Review

Treatment with Proprotein Convertase Subtilisin/Kexin Type 9 Inhibitors (PCSK9i): Current Evidence for Expanding the Paradigm?

Journal of Cardiovascular Pharmacology and Therapeutics
Vol. 28: 1-13

DOI: 10.1177/10742484231186855
journals.sagepub.com/home/cpt
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Abstract

Background: Proprotein convertase subtilisin/kexin type 9 inhibitors (PCSK9i) are low-density lipoprotein cholesterol (LDL-C)-lowering drugs that play a critical role in lipoprotein clearance and metabolism. PCSK9i are used in patients with familial hypercholesterolemia and for the secondary prevention of acute cardiovascular events in patients with atherosclerotic cardiovascular disease (CVD). **Methods:** We focused on the literature from 2015, the year of approval of the PCSK9 monoclonal antibodies, to the present on the use of PCSK9i not only in the lipid field but also by evaluating their effects on metabolic factors. **Results:** PCSK9 inhibits cholesterol efflux from macrophages and contributes to the formation of macrophage foam cells. PCSK9 has the ability to bind to Toll-like receptors, thus mediating the inflammatory response and binding to scavenger receptor B/cluster of differentiation 36. PCSK9i lower the entire spectrum of apolipoprotein B-100 containing lipoproteins (LDL, very LDLs, intermediate-density lipoproteins, and lipoprotein[a]) in high CVD-risk patients. Moreover, PCSK9 inhibitors are neutral on risk for new-onset diabetes mellitus and might have a beneficial impact on the development of nonalcoholic fatty liver disease by improving lipid and inflammatory biomarker profiles, steatosis biomarkers such as the triglyceride-glucose index, and hepatic steatosis index, although there are no comprehensive studies with long-term follow-up studies. **Conclusion:** The discovery of PCSK9i has opened a new era in therapeutic management in patients with hypercholesterolemia and high cardiovascular risk. Increasingly, there has been mounting scientific and clinical evidence supporting the safety and tolerability of PCSK9i.

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Manuscript submitted: June 11, 2023; **accepted:** June 19, 2023.

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Keywords
atherosclerosis, low-density lipoprotein cholesterol, low-density lipoprotein receptor, nonalcoholic fatty liver disease, proprotein convertase subtilisin/kexin type 9, transaminase

Introduction

Proprotein convertase subtilisin/kexin type 9 inhibitors (PCSK9i) are low-density lipoprotein cholesterol (LDL-C)-lowering drugs used to treat dyslipidemia in patients with familial hypercholesterolemia (FH) or atherosclerotic cardiovascular disease (ASCVD).[1] The PCSK9i were the first cholesterol-lowering drugs that target selected genes that promote ASCVD.[2] PCSK9 decreases LDL receptor (LDL-R) recycling by chaperoning LDL-R into the lysosome for destruction. Inhibiting PCSK9 leads to an increase in cell surface LDL-Rs for binding and removal of circulating LDL particles.[3] Some patients with FH have a gain of function mutations in PCSK9 with often dramatic elevations in LDL-C.[4] There are two monoclonal antibodies (mAbs) directed against PCSK9, alirocumab, and evolocumab. These drugs are administered every 2 weeks or monthly via subcutaneous injection, with data from cardiovascular (CV) outcomes studies supporting their use.[5] These drugs are used in patients with genetic hypercholesterolemic disorders with very high LDL-C levels and are also indicated for use in secondary prevention of ASCVD-related events following determining recommendations.[6]

Guidelines recommend the use of PCSK9i in very high-risk patients who fail to achieve their LDL-C goal while on maximally tolerated statin therapy with or without ezetimibe; if there is intolerance to the use of statins they can be used as monotherapy or in combination with ezetimibe.[7] The purpose of this review is to provide an overview of the use of these drugs in various metabolic scenarios and their capacity to reduce CV risk.

PCSK9 Mechanism of Action

A proprotein convertase proteolytically converts an inactive precursor enzyme to an active one by hydrolyzing an amino acid sequence that either blocks the active site or facilitates a change in three-dimensional conformation. This can be illustrated by a proenzyme or zymogen that is converted into a catalytically active enzyme poised to perform a specific reaction in intermediary metabolism. The proprotein convertase subtilisin/kexin (PCSK) family includes 9 serine proteases, 8 of which are catalytically active immediately after biosynthesis and convert receptors, transcription factors, hormones, and enzymes into their biologically active species. The 9th member of the PCSK family (PCSK9) plays a critical role in lipoprotein clearance and metabolism. Upon synthesis in the endoplasmic reticulum (ER), its signal peptide is hydrolyzed to produce the zymogen proPCSK9.[8] This zymogen exits the ER and enters the hepatocyte cytosol by catalyzing the autocleavage of its prosegment. This step eliminates the potential for catalytic activity; the prosegment remains associated with PCSK9 and sterically hinders PCSK9's active site. The only catalytic target of PCSK9 is its own prosegment. PCSK9 is chaperoned to the extracellular space by sortilin-1.[9]

LDL particles (LDL-P) constitute the end product of lipoprotein metabolism and are principally cleared from the circulation by LDL-R.[10] LDL-R are expressed on the hepatocyte surface and are concentrated in clathrin-coated pits within the cell membrane. As an LDL-R binds to LDL-P, it is aligned within the clathrin-coated pit by LDL-R adaptor protein 1 (aka clathrin-associated sorting protein); there is, however, evidence that the protein-disabled homolog adaptor protein 2 also performs this function.[11,12] An endosome forms and is covered by a clathrin polyhedral lattice.[13] The endosome undergoes endocytosis and translocates into the cytosol, clathrin dissociates, and the endosome is acidified.[14] The pH reduction potentiates the dissociation of LDL-R from LDL-P. Through a mechanism that is yet to be defined, the LDL-P is specifically translocated into the lysosome for destruction by cathepsins and lipases. Some of the LDL-derived cholesterol can also be rerouted for biliary clearance or conversion to bile salts via 7-α-hydroxylase. The LDL-R is recycled to the hepatocyte surface to initiate another round of LDL-P uptake and catabolism.

PCSK9 controls the expression of LDL-R on the surface of hepatocytes. After secretion into the extracellular milieu, PCSK9 can bind to the epidermal growth factor-like repeat A domain of LDL-R.[15] LDL-R bound to both an LDL-P and PCSK9 are concentrated in clathrin-coated endosomes. The endosomes carry LDL-R–PCSK9–LDL-P complexes into the cytosol. The PCSK9 holds the LDL-R and LDL-P tightly together and they do not dissociate as the intraendosomal pH decreases.[16] The PCSK9 chaperones the LDL-R–LDL-P complex into the lysosome for proteolytic destruction. This results in (a) fewer LDL-Rs recycling to the cell membrane and (b) decreased hepatic capacity to clear systemic LDL-P.

Hepatocyte lipoprotein trafficking and receptor physiology are complex. PCSK9 also regulates cell surface expression of lipid and lipoprotein receptors other than LDL-R, thereby impacting serum levels of multiple lipoprotein classes and their subfractions.[17,18] PCSK9 exerts posttranscriptional control over the expression of the very LDL-R (VLDL-R), the apolipoprotein E2 receptor, and the LDL-R-related protein 1, which participate in the binding and uptake of various apolipoprotein B (apoB)-containing lipoprotein.[19-22] In addition, PCSK9 controls the activity of cluster of differentiation 36 (CD36), a fatty acid transport protein expressed by hepatocytes and adipocytes.[23] PCSK9 also increases jejunal expression of Niemann-Pick C1-like-1 protein, microsomal triglyceride transfer protein, and acyl-coenzyme A: cholesterol acyltransferase 2, which collectively results in greater cholesterol absorption from dietary and biliary sources and increased production of chylomicrons.[19,24]

Monoclonal Antibodies for Inhibiting PCSK9

A mAb is a highly specific antibody targeted against a single antigen.[25] Since PCSK9 is a secreted protein and is active in the extracellular milieu, it can be neutralized by a mAb. Two fully human mAbs (evolocumab and alirocumab) targeting PCSK9 have been developed for treating hyperlipidemia and both have been shown to reduce the risk for CV events in prospective randomized trials.[26,27]

Because they are fully human mAbs, the risk for both autoimmune responses and tachyphylaxis is minimized. These mAbs can be used irrespective of baseline renal and hepatic function because they have no dependence on hepatic uptake and metabolism for activation or biotransformation, and they do not require renal elimination.[28-30] The antibody complexes formed between these agents and extracellular PCSK9 are removed from serum by the reticuloendothelial system (ie, Kupffer cells in the liver, spleen, lymph nodes, and bone marrow). The PCSK9 mAbs have no drug interactions since they do not impact the activity of organic anion transport proteins, cytochrome P450 isozymes, or conjugation reactions (glucuronic acid, glutathione, glycine, etc).

Effect of PSCK9i on Lipid Parameters

PCSK9 is one of the most important regulators of lipid metabolism.[31] Given its capability to bind and to reduce their number on the hepatocyte cell surface, increased expression of PCSK9 leads to an increase in LDL-C level in the circulation.[32] PCSK9 also exerts other proatherosclerotic effects and contributes to the onset and progression of ASCVD.[33-35]

Some of the properties attributed to PCSK9 include (but are not limited to) the stimulation of intestinal production of triacylglycerol-rich lipoproteins due to the promotion of triacylglycerol and apoB48 synthesis.[35,36] PCSK9 has the ability to bind to Toll-like receptors, thus mediating the inflammatory response, and to bind to class B scavenger receptors/CD36, thus accelerating platelet activation and thrombosis.[37] Additionally, it binds to VLDL-R, apolipoprotein E receptor-2, and LDL-R-related protein 1 and stimulates their degradation which further leads to increases in serum lipid/lipoprotein levels and endothelial dysfunction.[37] PCSK9 activates inflammation and LDL-C scavenging by macrophages leading to increased arterial white cell recruitment and foam cell generation.[33,38] Moreover, PCSK9 inhibits cholesterol efflux from macrophages, further exacerbating foam cell formation.[33] Given these observations, the identification of PCSK9 as a therapeutic target was reasonable.[39]

The statins (inhibitors of hydroxymethylglutaryl-coenzyme A reductase) are highly established lipid-lowering agents and have been used for decades to treat hypercholesterolemia. However, these medications have some drawbacks that limit their efficacy and safety in terms of lowering LDL-C in individuals with high CV disease (CVD) risk.[35,39] Diminished therapy adherence, adverse effects (ie, myopathies and myalgias), and the fact that about 70% of individuals that use statin monotherapy do not achieve target LDL-C levels point out the urgent need for the discovery of novel agents that improve long-term therapy adherence and are better tolerated.[39]

Ezetimibe blocks the jejunal absorption of cholesterol by inhibiting Niemann-Pick C1-like-1 protein and is recommended as a second-line therapy in subjects who cannot achieve targeted LDL-C values even though they use maximally tolerated doses of statin or if they manifest intolerance to statin.[5,6,40]

PCSK9i, such as PCSK9 mAbs (PCSK9 mAbs, alirocumab, and evolocumab), and a small interfering RNA molecule (siRNA; inclisiran) promote large reductions in circulating LDL-C levels.[37,39] These therapeutics have shown beneficial properties in clinical trials and are recommended for use on a background of the maximum tolerated dose of statin and ezetimibe.[37,39] Alirocumab and evolocumab neutralize PCSK9 in the extracellular space.[37,39] Inclisiran inhibits hepatic PCSK9 synthesis by silencing PCSK9 messenger RNA (mRNA) translation. Each of these therapeutic approaches increases LDL-R on the hepatocyte surface resulting in enhanced uptake of LDL-C and reductions in LDL-C.[32,35,39] Meta-analyses demonstrate that alirocumab and evolocumab[41,42] and inclisiran[43] are well tolerated and safe.

Not only are PSCK9i highly effective drugs for reducing LDL-C, but they also affect the serum levels of other lipids and lipoproteins.[44,45] Treatment with a PCSK9i is associated with reductions in triglycerides (TGs), VLDL cholesterol (VLDL-C) and VLDL remnants, intermediate-density lipoprotein cholesterol (IDL-C), non-high-density lipoprotein cholesterol (non-HDL-C), apoB and lipoprotein(a) (Lp[a]).[17,18,41,43,46-48] Non-HDL-C (containing VLDL, IDL, LDL, chylomicron remnants, and Lp[a]) is advantageous over LDL-C since it is not influenced by TG variability and contains lipoprotein with remnant cholesterol.[35,41] The administration of PCSK9i also increases apolipoprotein A-I (apoA-I) and HDL-C.[41]

Evolocumab

There are 2 recommended dosage regimens of evolocumab administration, and both are equally satisfactory, that is, an injection of 420 mg monthly (ie, every 4 weeks) or 140 mg bi-monthly (ie, every 2 weeks).[35] The Further CV Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk (FOURIER) trial was a large randomized double-blind, placebo-controlled study that included 27 564 patients with ASCVD and LDL-C $\geq$ 70 mg/dL[26] and examined the clinical efficacy and safety of evolocumab. After a median of 12 weeks follow-up, evolocumab reduced LDL-C by 63%, as compared with placebo (ie, to 26 mg/dL from a median baseline value of 92 mg/dL) when added to conventional statin therapy at maximum tolerated doses. At 48 weeks, evolocumab reduced non-HDL cholesterol levels by 51.2%, Lp(a) by 26.9%, apoB levels by 46%, total cholesterol (TC) by 35.5%, and TG by 16.2% ($P < .001$ for all). HDL-C (8.4%) and apoA-I (6.5%) both increased in the evolocumab group versus the placebo group ($P < .001$ for both). In the FOURIER trial,

treatment with evolocumab reduced the risk of the primary composite endpoint (consisting of myocardial infarction [MI], stroke, hospitalization for unstable angina, coronary revascularization, and CV death) by 15% (hazard ratio [HR], 0.85; 95% confidence interval [CI], 0.79–0.92; $P < .001$). The medication was well tolerated and no adverse effects on glucose metabolism were found.[26] A meta-analysis of over 6000 participants included in the PROFICIO program also showed evolocumab to be safe and well tolerated.[49]

The clinical efficacy and safety of evolocumab were also tested in the BERSON study that included 451 Chinese patients with type 2 diabetes mellitus (T2DM) and dyslipidemia on background atorvastatin therapy. After 12 weeks of treatment with the same dosage regimens as in the FOURIER study, evolocumab significantly lowered LDL-C by 74.8%–85%, and non-HDL-C by 63%–81%, as compared with placebo.[50] Significant reductions in apoB (by 51%–58%), Lp(a) (by 42.1%–48.3%), TG (by 5.4%–11.1%), VLDL-C (by 12.1%–13.6%), HDL-C (by 9.2%–12.4%), TC (by 36.7%–40.4%) were also observed.[50] Evolocumab was shown to be well tolerated and safe.[50]

Alirocumab

In the ODYSSEY Outcomes trial ($n = 18\,924$ ASCVD patients), the clinical efficacy and safety of alirocumab with a biweekly dose of 75–150 mg were tested when added to maximum tolerated statin doses.[27] After 48 months of follow-up alirocumab reduced LDL-C by more than 50% and reduced major adverse CV events by 15% versus placebo (HR, 0.85; 95% CI, 0.73–0.98).[27]

The efficacy and safety of alirocumab were also tested in the ODYSSEY KT study, which included 199 patients with high CVD risk from South Korea and Taiwan.[51] LDL-C was significantly reduced by 57.1% after 24 weeks of treatment with alirocumab in patients on maximum tolerated statin therapy, end at the end of the treatment 85.8% of patients treated with alirocumab reached LDL-C < 70 mg/dL (placebo: 14.2%; $P \leq .0001$ vs placebo).[51] There were no significant differences in adverse events (AEs) between the alirocumab and placebo groups after the treatment. A total of 58.8% of patients in the alirocumab group versus 61.8% in the placebo group were shown to have treatment-emergent AE, whereas 2.1% of patients in the alirocumab group versus 1.0% ceased the treatment due to treatment-emergent AE.[51] Similarly, the ODYSSEY Japan study ($n = 216$ patients with heterozygous FH [HeFH] or non-FH at high CV risk) showed a decrease in LDL-C levels of 62.5% after 24 weeks of treatment with alirocumab, when added to statin therapy (in the alirocumab group mean ± SE change in LDL-C from baseline was $-62.5 \pm 1.3\%$ vs $1.6 \pm 1.8\%$ in the placebo group, with a difference $-64.1 \pm 2.2\%$).[52] This decrease was maintained to week 52 (the difference was $-62.5 \pm 1.4\%$ in the alirocumab vs $3.6 \pm 1.9\%$ in the placebo group).[52]

Results from a meta-analysis (which included 52 586 high-risk patients from 14 trials) showed the beneficial effects of PCSK9i on the reduction of different proatherogenic lipid/lipoprotein parameters.[41] Evolocumab and alirocumab provide incremental reductions in LDL-C (mean difference [MD] = −46.86%), non-HDL-C (MD = −42.86%), apoB (MD = −38.44%), Lp(a) (MD = −26.69%), TC (MD = −31.92%), and TG (MD = −8.13%) levels in high CVD-risk patients on statins and other lipid-lowering medications. The PCSK9 mAbs increased levels on average of apoA1 (MD = 4.33%) and HDL-C (MD = 6.27%).[41]

Inclisiran

Although PCSK9 mAbs safely lowered LDL-C levels they require monthly or semimonthly injections.[53] The need for less-frequent administration may increase patient compliance.[35] In the context of such a need, inclisiran, an inhibitor of PCSK9 synthesis in the liver, was developed. It is a siRNA that binds to PCSK9 mRNA and promotes its destruction by RNases in the cytosol of hepatocytes.[35] Its administration subcutaneously is required half yearly which enhances patient compliance.[35,53]

Inclisiran reduces both the intracellular and extracellular levels of PCSK9, unlike mAbs which only influence the extracellular PCSK9 levels.[46] The clinical efficacy and safety of inclisiran in lowering LDL-C have been explored in randomized, placebo-controlled, double-blind clinical trials.[54,55] ORION-9, ORION-10, and ORION-11 phase 3 trials have demonstrated that the inclisiran regimen of 284 mg (equivalent to 300 mg inclisiran sodium) has good efficacy for LDL-C level reduction. The ORION-9 study included patients with HeFH and LDL-C ≥ 100 mg/dL.[54] ORION-10 included patients with LDL-C ≥ 70 mg/dL and ASCVD, whereas ORION-11 included patients with LDL-C ≥ 70 mg/dL and ASCVD, as well as ASCVD risk equivalents such as HeFH, T2DM, or a 10-year Framingham risk score for CVD ≥ 20%.[55]

The ORION-9 study included 482 patients and showed a 39.7% reduction in LDL-C in the inclisiran group as compared to the placebo after 510 days of observation.[54] A reduction was also recorded in TC (by 26.1%), apoB (by 34%), non-HDL cholesterol (by 36.1%), and TG (by 11.1%) at day 510 in the inclisiran versus the placebo group.[54] ORION-10 evaluated patients in the USA and included 1561 participants. All patients were given inclisiran subcutaneously at a dose of 284 mg or placebo on day 1, month 3, and then every 6 months for 510 days of observation. It was shown that inclisiran lowered LDL-C by 52.3%.[55] ORION-11 included 1617 patients with the same regimen dose of inclisiran as in ORION-10 and decreased LDL-C by 49.9%, with a similar incidence of AE as in ORION-10.[55] Serious AEs were shown in 22.4% of patients (among them 1.5% deaths) in the inclisiran group and 26.3% of patients (among them 1.4% deaths) in the placebo group in the ORION-10 trial versus 22.3% of patients (among them 1.7% deaths) in the inclisiran group and 22.5% of patients (among them 1.9% deaths) in the placebo group in the ORION-11 trial.[55]

A pooled analysis of all patients ($n = 3660$) from the ORION-9, ORION-10, and ORION-11 trials showed that

subcutaneously administered inclisiran at day 1, day 90, and then every 6 months provides effective and safe LDL-C lowering when added to maximally tolerated doses of oral lipid-lowering drugs. A mean LDL-C reduction of 50.7% at day 510 with inclisiran therapy was demonstrated. The treatment with inclisiran also reduced non-HDL-C (by 46.3%), apoB (by 41.7%), and TC (by 32.4%) as compared with placebo, but with similar safety in both groups.[56]

Unlike the previously mentioned pool analysis of phase III global studies,[56] another pooled analysis (from the ORION-9, ORION-10, and ORION-11 trials), but specifically focused on patients from South Africa, was conducted as ORION phase 3 clinical trials.[57] Of the 3660 participants, 298 were recruited from South Africa. It also showed that inclisiran treatment in addition to maximally tolerated lipid-lowering therapy is safe and effective in patients with high CVD risk.[57] In addition to maximally tolerated standard lipid-lowering therapy, inclisiran dosing at days 1, 90, 270, and 450 reduced LDL-C levels by 54.2% (95% CI, −61.3 to −47.2) compared with placebo by day 510. The mean reductions in TC were 29.5% (95% CI, −32.7 to −26.3), apoB by 39.8% (95% CI, −43.2 to −36.5), and non-HDL-C levels by 41.5% (95% CI, −45.6 to −37.5) in the inclisiran group compared with placebo.[57] The LDL-C decrease observed in the patients from South Africa was similar to that demonstrated in the previous (LDL-C decrease of 50.7% (95% CI, −52.9 to −48.4) pooled analysis.[56]

A meta-analysis that included 3 randomized control trials (RCTs) (with 3660 patients with hypercholesterolemia) demonstrated mean reductions in LDL-C levels by 51%, non-HDL-C by 45%, apoB by 41% and TC by 37% in patients receiving inclisiran treatment compared with placebo. A mild skin reaction at the injection site was observed as the only side effect.[43]

A Bayesian network meta-analysis (NMA) included 23 RCTs that examined differences in LDL-C with patients with ASCVD, HeFH, and/or high risk of CVD receiving maximum tolerated statins.[58] This NMA is the first to include a larger number of treatment comparisons (evolocumab, alirocumab, inclisiran, ezetimibe, and bempedoic acid) and evaluated time points for LDL-C change at 24 weeks. Alirocumab, inclisiran, and evolocumab were shown to be superior versus placebo, bempedoic acid, and ezetimibe in terms of LDL-C reduction in patients who do not achieve LDL-C reductions with statins as a monotherapy. Moreover, inclisiran, evolocumab, and alirocumab were comparable when LDL-C % change is regarded (MD for evolocumab vs inclisiran: 8.16% [95% credible interval (CrI): −1.82, 18.49]) and alirocumab versus inclisiran (0.78% [95% CrI: −8.35, 9.88]).[58] In an NMA of randomized trials evaluating the comparative efficacy of lipid-lowering drugs added to maximally tolerated doses of statins for the reduction of LDL-C, evolocumab and alirocumab were found to be superior to inclisiran, and this was followed by a combination of bempedoic acid/ezetimibe, ezetimibe monotherapy, and bempedoic acid monotherapy.[59] Evolocumab (140 mg/420 mg) and alirocumab (150 mg) lowered LDL-C (mean percentage change) by approximately 64.7% and 62.7%, respectively, while inclisiran reduced LDL-C by 50.2%.[59]

Gender/Sex Differences

Recent findings pointed out the need for the development of sex-specific strategies and recommendations for the management of ASCVD in females, considering the fact that several RCTs as well as animal studies reported sex-dependent changes of PCSK9 on LDL-C.[60-62] A recent meta-analysis that included six real-life studies (641 women and 1216 men) explored the effects of sex related to PCSK9 mAbs on LDL-C reduction.[63] Although women displayed higher LDL-C at baseline, the treatment with PCSK9 mAbs lead to greater reductions in men (mean LDL-C difference = 7.6 mg/dL, $P = .002$) as compared to women.[63] In an effort to exclude potential confounding variables (such as psychosocial and environmental factors), the investigators examined the level of LDL-C decrease in both sexes in connection with genetic inhibition of PCSK9 loss of function variant (R46L) in 382 813 subjects who did not receive lipid-lowering drugs (163 512 men and 219 301 women) and revealed a sex-dependent association between PCSK9 R46L and LDL-C in the general population. The magnitude of LDL-C reduction was lower in women than in men (mean LDL-C difference: −26 mg/dL vs −35 mg/dL, in homozygous carriers vs noncarriers in women and men, respectively).[63]

Evolocumab and alirocumab robustly reduce LDL-C in both sexes, but a larger decrease in LDL-C was reported in men, as compared to women (−58% vs −52%, respectively) in the FOURIER clinical trial[60] and in the 10 ODYSSEY phase 3 clinical trials (−60% and −48.3%, respectively),[61] respectively. Preclinical animal experimental studies showed a sex difference in the management of LDL-R expression on the hepatocyte cell surface with regard to pharmacological inhibition (using PCSK9 mAbs) or genetic deficiency (with PCSK9 knockout mouse models) in mice and suggested a potential mechanism that might explain such differences.[62] Namely, as a consequence of PCSK9 inhibition in female mice, sex-specific shedding of excess hepatic LDL-Rs occurs, which favors the hypothesis of PCSK9 sex-dependent effect on LDL-C metabolism.[62] More investigations are needed to further elucidate the sex-dependent effects of PCSK9i in patients with high or very high CVD risk.

Effect of PCSK9i on Risk for Diabetes Mellitus

After three decades of use, it is recognized that the statins are modestly diabetogenic,[64] though mechanistically it is not clear why.[65,66] In the Justification for the Use of Statin in Prevention: An Intervention Trial Evaluating Rosuvastatin trial, rosuvastatin therapy increased risk for incident diabetes mellitus (DM).[67] However, when compared to placebo, rosuvastatin accelerated time to new-onset DM on average by only 5.5 weeks. A meta-analysis showed the following: one would have to treat 1000 patients per year to see 1 new

case of DM with low-dose statin therapy and 500 patients per year to observe 1 new case of DM with moderate- to high-dose statin therapy.[68] Risk for DM among statin-treated patients also depends on the number of components of the metabolic syndrome a patient has (ie, the greater the number of components, the higher the risk) and whether or not they are already prediabetic.[69] It is widely recognized that overall the benefits of statin therapy far outweigh its risks, including that for DM.

Given these observations with statins, it has been of interest to investigate whether or not the inhibition of PCSK9 and the large reductions of LDL-C were associated with new-onset DM. In a Mendelian randomization study that included over 550 000 persons, the impact of PCSK9 loss of function variants (rs11583680, rs11591147, rs2479409, and rs11206510) scaled to 1 mmol/L lower LDL-C associated with increased fasting glucose (0.09 mmol/L; 95% CI, 0.02–0.15), body weight (1.03 kg; 95% CI, 0.24–1.82), waist-to-hip ratio (0.006; 95% CI, 0.003–0.010), and an odds ratio for incident diabetes of 1.29 (95% CI, 1.11–1.50). This analysis suggested that the association between PCSK9 loss of function mutations with diabetes may be clinically meaningful.

A number of analyses have been performed on patients treated with PCSK9 mAbs in an effort to discern if these agents are diabetogenic. In 1 analysis of alirocumab treatment (10 trials, 4974 participants), the HR associated with a transition from prediabetes to new-onset DM was 0.90 (0.63–1.29) versus placebo and 1.10 (0.57–2.12) versus ezetimibe. Mean changes in fasting glucose and HbA1c levels showed no difference between treatment groups in patients without diabetes.[70] Among 4802 patients treated with either evolocumab or standard of care for 1 year, there were no between treatment group difference in fasting plasma glucose or HbA1c. In the ODYSSEY Outcomes (Evaluation of CV Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab) trial, when evaluated over a median of 2.8 years, alirocumab did not increase the risk of new-onset DM (HR, 1.00; 95% CI, 0.89–1.11). Among patients without diabetes at baseline, 676 (10.1%) developed diabetes treated with a placebo, compared with 648 (9.6%) treated with alirocumab.[71] In the FOURIER trial, evolocumab therapy did not increase the risk for new-onset DM compared to placebo.[72] Among participants with metabolic syndrome in the FOURIER trial, evolocumab did not impact the transition to new-onset DM.[73] Evolocumab does not adversely affect fasting plasma glucose, HbA1c, C-peptide, serum insulin, HOMA-B cell function or HOMA-insulin resistance in persons who were normoglycemic or had metabolic syndrome, impaired fasting glucose, or were diabetic.[74] The data for glycemic indices and measures of insulin resistance are similar for alirocumab.[75]

A meta-analysis of 18 studies (26 123 participants) without diabetes treated with either evolocumab or alirocumab showed no significant differences between placebo and PCSK9-mAb treatment groups, including new-onset DM (risk ratio [RR], 1.05; 95% CI, 0.95–1.16), fasting plasma glucose (MD, 0.00 mmol/L; 95% CI −0.02 to 0.02) or HbA1c (MD, 0.00% [0 mmol/L]; 95% CI, −0.01 to 0.01). Neither treatment duration nor intensity of LDL-C reduction correlated with elevations in risk for new-onset DM. Sensitivity analyses did not alter the results. Another meta-analysis (20 studies, 68 123 participants) of patients treated with PCSK9 mAbs over a median follow-up of 78 weeks showed that PCSK9 inhibition nominally increased fasting blood glucose (weighted MD, 1.88 mg/dL [95% CI, 0.91–2.68]; $P < .001$) and HbA1c (0.032% [95% CI, 0.011–0.050]; $P < .001$) when compared to placebo. These changes in glucose and HbA1c were too small to increase the incidence of diabetes (RR, 1.04 [95% CI, 0.96–1.13]; $P = .427$). These findings are reassuring and make it likely that it will require very large numbers of patients to detect a signal for heightened hazard for new-onset DM with the PCSK9 mAbs. Surveillance of this issue continues. To date, however, it is concluded that the PCSK9 mAbs are neutral on risk for new-onset DM.

Effect of PCSK9 Inhibition on Nonalcoholic Fatty Liver Disease

Nonalcoholic fatty liver disease (NAFLD) is a liver disorder characterized by the accumulation of at least 5% fat in the liver in the absence of secondary causes such as excessive alcohol consumption, viral hepatitis, and other chronic liver diseases. It is a diagnosis of exclusion.[76] NAFLD has a wide spectrum of histological variations ranging from NAFL, nonalcoholic steatohepatitis (NASH), fibrosis, NASH cirrhosis, and NASH-related hepatocellular carcinoma. The global prevalence of NAFLD is estimated to be around 1 billion, and a significant proportion of NAFLD patients progress to NASH.[76] By 2030, NAFLD is predicted to be the most common indication for liver transplantation.[77] NAFLD is a complex disease driven by the interplay between host genetics, environmental factors such as diet and coexisting comorbidities.

Although NAFLD can be managed successfully with diet and lifestyle changes, pharmacological intervention is warranted to mitigate the pathophysiology and clinical course of the disease in refractory or clinically significant advanced cases such as those with bridging fibrosis. Despite the unrelenting increase of NAFLD and the enormous healthcare and economic burden, there are no universally approved pharmacotherapies for NAFLD. Data supporting the use of newer therapies are emerging. NAFLD is associated with insulin resistance, elevated TGs/remnant lipoproteins, hypobetalipoproteinemia, and small dense LDL[78]. PCSK9 inhibitors have the potential to improve NAFLD through the beneficial effects on lipid metabolism and modification of risk factors such as hyperglycemia and insulin resistance as described in other sections of the paper. In the following section, we will review the current evidence from preclinical and clinical studies describing the impact of PCSK9 inhibition in the pathogenesis and treatment of NAFLD.

Preclinical Evidence

Preclinical studies indicate that PCSK9 inhibition might have a beneficial impact on the development of NAFLD. PCSK9 knockout mice compared to wild-type mice on high-cholesterol diets were found to have increased hepatic free cholesterol and fibrosing steatohepatitis with a higher predisposition to liver cancer.[76] C57BL/6J mouse models with high-fat diet-induced hepatic steatosis have been found to increase de-novo hepatic PCSK9 expression and elevate circulating PCSK9 levels.[77] PCSK9 enhances LDL-R degradation, resulting in LDL accumulation in plasma. PCSK9-deficient mice have been shown to have resistance to liver steatosis under both chow and high-cholesterol diets.[79] Alirocumab reduces fibrosis and NASH severity in methionine-choline-deficient diet male mice.[80] These observations suggest that PCSK9 inhibition may be a ground-breaking approach for NAFLD. In contrast, PCSK9 deficiency has also been found to be associated with NASH development in mice. PCSK9-deficient mice are resistant to hepatic steatosis under NAFLD-inducing challenge.[81] In a study of C57Bl/6J mice fed with a NASH-inducing diet higher hepatic steatosis and fibrosis with a noticeable inflammatory phenotype were seen with decreased PCSK9 levels.[82] PCSK9 deficiency and NASH development in mice can also be explained by the role of PCSK9 in modulating the transport of fatty acids to the liver and TGs storage in hepatocytes.[23] Similarly, a more severe form of steatohepatitis with exacerbated liver fibrosis was noted in $PCSK9^{-/-}$ C57BL/6J mice compared to $PCSK9^{+/+}$ controls.[81] PCSK9 is a key pathway in liver regeneration and the effect of PCSK9 deficiency in the NASH progression might be due to impaired liver regeneration as shown in $PCSK9^{-/-}$ mice.[79]

Genomics-Based Evidence

The evidence from studies investigating the association of PCSK9 loss of function mutations has reported controversial results. In a study of 2113 Chinese Han population, the "A53V" LOF PCSK9 variant correlated with a reduced risk of NAFLD.[83] In a European cohort of 1874 individuals at risk for NASH, the PCSK9 rs11591147 LOF variant was found to be protective against all types of NAFLD including NAFL, steatosis, and NASH and fibrosis even after adjustment for clinical metabolic risk factors.[84] Similarly, in an analysis of the UK Biobank and Electronic Medical Record and Genomics cohort, the LOF variant PCSK9-p.Arg46Leu was protective against NAFLD and elevated liver enzymes.[85] In contrast, carriers of the PCSK9 R46L variant were found to have excess ectopic fat deposition in the liver with a 2-fold increased prevalence of hepatic steatosis and epicardial fat thickness in a small sample size Italian cohort of 13 patients.[86] Hence the current landscape of genomics studies examining the role of PCSK9 in NAFLD remains unsettled.

PCSK9 Levels and NAFLD

The levels of circulating PCSK9 have been studied to assess the association between liver fat accumulation and hepatic steatosis. In a study of 2027 subjects from the Dallas Heart Study cohort, PCSK9 levels were shown to have a positive association with circulating PCSK9 and liver fat content.[87] Similarly, a report from 698 Kenya Diabetes Study participants and 201 Italian patients demonstrated circulating levels of PCSK9 were correlated with hepatic steatosis.[88,89] In contrast, circulating levels of PCSK9 failed to show a significant association in 478 patients with advanced stages of NASH with T2DM or metabolic syndrome suggesting a lack of deleterious impact of plasma PCSK9 levels on advanced stages of NAFLD.[90] Interestingly, it has been suggested that a correlation between circulating PCSK9 levels and liver fat content changes with the severity of hepatic steatosis.[90]

Therapeutic Benefits and Safety Profile of PCSK9i in NAFLD

There are currently two types of PCSK9i: one acting on circulating PCSK9 in the form of mAbs (alirocumab and evolocumab) and the other is a small interfering mRNA inhibiting the intracellular synthesis of PCSK9 (inclisiran).[27,91,92] While there are no comprehensive studies with long-term follow-up studying the impact of PCSK9 inhibition in the development and progression of NAFLD, there are promising data.[93,94] In an observational study of 26 genetically confirmed FH patients with NAFLD on background therapy of statins and ezetimibe, PCSK9 inhibition led to a significantly improved biomarker profile of hepatic steatosis after 6 months of therapy.[94] There was an improvement in lipid and inflammatory profiles, as well as steatosis biomarkers such as triglyceride-glucose index and hepatic steatosis index. In another retrospective chart review of 29 patients with NAFLD patients, 18 months of PCSK9 inhibition was associated with full resolution of hepatic steatosis/NAFLD computed tomographic features in 8 out of 11 patients with hepatic steatosis and improvement of serum alanine transaminase (from 21.8 ± 11.9 at pretreatment to 17.7 ± 8.00 at posttreatment, $P = .042$).[93] A study group of 40 HeFH patients with metabolic syndrome (13 with NAFLD/NASH) had significant reductions of serum liver biomarkers and improvement of NAFLD/NASH profile after 1 year of PCSK9i with either evolocumab or alirocumab.[95] Data surrounding the beneficial effects of intracellular PCSK9 inhibition using agents such as inclisiran are lacking.

There has been mounting scientific and clinical evidence supporting the hepatic safety and tolerability profile of PCSK9i.[56,91,92,96,97] No significant increase in risk for AEs of hepatic transaminase elevation was reported in a prespecified secondary analysis of the FOURIER trial with evolocumab.[92] A similar safety profile was noted in a study by Ji et al[97] investigating 31,475 AE reports regarding PCSK9i (alirocumab and evolocumab) from the Food and Drug Administration Adverse Event Reporting System database. Given that inclisiran remains active and causes PCSK9

inhibition for >1 year, liver safety is critical.[96] In another study of more than 340 000 individuals from UK Biobank, it was reported that the variant PCSK9-p.Arg46Leu is not associated with higher levels of circulating liver enzymes. Hence supporting that the genetic inhibition of PCSK9 in humans is not associated with an increased risk of NAFLD.[85] In a small cohort of 28 participants, inclisiran was safe and well tolerated in patients with mild or moderate hepatic impairment similar to those with normal hepatic function.[98] In a 4-year open-label extension of the ORION-1 trial reporting the safety of inclisiran in 382 patients, participants experienced the following hepatic events: increases in hepatic enzymes (3 cases) and hepatic fibrosis (1 case) and 36 patients experienced at least 1 treatment-emergent hepatic event.[91] In a pooled analysis of 3660 participants from 3 phase–3 studies (ORION-9, ORION-10, and ORION-11), analysis of liver enzyme markers demonstrated no significant differences between Inclisiran and the placebo arm.[56]

The abovementioned preclinical and human data support a potential protective role of PCSK9 inhibition in NAFLD at various stages. However, the data are inconsistent and point toward caution, particularly with respect to NASH pathology. Overall, there are significant gaps in knowledge and literature with no randomized clinical trials focusing on the NAFLD patient subgroups that might respond best to PCSK9 pathway inhibition.

Effect of PCSK9i on CV Outcomes

Patients with FH are at high risk of ASCVD events due to lifelong exposure to high levels of LDL-C; the risk of CV events is increased because many individuals with FH also have elevated Lp(a).[99] Patient safety and the reduction of medication errors are a priority in healthcare. Incorrect use of drugs can lead to AEs with serious consequences for patients. Some guidelines represent a complete tool to support operators to be implemented in all healthcare facilities to avoid or minimize the risk of the onset of a particularly serious, potentially avoidable AE.

The European guidelines provide, both in FH patients with ASCVD and in FH patients in primary prevention, with another CV risk factor (DM and/or advanced chronic kidney disease) at very high risk, a recommended goal of LDL-C ≥ 50% from baseline and an LDL-C < 1.4 mmol/L (<55 mg/dL); for FH patients without ASCVD or another high-risk CV risk factor, the goal of LDL-C ≥ 50% of LDL-C from baseline and an LDL-C < 1.8 mmol/L (<70 mg/dL) is recommended.[6] In patients with FH, statins remain the cornerstone of lipid-lowering therapy, and early initiation may reduce the progression of atherosclerosis and the risk of future CV events.[100] When adequate LDL-C reduction cannot be achieved with statin therapy alone, the addition of ezetimibe and PCSK9i may be necessary. The American Heart Association/American College of Cardiology Multisociety guidelines recommend the use of PCSK9i in HeFH patients between 30 and 75 years of age with an LDL-C level ≥2.6 mmol/L (100 mg/dL) during treatment on maximally tolerated statins in combination with ezetimibe.[101] Treatment with evolocumab or alirocumab significantly reduces LDL-C in patients with HeFH without major adverse side effects; there are no studies targeting outcomes of PCSK9i on ASCVD for patients with HeFH. Both European and North American guidelines provide recommendations for the use of PCSK9i in these very high-risk primary prevention patients.

The RUTHERFORD study demonstrated that evolocumab reduced LDL-C by 60% at 12 weeks, and sustained reductions in LDL-C by an average of 53.6% after 48 weeks.[101] The ODYSSEY FH1 and FH2 study showed a 50%–60% reduction in LDL-C in the PCSK9 group compared to the placebo after 24 weeks, with the effect persisting at 78 weeks.[102] The TESLA study evaluated the effect of evolocumab on homozygous FH (HoFH);[103] patients with HoFH may have very low or zero LDL-R expression in the liver and have an inferior response to PCSK9 inhibition.[104] However, many patients with HoFH have some residual capacity for functional LDL-R expression. The TESLA study showed at 12 weeks a significant reduction in LDL-C of 30.9% compared to placebo. Very high-risk primary prevention patients benefit from PCSK9i when LDL-C goals are not met through treatment with statins and ezetimibe. Efficacy in PCSK9 inhibition for secondary prevention of secondary CV events is supported by 2 well-known CV outcome studies: FOURIER for evolocumab and ODYSSEY OUTCOMES for alirocumab.

In FOURIER subgroup analyses, evolocumab significantly reduced the occurrence in primary prevention of stroke (HR, 0.79; 95% CI, 0.66–0.95) and in secondary prevention of ischemic stroke (HR, 0.75; 95% CI, 0.62–0.92), with no difference in hemorrhagic stroke.[105] In a prespecified analysis of FOURIER, participants with lower extremity peripheral artery disease (PAD), treatment with evolocumab reduced the relative risk of the primary composite endpoint ($P = .0098$).[105] The ODYSSEY OUTCOMES study showed a reduction in the risk of any stroke in primary prevention (HR, 0.72; 95% CI, 0.57–0.91) and ischemic stroke (HR, 0.73; 95% CI, 0.57–0.93) without increasing hemorrhagic stroke in secondary prevention.[106] Among patients with a history of PAD, treatment with alirocumab resulted in a significant relative risk reduction of extremity events of 41% and an absolute risk reduction of 8.6%.[107]

The evolocumab for early reduction of LDL cholesterol levels in patients with acute coronary syndromes (EVOPACS) study evaluated the feasibility, efficacy, and safety of initiating evolocumab during hospitalization for very high-risk patients with recent-onset acute coronary syndromes. The study demonstrated that evolocumab added to a high-intensity statin was safe and resulted in a significant percentage of reaching target LDL-C levels (from 3.61 to 0.79 mmol/L at week 8; 95% CI, −45.2 to −36.2; $P < .001$).[108]

The global assessment of plaque regression with a PCSK9 antibody as measured by intravascular ultrasound

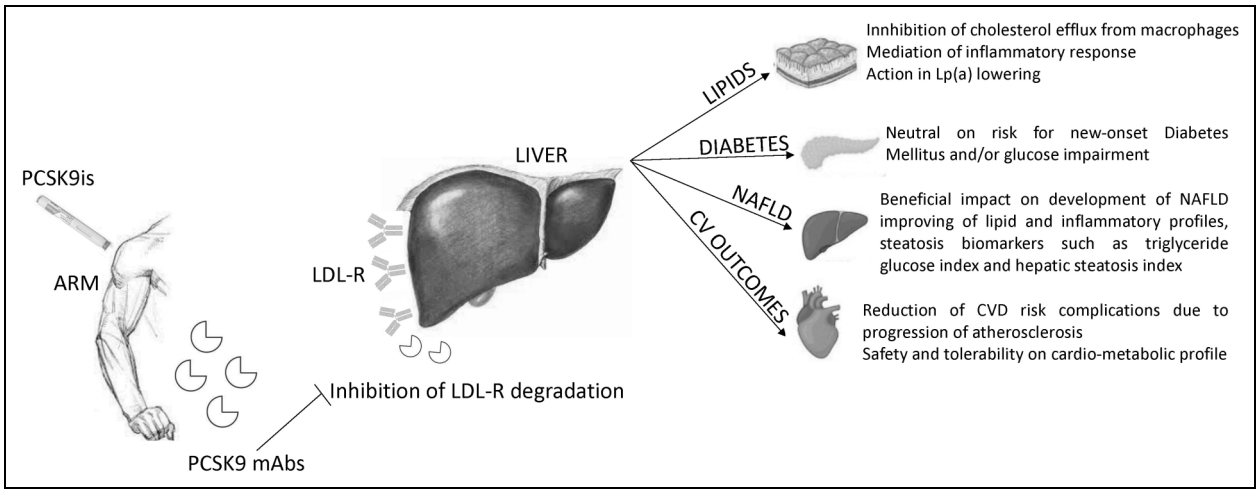

Figure 1. Effects of proprotein convertase subtilisin/kexin type 9 inhibitors (PCSK9i) on metabolic factors.

(GLAGOV) study evaluated the action of evolocumab treatment on coronary artery plaque among statin-treated individuals after 76 weeks of treatment and found a significant reduction in percent atheroma volume in the evolocumab treatment group versus placebo (−1.0%; 95% CI, −1.8% to −0.64%). Moreover, it was found that a higher percentage of patients showed plaque regression in the evolocumab group compared to the placebo (64.3% vs 47.3%).[109] The PACMAN-AMI study evaluated alirocumab treatment after the urgent percutaneous coronary intervention of the causative plaque in patients with acute MI. Patients receiving alirocumab had significantly greater coronary plaque regression in nonculprit vessels after 52 weeks: at 52 weeks the mean change in percent atheroma volume was −2.13% with alirocumab versus −0.92% with placebo (difference, −1.21%; 95% CI, −1.78% to −0.65%; $P < .001$), mean change in maximum lipid core burden index within 4 mm was −79.42 with alirocumab versus −37.60 with placebo (difference, −41.24; 95% CI, −70.71 to −11.77; $P = .006$), mean change in minimal fibrous cap thickness was 62.67 µm with alirocumab versus 33.19 µm with placebo (difference, 29.65 µm; 95% CI, 11.75–47.55; $P = .001$).[110]

Conclusion

The discovery of PCSK9i has opened a new era in therapeutic management in patients with hypercholesterolemia and high risk of ASCVD,[111-114] in combination with or as an alternative to statins in cases of incomplete or complete intolerance.[115,116] The PCSK9i do not increase the risk of new-onset diabetes.[69,117] PCSK9 inhibition favorably impacts all of the lipid and lipoprotein fractions that drive atherogenesis. PCSK9 inhibition also greatly improves LDL-C goal attainment rates in patients with or without FH. PCSK9 inhibition may have a beneficial impact on the development of NAFLD, but more comprehensive studies with long-term follow-up are required.[3,92] Notably, conflicting data from preclinical studies exist raising controversy and calling for more research endeavors to support or refute the favorable role of PCSK9 inhibition in NAFLD. The PCSK9 mAbs have dramatically increased our capacity to lower LDL-C to levels not previously anticipated. They are safe, effective and well tolerated (Figure 1); they reduce ASCVD-related event rates in men and women and in patients with diabetes, ASCVD, or PAD. The addition of inclisiran to our dyslipidemia armamentarium now allows clinicians to treat patients with an intervention that requires only 2 doses annually, thereby improving adherence. We eagerly await the results of the ORION outcomes trial.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: This article has been written independently and reflects the opinion of the authors, without any role of the industry. Everyone must disclose. PPT: speaker's bureau, Amgen and Novo-Nordisk. Consultant: Novartis.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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