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REVIEW ARTICLE

PCSK9 inhibition as an emerging lipid lowering therapy: Unanswered questions

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Abstract Although statins have been used for the treatment of hypercholesterolemia for more than two decades, cardiovascular disease (CVD), which is related at least in part to high levels of low-density lipoprotein cholesterol (LDL-C), is the number one cause of death in Europe and the USA. Several studies have shown that the reduction in cardiovascular (CV) events is proportional to the absolute LDL-C lowering achieved with statins. In the quest for further reduction in LDL-C and CV events, new drugs that mainly support statin action have emerged. Since 2003, with the discovery of proprotein convertase subtilisin/kexin type 9 (PCSK9), which is a key factor in the LDL clearance pathway, new modalities, mainly in the form of monoclonal antibodies that block this protein (PCSK9 inhibitors), have reached phase III of clinical development with very promising efficacy and safety data. With a mean further reduction of LDL-C levels of ~60% beyond that achieved with statins, the PCSK9 inhibitors set the bar even lower in terms of LDL-C levels. This review manuscript addresses important questions about the efficacy, safety and clinical use of PCSK9 inhibitors to evaluate the role of these agents in reducing CV risk.

1. Introduction

Cardiovascular disease (CVD) is the leading cause of death in Europe and the USA.[1,2] Over the past few years, studies have shown a very strong correlation between low-density lipoprotein cholesterol (LDL-C) levels and the development of CVD, mainly due to the key role of LDL-C in the atherosclerotic process.[3,4] The treatment of hypercholesterolemia has been primarily based on statin use. Indeed, statins have successfully served their purpose as a very effective lipid lowering medication class for 25 years since their introduction. However, a significant number of very high risk patients fail to achieve the LDL-C targets despite statin treatment necessitating the development of new agents for additional LDL-C lowering.[5]

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Following the discovery of the LDL receptor (LDLR) by Goldstein and Brown,[6] a new part of the puzzle of LDL clearance and homeostasis was elucidated by Abifadel and her coworkers in 2003.[7] The discovery of proprotein convertase subtilisin/kexin type 9 (PCSK9) by this group not only identified a new important and unknown element of the LDL clearance mechanism but also explained the limitations of statin therapy. PCSK9 is the last identified member of the proprotein convertase family. It is produced mainly in hepatocytes, and after an auto-processing cleavage reaction, it is secreted in the plasma where it binds with the LDLR. This does not interfere with the LDLR's ability to bind with LDL, but it incapacitates the LDLR's ability to return to the surface of the hepatocyte and bind to a new LDL molecule (Fig. 1). In particular, the direct interaction between PCSK9 and LDLR leads to the destruction of the receptors, a decrease in their concentration at the surface of the hepatocytes and a reduction in LDL clearance from the plasma. An important aspect of this process is that at the transcriptional level, PCSK9 production is upregulated by the activity of sterol regulatory element binding protein-2 (SREBP-2). SREBP-2 activity is increased in very low levels of intracellular cholesterol in the hepatocytes, and its main role is to promote the transcription of LDLR and PCSK9. As statins diminish intracellular cholesterol synthesis, they indirectly promote both LDLR and PCSK9 production through SREBP-2. This explains the statin treatment plateau that is achieved even at maximal doses.[8] Although PCSK9 was initially discovered through the analysis of familial hypercholesterolemia (FH) patients with a gain of function (GOF) mutation of the *PCSK9* gene, further studies have revealed naturally occurring loss of function (LOF) mutations. Homozygotes or compound heterozygotes for two LOF mutations in the *PCSK9* gene have minimal or even no PCSK9 production; their LDL-C levels are $\leq$20 mg/dL, and they are healthy.[9] These findings, in conjunction with the extracellular PCSK9 mode of action and the identification of its crystal structure and its active binding site with the LDLR,[10,11] have led to the development of pharmaceutical agents aimed at PCSK9 inhibition. To date, one of these modalities has been extensively tested and reached phase III of clinical development with great success. The use of monoclonal antibodies (mAbs) as a means of inhibiting PCSK9 action has shown consistent efficacy regarding the reduction of LDL-C levels (~60%) and a good safety profile with short-term administration.

To date, two of these fully human mAbs (evolocumab developed by Amgen and alirocumab developed by Sanofi/Regeneron) have completed most of their phase III programs, whereas a third PCSK9 inhibitor, bococizumab developed by PFIZER, is currently in phase III trials. On July 21st, 2015 and August 27th, 2015, the European Commission (EC) and the US Food and Drug Administration (FDA), respectively, announced the approval of evolocumab (Repatha) as an adjunct to diet and maximally tolerated statin therapy in adult patients with FH who failed to achieve LDL-C treatment goals, adult patients who were unable to tolerate statin therapy, and in homozygous FH adults and adolescents ($\geq$12 years old). On July 25th, 2015, the FDA, and one month later the EC, approved alirocumab (Praluent) as an adjunct to diet and maximally tolerated statin therapy for the treatment of adults with heterozygous FH or CVD, who require additional lowering of LDL-C and in patients who cannot tolerate statins.

Recently, a reduction of cardiovascular (CV) events with the administration of PCSK9 inhibitors was reported. In the ODYSSEY LONG TERM trial, 2341 high-risk patients with LDL-C levels $\geq$70 mg/dL while on a maximally tolerated statin dose were randomized in a 2:1 ratio to 150 mg alirocumab every two weeks subcutaneously or placebo over a period of 78 weeks. Alirocumab reduced LDL-C levels by an additional 62%, and post hoc analysis showed that this was associated with a 48% relative risk reduction of CV events (1.7% in the alirocumab group vs. 3.3% in the placebo group, $p = 0.02$).[12]

In addition, the OSLER (Open-Label Study of Long-Term Evaluation against LDL Cholesterol)-1 and OSLER-2 trials reported similar results. The participants of the OSLER-1 trial had already completed one of the five phase 2 parent evolocumab studies, whereas the OSLER-2 participants had participated in at least one of the seven phase III evolocumab studies.[13] Patients (n = 4465) were randomly assigned in a 2:1 ratio to receive either subcutaneous evolocumab 140 mg every two weeks or 420 mg monthly in addition to their standard therapy or standard therapy alone. The use of evolocumab plus standard therapy reduced the levels of LDL-C by 61% compared to standard

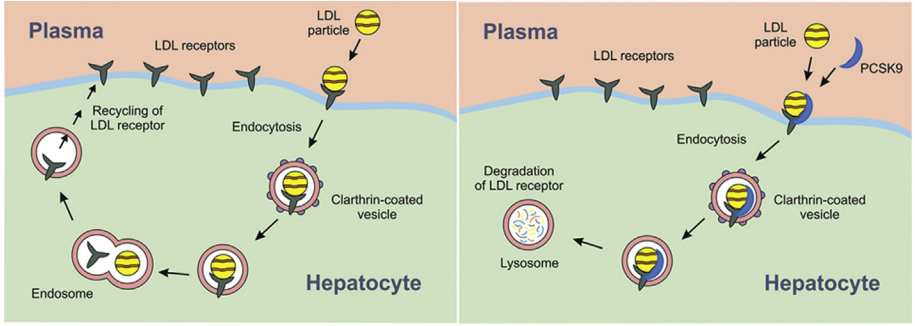

Figure 1 **Left panel**: LDL receptors carry LDL particles into hepatocytes via clathrin-coated vesicles that fuse with endosomes allowing the recycling of LDL receptors to the cell membrane up to 150 times. **Right panel**: Proprotein convertase subtilisin/kexin type 9 (PCSK9) binds to LDL receptors, and the complex LDL receptor-PCSK9 is degraded in the lysosomes. Therefore, increased plasma concentrations of PCSK9 result in low levels of LDL receptors at the cell surface and increased levels of circulating LDL cholesterol.

therapy alone and reduced the incidence of CV events by 53% in a prespecified exploratory analysis. In particular, the rate of CV events at one year was reduced from 2.18% in the standard therapy group to 0.95% in the evolocumab plus standard therapy group ($p = 0.003$).

Although these results are encouraging, it should be emphasized that the number of CV events was relatively low and the duration of follow-up was short for a treatment of a chronic disease. Before this novel lipid lowering treatment takes its place among the other hypolipidemics, several questions should be addressed:

1) **How safe is the chronic exposure to monoclonal antibodies?**

The viability of PCSK9 inhibition with mAbs in clinical practice is highly dependent on their safety profile. The chronic exposure to mAbs raises some concerns regarding mild hypersensitivity reactions and immune responses. The fact that evolocumab and alirocumab are fully human mAbs minimizes the possibility of such reactions but may not completely eliminate the risk. This is because several other factors that are unrelated to the primary sequence may contribute to the immunogenic potential, such as aggregation of mAbs induced upon storage, protein conformation, impurities arising from the production method, etc.[14] It should be noted here that real-life data regarding the use of mAbs for rheumatoid arthritis and Crohn's disease have shown that they are well tolerated over the long-term.[15]

2) **How safe are the very low LDL-C levels achieved by the combination of PCSK9 inhibitors with statins?**

The safety issue of low LDL-C levels has been raised since the publication of the first randomized studies with intensive statin therapy where LDL-C levels beyond the target had been achieved.[16,17] This concern is justified by the fact that cholesterol is an essential component of cellular membranes and neurons and is also necessary for steroid hormones and vitamin D synthesis. However, LDL-C levels in the range of 40 to 50 mg/dL that currently seem very low are possibly the genetically determined "ideal" LDL-C levels for humans. This is based on the fact that LDL-C levels are ~40 mg/dL in healthy neonates and 50 to 75 mg/dL in hunter/gatherer populations (e.g., Pygmies) that continue to live primitively and in whom coronary atherosclerosis is rare or non-existent.[18] Hence, the issue is whether very low LDL-C levels achieved by hypolipidemic agents are safe.

The answer to this question is not straightforward because only few data exist regarding the safety of very low levels of LDL-C as achieved with intensive lipid lowering therapy.

Subgroup analysis of the PROVE IT-TIMI 22 (Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocardial Infarction 22) trial according to achieved LDL-C levels while on statin treatment has shown that patients with very low LDL-C levels (<40 mg/dL) had a similar safety profile compared with those with LDL-C levels ≥40 mg/dL, and there was no case of rhabdomyolysis or brain haemorrhage.[19] However, only a limited number of patients (n = 193) had LDL-C levels <40 mg/dL, and the follow-up period was only two years.

A post hoc analysis of 16,304 participants enrolled in the JUPITER (Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin) trial raised some concerns regarding the safety of very low LDL-C levels achieved by the administration of 20 mg rosuvastatin daily.[20] In particular, 767 patients who had LDL-C levels <30 mg/dL, despite the clinical benefits, showed an increase in the rates of physician-reported diabetes mellitus, haematuria, hepatobiliary disorders, and insomnia compared with participants taking rosuvastatin who had LDL-C levels ≥30 mg/dL.

When a PCSK9 inhibitor is added to statin therapy, very low LDL-C levels are expected in a significant proportion of patients whose initial LDL-C levels are in the range of 70 to 100 mg/dL. This was shown in the LAPLACE-2 (LDL-C Assessment with PCSK9 Monoclonal Antibody Inhibition Combined with Statin Therapy) trial where evolocumab added to moderate or high-intensity statin therapy and compared with ezetimibe or placebo in hypercholesterolemic patients for 12 weeks resulted in very low LDL-C levels.[21] Particularly, ~20% of the moderate-intensity statin group and ~40% of the high-intensity statin group achieved LDL-C levels <25 mg/dL. Despite these very low LDL-C levels, adverse events occurred at similar proportions in the three studied groups i.e., in 36% of evolocumab-treated patients, 40% of ezetimibe-treated patients, and 39% of placebo-treated patients.

The OSLER studies also suggested no significant adverse effects of intensive LDL-C lowering.[13] After approximately one year of therapy, the risk of adverse events, including neurocognitive events, did not vary significantly across subgroups formed by achieved LDL-C levels, i.e., LDL-C <25 mg/dL, LDL-C between 25 and <40 mg/dL, and LDL-C ≥40 mg/dL compared to the standard of care alone arm.

Efficacy and safety data regarding the administration of alirocumab were also reported by the COMBO II study.[22] This was a double-blind, active-controlled, parallel-group, 104-week study of alirocumab vs. ezetimibe. In particular, 720 patients with high CV risk and elevated LDL-C levels despite maximal doses of statins were randomized with a 2:1 allocation ratio to subcutaneous alirocumab 75 mg every two weeks (plus oral placebo) or oral ezetimibe 10 mg daily (plus subcutaneous placebo). At week 24, the mean reduction in LDL-C from baseline was ~51% for the alirocumab group and ~21% for the ezetimibe group, and these reductions remained largely constant up to 52 weeks. Alirocumab was generally well tolerated with no evidence of an excess of adverse events. A similar safety profile showed 105 patients (22.8% of 460) in the alirocumab arm who had two consecutive LDL-C values <25 mg/dL during the treatment period.

To date, the side effect profile of PCSK9 inhibitors is comparable to the placebo group. However, the intervention period is short (3-12 months), and long-term follow-up is mandatory to prove the safety of PCSK9 inhibition. There is some reassurance in knowing that the few people who were compound heterozygotes for two LOF *PCSK9* gene mutations appear to be in good health despite the strikingly low LDL-C levels (~15 mg/dL).[9] However, safety data

regarding genetic PCSK9 deficiency cannot be extrapolated to individuals with genetically normal PCSK9 suppressed by a pharmaceutical intervention. In addition, the impairment of neurocognitive function remains a concern with such drastic reductions in LDL-C levels. Despite the low overall rate of neurocognitive adverse events in the OSLER (0.9%) and the ODYSSEY LONG TERM (1.2%) studies, the rate of self-reported neurocognitive events was slightly higher than in the placebo groups. The issue of neurocognitive adverse effects will be addressed by a dedicated neurocognitive substudy of the FOURIER study. This substudy (Evaluating PCSK9 Binding antiBody Influence oN coGnitive HeAlth in High cardiovascUlar Risk Subjects (EBBINGHAUS) (ClinicalTrials.gov Identifier: NCT02207634) will objectively determine neurocognitive function via neurocognitive testing.

3) Do PCSK9 inhibitors have effects beyond LDL-C lowering?

Statins have several CV effects, collectively called pleiotropic effects, most likely not entirely explained by LDL-C lowering, such as amelioration of endothelial dysfunction, halting or retardation of atheroma development, antithrombotic effects and the reduction of inflammation and oxidation.[23] These effects may, at least partly, arise from the inhibition of several mevalonate-derived metabolites, which are involved in the control of various cellular functions. It has been hypothesized that these additional effects contribute, at least partly, to the clinical benefits of statins.

PCSK9 inhibitors have an entirely different mechanism of action. Because the metabolic pathway of cholesterol synthesis remains intact, the pleiotropic effects of statins, which might be related to mevalonate-derived metabolites, do not appear with PCSK9 inhibition. Conversely, PCSK9 inhibitors show some additional lipid effects, such as the reduction in lipoprotein (a) [Lp(a)]. In particular, a ~30% reduction in Lp(a) has been reported with the administration of evolocumab[24] or alirocumab.[25] Whether this Lp(a) lowering effect is translated into clinical benefit remains to be elucidated. In addition, because the exact functional role of PCSK9 is unknown, it cannot be predicted whether chronic inhibition of PCSK9 will uncover new off-target effects with unknown impact on the atherogenic process. PCSK9 appears to exhibit several metabolic effects that need to be further explored.[26] It has been proposed that PSCK9 may play roles in neuronal apoptosis, regulation of sodium channels, nervous system development, septic pathogen lipid transport and clearance, etc.[27,28] In addition, circulating PCSK9 has been reported to be associated with plasma glucose, body mass index and blood pressure.[29]

4) Are lower LDL-C levels better?

Large randomized, outcome trials support a treatment target of LDL-C levels in very high risk patients of less than 70 mg/dL, as this is associated with improved clinical outcomes. To date, it is unknown whether there is a threshold below which patients do not benefit from lower LDL-C levels. To assess the benefit of even lower LDL-C, it is important to recognize that CV risk reduction is not proportional to the percent change but rather to the absolute reduction in LDL-C levels.[30] Therefore, the risk reduction for the same relative LDL-C lowering diminishes with lower baseline LDL-C levels.[31]

A meta-analysis that included 38,153 patients from eight randomized controlled statin trials showed that patients achieving an LDL-C level <50 mg/dL were at significantly lower risk for major CV events compared with those reaching LDL-C levels of 75 to <100 mg/dL (adjusted hazard ratio: 0.81; 95% confidence interval: 0.70 to 0.95).[32]

The recently published IMPROVE-IT (IMProved Reduction of Outcomes: Vytorin Efficacy International Trial) also reinforces "the lower the better" hypothesis.[33–35] In that study, a total of 18,144 patients with a recent acute coronary syndrome and LDL-C levels ≤125 mg/dL (≤100 mg/dL if they had taken previous hypolipidemic medication) were randomized to either 40 mg simvastatin alone or 40 mg simvastatin plus 10 mg ezetimibe daily. The mean level of LDL-C achieved in the simvastatin group was 69.5 mg/dL compared with 53.7 mg/dL in the combined treatment group. The ~16 mg/dL reduction of LDL-C achieved by the addition of ezetimibe to simvastatin was associated with a further modest clinical benefit, i.e., a 6.4% relative reduction of all CV events.

Although there are encouraging findings supporting "the lower the better", this hypothesis will be confirmed or rejected when the clinical outcome trials with PCSK9 inhibitors (FOURIER [Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk] and ODYSSEY LONG-TERM trials) will be completed in ~2 years.

5) Which patients are candidates for PCSK9 inhibitors?

The introduction of statins 25 years ago changed the landscape of lipid lowering therapy. Statins have been incorporated into all national and international guidelines for the treatment of hypercholesterolemia in coronary artery disease (CAD). The benefits of statins have been shown to be significant, both in the primary and secondary prevention of CAD. Despite the large benefit, a significant number (~15-20%) of CAD patients on statin treatment still develop CV events within a five-year period. This may be due to failure to attain LDL-C goals, to co-existence of other risk factors, such as diabetes mellitus, Lp(a), and atherogenic dyslipidaemia, that are not treated sufficiently with existing drugs, or to the presence of other, as yet unknown, CAD risk factors .[36]

Failure to achieve LDL-C levels was shown in the EUROASPIRE (European Action on Secondary and Primary Prevention by Intervention to Reduce Events) IV trial,[37] which recorded risk factors from 24 European countries in individuals with established CAD. This survey, conducted from May 2012 to April 2013, showed that despite the high reported use of statins in secondary prevention (~86%), only one of five patients achieved the therapeutic target for LDL-C, <70 mg/dL. This therapeutic deficit may be due to inadequate up-titration of the statin dose or the failure of high dose statins to attain LDL-C goals because of very high initial LDL-C levels.

Table 1 Potential candidates for PCSK9 inhibitors may include:

1) Patients with CVD and LDL-C ≥100 mg/dL despite maximal tolerated dose of effective statin (20/40 mg rosuvastatin or 40/80 mg atorvastatin daily) + ezetimibe (10 mg daily)
2) Heterozygous FH patients without CVD and LDL-C ≥130 mg/dL despite maximal tolerated dose of effective statin (20/40 mg rosuvastatin or 40/80 mg atorvastatin daily) + ezetimibe (10 mg daily)
3) Diabetic patients without CVD but with ≥1 cardiovascular risk factors and/or target organ damage and LDL-C ≥100 mg/dL despite maximal tolerated dose of effective statin (20/40 mg rosuvastatin or 40/80 mg atorvastatin daily) + ezetimibe (10 mg daily)
4) Statin-intolerant patients (documented intolerance to ≥2 statins) who, despite ezetimibe ± bile acid sequestrants use have:
   a) LDL-C ≥100 mg/dL (very high risk patients) or
   b) LDL-C ≥130 mg/dL (high risk patients).
5) Homozygous FH patients aged ≥12 years in combination with other lipid-lowering therapies (only evolocumab has been approved).

CVD = cardiovascular disease, LDL-C = low density lipoprotein-cholesterol, FH = familial hypercholesterolemia.

Another issue hampering the use of statins is the development of statin-associated muscle symptoms. In clinical practice, ~10% of patients receiving statins develop muscle symptoms.[38,39] The PRIMO (Prediction of Muscular Risk in Observational conditions) study showed that 17% of patients on statins with muscular symptoms decreased the dose of statins, while 20% of them discontinued it.[40] For those who discontinued the statins, 70% of them can tolerate lower doses of statins.[38]

Promising results for treating hypercholesterolemia in patients who cannot tolerate statins due to muscle symptoms yielded the GAUSS-2 (Goal Achievement After Utilizing an Anti-PCSK9 Antibody in Statin Intolerant Subjects-2) trial.[41] This study selected 307 patients who were intolerant to ≥2 statins due to muscle symptoms and randomized them to evolocumab (140 mg every two weeks or 420 mg once monthly) or ezetimibe 10 mg daily for 12 weeks. Muscle adverse events occurred in only 12% of the evolocumab-treated patients and 23% of the ezetimibe-treated patients.

Therefore, potential candidates for PCSK9 inhibition may be high risk or very high risk patients who are unable to reach the LDL-C target despite treatment with the highest tolerable statin dose plus ezetimibe or statin-intolerant patients (very high or high risk). However, taking into account the fact that long-term CV outcome data are not available yet and the presumed high cost of these medications, we currently suggest a more conservative treatment approach (Table 1).[42] Applying these criteria, we estimate that approximately <5% of all very high risk patients with hypercholesterolemia will be candidates for PCSK9 inhibitors.

2. Conclusions

The discovery of PCSK9 in 2003 considerably changed the therapeutic reality in the lipid field. PCSK9 reduces LDLR recycling and increases LDL-C levels. Fully human mAbs are currently the most advanced PCSK9 inhibitors. They result in a consistent decrease in LDL-C levels of ~60%, either on top of statins or as a monotherapy. Long-term randomized controlled clinical trials are underway to assess the safety, tolerability, and efficacy of PCSK9 inhibitors to reduce CV events. If these trials show positive results, PCSK9 inhibitors will offer a novel and powerful therapeutic option for patients with difficult-to-treat hypercholesterolemia.

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