70 mg 105 mg 140 mg EVO QM SC: 280 mg 350 mg 420 mg PL	12 weeks	 – 48% – 64% – 69% – 50% – 62% – 62%	 – 41.8 %* – 60.2%* – 66.1% * – 41.8%* – 50%* – 50.3%*	 TC: (–) HDLc: (+) TG: (–) VLDLc: (–) non-HDLc: (–) apoB: (–)

	MENDEL [99]	406 individuals (FH and non-FH) in the absence of concurrent lipid-lowering treatment	EVO Q2W SC 70 mg, 105 mg 140 mg matching PL; EVO QM SC 280 mg 350 mg 420 mg PL or EZE 10 mg OD	12 weeks	 — 41% — 43.9% — 50.9% — 39% — 43.2 — 48%	vs PL* vs EZE* – 37.3% – 26.7% – 40.2% – 29.6% – 47.2% – 36.7% – 43.6%, – 25.2% – 47.7% – 29.3% – 52.5% – 34.1%	 TC: (–) Lp(a): (—) VLDLc: (–) non-HDLc: (–) apoB: (–) apoA1: (+) HDLc: (+)[§ on EVO 140/420]
	RUTHERFORD [100]	167 HeFH individuals on statin combination therapy (± EZE)	EVO SC QM 350 mg, 420 mg PL	12 weeks	 — 42.7% — 55.2%	 — 43.8%[I] — 56.4%[I]	 TC: (–) HDLc: (+) Lp(a): (—) VLDVc: (-) non-HDLc: (-) apoB: (–) TG: (—)
	YUKAWA [101]	307 individuals with FH and non-FH on statin combination therapy (± EZE)	EVO SC 70 mg Q2W 140 mg Q2W 280 mg QM 420 mg QM PL Q2W or QM	12 weeks	 – 55.6% – 71.3% – 58.1% – 63.9%	 – 56.9%* – 71.7%* – 65.5%* – 68.7%*	 TC: (–) HDLc: (+) non-HDLc: (–) ApoB: (–) ApoA1: (+) TG: (—)

	OSLER-1 [102,103]	1324 individuals completed Phase II study (MENDEL-1, LAPLACE-TIME 57, GAUSS-1, RUTHERFORD-1, YUKAWA-1)	EVO 420 mg SC QM standard of care (SOC)	up to 5 years	─ 54%	─ 55%*	N/A
	TESLA Part A [104]	8 individuals with HoFH on stable drug therapy (statins, bile acid sequestrants, nicotinic acid, no lipoprotein apheresis)	EVO SC QM 420 mg followed by EVO SC Q2W 420 mg	2 treatment phases of 12 weeks	N/A N/A	−16.5% (p=0.0781) −13.9% (p=0.1484)	HDLc: (+) ApoB: (–) ApoA1: (+) TG: (─) Lp(a): (–) HDLc: (-) ApoB: (–) ApoA1: (+) TG: (─) Lp(a): (–)
Alirocumab (ALI)	**Study name/ Reference**	**N & population type**	**Medical treatment**	**Duration of treatment**	**% LDL-C change from baseline to study end**	**Difference between % LDL-C change (ALI vs EZE/PL/Statin)**	**Impact on other lipid parameters: increase (+) or reduction (-)[§]**
	Stein et al 2012 [117]	77 individuals with HeFH on statin therapy (with or without ezetimibe)	ALI SC QM 150 mg 200 mg 300 mg ALI 150 mg SC Q2W matching placebo Q2W	12 weeks	 – 28·8% – 31.5% – 42.5% – 67.9% + 10.6	 – 18.2%[¥] – 20.9%[¥] – 31.9%[¥] – 57.3%[¥]	 ApoB: (–) ApoA1: (+) non-HDLc: (─)

	Mc Kenney et al [116]	183 individuals at moderate to very high CV risk on statin therapy	ALI SC Q2W 50 mg 100 mg 150 mg ALI SC QM 200 mg 300mg matching PL Q2W	12 weeks	 – 39.6% – 64.2% – 72.4% – 43.2% – 47.7% -5.1	 – 34.5%* – 59.1%* – 67.3%* – 38,1%* – 42.6%*	TC: (–) ApoB: (–) Lp(a): (–) non-HDLc: (–) HLD ↓ on ALI200/300
	Roth et al [115]	92 individuals at moderate to very high CV risk on statin therapy	ALI 150 mg SC Q2W plus Atrovastatin 10 mg ALI 150 mg Q2W SC plus Atrovastatin 80 mg Atrovastatin 80mg plus placebo	8 weeks	– 66.2% - 73.2% - 17.3%	– 48.9%[l] – 55.9%[l]	 HDLc: (+) Lp(a): (–) TC: (–) apoA1: (-)

Phase III

Evolocumab (EVO)	Study name/ Reference	N & population type	Medical treatment	Duration of treatment	% LDL-C change from baseline to study end	Difference between % LDL-C change (EVO vs EZE/PL/Statin)		Impact on other lipid parameters: increase (+) or reduction (-)[$]
	GAUSS-2 [109]	307 individuals with statin intolerance (FH and non FH; non ezetimibe LLT)	EVO 140 mg Q2W	12 weeks	–56.1%	vs EZE: –38.1%[l]		ApoB: (–) Lp(a): (–) non-HDLc: (—)
			EVO 420 mg QM		–52.6%	–37.6%[l]		
			PL Q2W & EZE 10mg PO OD		–18.1%			
			PL QM and EZE 10mg PO OD		–15.1%			
	MENDEL-2 [107]	614 individuals (FH and non FH; non-background LLT)	EVO 140 mg SC Q2W	12 weeks	— 57%	vs. PL[l] — 57.1%	vs. EZE[l] — 39.3%	ApoB: (–) Lp(a): (–) non-HDLc: (-) HDLc: (+)
			EVO 420 mg QM		— 56.1%	— 54.6%	— 37.6%	
			PL Q2W					
			PL QM & EZE PO OD					

	DESCARTES [108]	901 individuals with varying range of CVR (FH and non FH) on diet ± statin (Atrovastatin 10mg/A10, Atrovastatin 80mg/A80) ± EZE	EVO 420 mg QM EVO 420 mg QM + diet EVO 420 mg QM + diet + A10 EVO 420 mg QM +diet +A80 EVO 420mg QM +diet +A80 +EZE PL SC QM	52 weeks	─57% ─ 55.7%, ─ 61.6% ─ 56.8% ─ 48.5% +6.8	─ 50.1%[I] ─ 51.5%[I] ─ 54.7%[I] ─ 46.7%[I] ─ 46.5%[I]	ApoB: (–) non-HDLc: (–) Lp(a): (–) TG: (–) HDL: (+) ApoA1: (+)
	TESLA Part B [111]	49 individuals with HoFH on stable drug therapy	EVO 420mg QM PL QM	12 weeks	– 23·1%	– 30·9% *	ApoB: (–)

	FOURIER [110]	27525 individuals with ASCVD on statin therapy (high, moderate or low intensity/ at least 20 mg Atrovastatin daily) ± EZE	EVO 140mg Q2W EVO 420mg QM PL	2.2 years	N/A	vs. PL: at 12 week – 61.1% – 56.9% Combined EVO pts at 68 weeks – 54%[l]	At 48 week non HDL: (-) apoB: (-) TG: (–) Lp(a): (–) TC: (-) HDL: (+) ApoA1: (+)

	LAPLACE-2 [106]	1896 individuals (with FH and non FH) on background statin therapy	EVO 140mg Q2W EVO 420mg QM PL and EZE PO	12 weeks	**EVO Q2W** A10 –61.6% A80 –61.8% S40 –65.9% R5 –60.1% R40 –58.9% **EVO QM** A10 –58.2% A80 –58.7% S40 –57% R5 –59.4% R40 –52.4%	**EVO Q2W** vs PL [§] A10 –71.4% A80 –76.3% S40 –70.6% R5 –68.2% R40 –68.3% **EVO Q2W vs. EZE** [§] A10 –39.6% A80 –47.2% **EVO QM** vs PL [§] A10 –59.2% A80 –70.5% S40 –60.4% R5 –64.5% R40 –55% **EVO QM vs. EZE** [§] A10 –41.1% A80 –38.9% :	Non-HDL: (–) ApoB: (–) HDLc: (+) TC: (–) Lp(a): (–)

	RUTHERFORD-2 [113]	329 individuals with HeFH on statin therapy ± EZE	EVO 140mg Q2W EVO 420mg QM PL	12 weeks	─ 61.3% ─ 55.7%	vs. PL: ─ 59.2%* ─ 61.3%*	Non-HDL: (-) ApoB: (-) TG: (-) HDLc: (+) Lp(a): (–) ApoA1: (+) HDL: (+)
	OSLER-2 [114]	3514 individuals completed phase III parent study	EVO 140mg Q2W EVO 420mg QM	up to 2 years	N/A	At 12 week ─64% Osler 1 &2 At week48 ─58.4%[l]	non-HDLc: (-) ApoB: (─) TC: (─) TG: (─) Lp(a): (─) HDL: (+) ApoA1: (+)
	TAUSSIG [112] (data from interim subset analysis of HoFH group N= 95)	310 individuals with severe FH (including HoFH) on background LLT (statin ± EZE ± lipoprotein apheresis)	**if not on apheresis:** EVO 420 mg QM Increase dosing to EVO 420 mg Q2W after 12 week **if on apheresis:** EVO 420 mg Q2W	up to 5 years	At 42 week: ─ 20.1% ─ 28.3% ─ 26.7%	At 42 week On apheresis & not on apheresis: -23.3%*	 Lp(a): (─) HDLc: (+)

	YUKAWA 2 [105]	202 individuals at high CVR randomized to Atrovastatin 5mg/A5 or Atrovastatin 20 mg/A20	EVO 140 mg Q2W EVO 420 mg QM PL	12 weeks	N/A	─ 74.9% to - 75.9%[I] ─ 69.9% to - 66.9%[I]	HDL: (+) ApoB: (─) Lp(a): (─) TG: (─) ApoA1: (+)
Alirocumab (ALI)	**Study name/ Reference**	**N & population type**	**Medical treatment**	**Duration of treatment**	**% LDL-C change from baseline to study end**	**Difference between % LDL-C change (ALI vs EZE/PL/Statin)**	**Impact on other lipid parameters: increase (+) or reduction (-)[§]**
	ODYSSEY MONO [118]	103 individuals at moderate CVR with no background LLT	ALI 75 mg titrated to 150 mg Q2W, if LDL target were not achieved at 12 week EZE PO 10 mg OD	24 weeks	─ 47.2% ─ 15.6%	─31.6%* (Excluding Pts post up-titration: ─ 28.7%)	ApoB: (-) non-HDLc: (-) TC: (-)
	ODYSSEY ALTERNATIVE [119]	314 Individuals at moderate to high CVR with statin intolerance on LLT	ALI 75 mg/150 mg Q2W if LDLc targets were not achieved at 12 week EZE PO 10 mg OD	24 weeks	─ 45% ─ 14.6%	─ 30.4%*	ApoB: (-) non-HDLc: (-) TC: (-) Lp(a): (-)

	ODYSSEY CHOICE II [120]	233 Individuals at moderate to high CVR with statin intolerance on LLT	ALI 150 mg ALI 75 mg/150 mg Q2W if LDLc target not achieved at 12 week PL Q2W	24 weeks	─ 51.7% ─ 53.5% + 4.7%	─ 56.4%* ─ 58.2%*	ApoB: (-) non-HDLc: (-) TC: (-) HDLc: (+) ApoA1: (+) Lp(a): (-)
	ODYSSEY COMBO I [121]	316 Individuals at moderate to very high CVR on statin ± LLT	ALI 75 mg/150 mg Q2W if LDLc target not achieved at 12 week; PL Q2W	52 weeks	─ 48.2% ─ 2.3%	─ 45.9%*	ApoB: (─) non-HDLc: (─) TC: (─) HDLc: (+) Lp(a): (-)
	ODYSSEY COMBO II [121]	720 Individuals at moderate to high CVR on statin therapy	ALI 75 mg/150 mg Q2W if LDLc target not achieved at 12 week; EZE PO 10 mg OD	104 weeks	─ 50.6% ─ 20.7%	─ 29.8%*	ApoB: (-) non-HDLc: (-) TC: (-) HDLc: (+) Lp(a): (-) ApoA1: (+)

	OSDYSSEY OPTIONS I [122]	355 Individuals at moderate to high CVR on statin therapy (Atrovastatin 20 mg/A20 or Atrovastatin 40 mg/A40)	ALI 75 mg/150 mg Q2W if LDLc target not achieved at 12 week EZE PO 10 mg OD (A20/A40) A40 (double A20) A80 (double A40) R40 (switch from A40)	24 weeks	─ 49% ─ 21.5% ─ 5% ─ 4.8% ─ 21.4%	N/A ─ 27.5%[l] ─ 44%[l] ─ 44.2%[l] ─ 27.6%[l]	N/A ApoB: (-) non-HDLc: (-) Lp(a): (-)
	ODYSSEY OPTIONS II [123]	305 Individuals at moderate to high CVR on statin therapy (Rosuvastatin 10mg/R10 or Rosuvastatin 20 mg/R20)	ALI 75 mg/150 mg Q2W if LDLc target not achieved at 12 week EZE PO 10 mg OD R20 (double R10) R40 (double R20)	24 weeks	ALI 75/150 R10/20 ─ 43.5% ─ 12.7% ─ 16.3% ─ 15.9%	N/A ─ 30.8[§] ─ 27.2%* ─ 27.6% (p=0.0453)	N/A ApoB: (-) non-HDLc: (-) Lp(a): (-)

	ODYSSEY JAPAN [124]	216 Individuals (including HeFH) at moderate to very high CVR on statin ± LLT	ALI 75 mg/150 mg Q2W if LDLc target not achieved at 12 week PL Q2W	52 weeks	─ 62.5% +1.6%		─ 64.1%* (to 24 week)		ApoB: (-) non-HDLc: (-) TC: (-) HDLc: (+) Lp(a): (-) TG: (-) ApoA1: (+)
	ODYSSEY LONG TERM [125]	2341 Individuals (including HeFH) at very high CVR on statin ± LLT	ALI 75 mg/150 mg Q2W if LDLc target not achieved at 12 week PL Q2W	78 weeks	─ 52.4% ─3.8%		─ 56.2%[I]		ApoB: (-) non-HDLc: (-) TG: (-) HDLc: (+) Lp(a): (-) TG: (-) ApoA1: (+)
	ODYSSEY CHOICE I [126]	803 Individuals (including HeFH) at moderate to very high CVR 68% on statin ± LLT (S^+) 32% ± LLT (S^-)	ALI 300 QM ALI 75 mg/150 mg Q2W if LDLc target not achieved at 12 week PL Q2W	48 weeks	S^+ ─ 51.9% ─ 47% 45.7% ─ 6.2%	S^- ─ 45.7% ─ +1%	S^+ ─ 58.1%* ─ 53.1%*	S^- ─ 44.7%* ─ 44.8%*	ApoB: (-) non-HDLc: (-) TC: (-) Lp(a): (-)

	ODYSSEY FH I [127]	486 Individuals with HeFH at high/very high CVR on statin ± LLT	ALI 75 mg/150 mg Q2W if LDLc target not achieved at 12 week PL Q2W	78 weeks	At 24week: − 48.8% +9.1%	At 24 week: − 57.9%*	ApoB:(−), Non-HDL: (−) Lp(a): (-) TG: (−) HDLc: (+) ApoA1: (+)
	ODYSSEY FH II [127]	249 Individuals with HeFH at high/very high CVR on statin ± LLT	ALI 75 mg/150 mg Q2W if LDLc target not achieved at 12 week PL Q2W	78 weeks	At 24 week: − 48.7% +2.8%	At 24 week: − 51.4%*	ApoB:(−), Non-HDL: (−) Lp(a): (-) TG: (−) HDLc: (+) ApoA1: (+)
	ODYSSEY HIGH FH [128]	105 Individuals with HeFH at high/very high CVR on statin ± LLT	ALI 150 mg Q2W PL Q2W	78 weeks	At 24 week: − 45.7% − 6.6%	At 24 week: − 39.1%*	ApoB:(−), Non-HDL: (−) Lp(a): (-) TC: (−)
	ODYSSEY ESCAPE [129]	62 Individuals with HeFH on frequent apheresis at high/very high CVR combined with statin ± LLT	ALI 150 mg Q2W PL Q2W	18 weeks	− 42.5% − 3.9%	− 46.4%*	At week 6: Lp(a): (-) TG: (−)

ApoA1: apolipoprotein A1; ApoB: Apolipoprotein B; A: Atrovastatin CVR: cardiovascular risk; EZE: ezetimibe; FH: Familial Hypercholesterolemia; HDL-C: high-density lipoprotein cholesterol, HeFH: Heterozygous Familial Hypercholesterolemia; HoFH: Homozygous Familial Hypercholesterolemia; LDL-C: low-density lipoprotein cholesterol; LLT: lipid lowering treatment; Lp(a): lipoprotein a; N/A: Not Applicable; OD: once a day; PL: placebo; QM: once a month; QW: once a week; Q2W: twice a week; Rosuvastatin TC: Total Cholesterol; TG: Triglycerides § $p<0.05$, *$p<.0001$, $^{|}$ $p<0.001$, $^{¥}$ $p<0.01$