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Proprotein Convertase Subtilisin Kexin 9 (PCSK-9) - Targeting Therapy as A New Line for The Treatment of Hyperlipidemia

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ABSTRACT

Subtilisin/Kexin type 9 proprotein convertase (PCSK9) is an enzyme produced in the liver. PCSK9 binds to low-density lipoprotein (LDL) receptors on the surface of hepatocytes, which are then degraded and result in elevated plasma LDL-cholesterol (LDL-C) levels. Blocking PCSK9 with antibodies lowers plasma LDL-C levels. Alirocumab and evolocumab are fully humanized monoclonal antibodies that bind to PCSK9-free plasma. They have been approved for clinical use by regulatory agencies. PCSK9 antibody levels of LDL-C were significantly lower. its use is associated with lower rates of myocardial infarction and stroke. Alirocumab in addition to intensive statin therapy may lead to a reduced risk of overall death after acute coronary syndrome in the long term. The most common side effect of evolocumab and alirocumab is injection site reactions. Other rates of side effects were comparable to those seen with placebo therapy. An attractive alternative to monoclonal antibodies for PCSK9-derived is PCSK9-small molecule interfering RNA (siRNA). Theoretically, these molecules offer profoundly lowering PCSK9 (intra and extracellular) at low dose frequencies and potentially at lower cost.

Introduction

Elevated circulating levels of the proprotein subtilisin/kexin type 9 (PCSK9) convertase are associated with increased low-density lipoprotein (LDL) and poorer cardiovascular outcomes. Antibodies to PCSK9 are approved by regulatory agencies for the treatment of individuals with inadequately treated LDL cholesterol (LDL-C) levels. They were able to lower LDL-C as much as 60 percent in patients undergoing statin therapy. In addition, they yield clinical benefits, such as reduced rates of stroke or myocardial infarction. A second therapy to degrade PCSK9, a small interfering RNA, is being actively activated.

Discussion

Mode of action

PCSK9, an enzyme (serine protease) encoded by the PCSK9 gene, is mainly produced in the liver.[1-3] PCSK9 binds to the LDL receptor (LDL-R) on the surface of hepatocytes, causing degradation of LDL-R and subsequently increasing plasma LDL-C levels.[4,5]

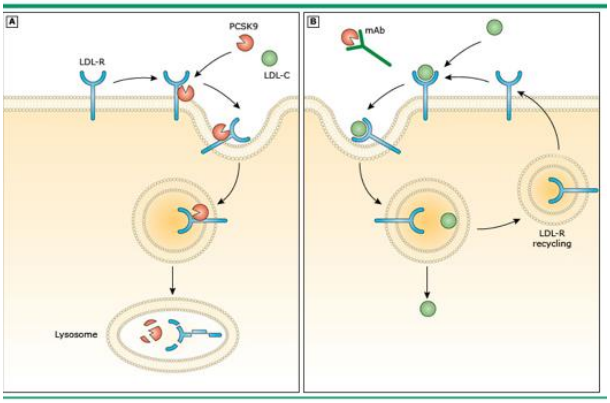

Figure 1. PCSK9 pathway and effect of PCSK9 antibody on LDL-R [6, 7]

Antibodies to PCSK9 interfere with LDL-R binding, leading to higher hepatic LDL-R expression and lower plasma LDL-C levels (figure 1).[6,7] Alirocumab and evolocumab are fully humanized monoclonal antibodies that bind to free plasma PCSK9, promoting the degradation of this enzyme.[8-11] As a result, less PCSK9 is available in plasma to bind LDL-R. This results in a higher fraction of LDL-R recycling towards the hepatocyte surface. As a consequence, the liver has the capacity to remove more LDL-C from the circulation, resulting in lower plasma LDL-C levels. This antibody is

specific for PCSK9 and does not bind to other members of the enzyme superfamily PCSK.[7,12,13] Another method that can interfere with PCSK9 is by blocking its synthesis, which is dependent on messenger RNA.[14] Inclisiran is one such antisense, silencing mRNA. Circulating levels of PCSK9 are upregulated in the presence of statins, suggesting that inhibition of the PCSK9 pathway may complement the LDL-C-lowering effect of statins.

PCSK9 monoclonal antibodies

PCSK9 monoclonal antibody is approved for use in many regions of the world. They are very effective in lowering LDL-C but have the disadvantage of being administered by frequent subcutaneous injection (once or twice a month) and high cost. The survey showed a high level of satisfaction with PCSK9 injection therapy, with few on-site reactions, and an interest in self-injecting using a subcutaneous pen injection device.[15]

Clinical impact

Post-hoc analysis of the initial randomized trial suggested mortality for this agent, the results of the FOURIER study with evolocumab (2.2 year follow-up) found no mortality benefit. However, a post-hoc analysis of the ODYSSEY RESULTS showed that alirocumab over intensive statin treatment after acute coronary syndrome could reduce all-cause mortality in the long term (hazard ratio [HR] 0.85, 95% CI 0.73-0.98; p = 0.03).[16] Despite the lack of a death benefit in FOURIER, the risk of myocardial infarction (HR 0.73, 95% CI 0.65-0.82; p<0.001) and stroke (HR 0.79, 95% CI 0.66-0.95; p = 0.01) was significantly reduced.

The relevance of inhibitory PCSK9 levels has been demonstrated by the Mendelian randomization study, in which the functional alteration of the PCSK9 mutation decreased with a decrease in LDL-C, as well as a significantly lower risk of occurrence.[17-19] In contrast, PCSK9 gain-of-function mutations with increased LDL-C levels and a higher risk of cardiovascular events [20–22]. Another study reported that HMG-CoA reductase mutations and PCSK9 mutations leading to LDL-C levels were more likely to reduce the impact of cardiovascular events, reflecting the reduced effect of PCSK9 antibodies over statins (figure 2).[23,24]

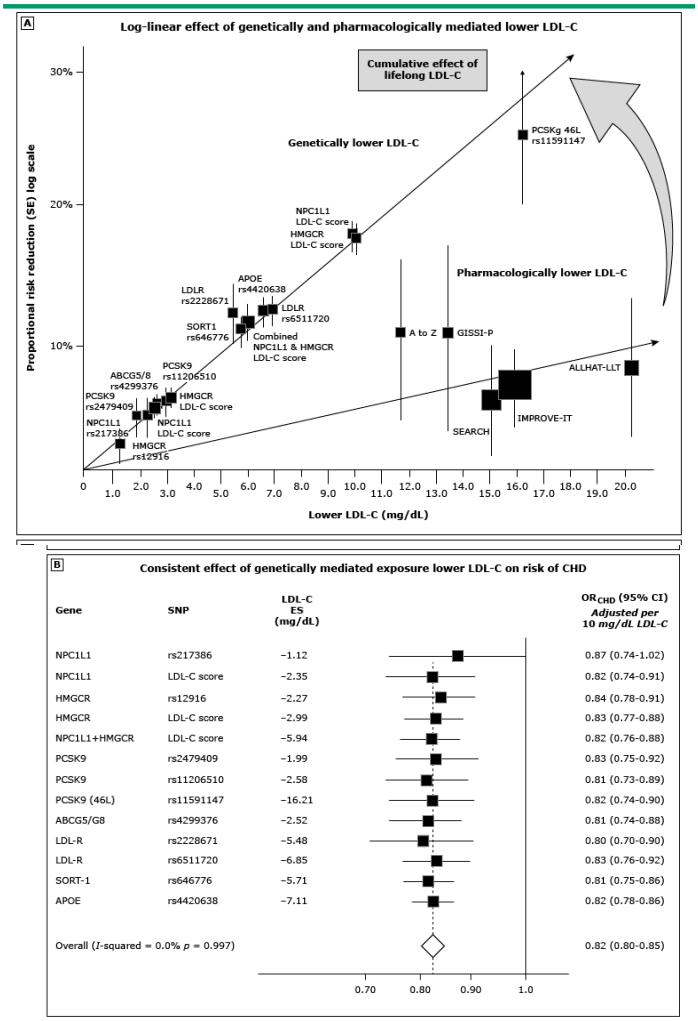

Figure 2. Log-linear effect of genetically and pharmacologically mediated lower LDL-C.[23, 24]

The benefits of PCSK9 monoclonal antibody include:

- LDL-C reduction in a dose-dependent manner, by as much as 70 percent, and as much as 60 percent in patients on statin therapy.[25-35]
- Decreases in lipoprotein(a) levels by 18 to 36 percent, triglyceride levels by 12 to 31 percent, and slight increases in density lipoprotein cholesterol as high as 5 to 9 percent.[34,36–41]
- Percent reduction in atheroma volume.[42]
- Significant reduction (up to 50 percent) in the risk of cardiovascular events in post-hoc analyzes of the initial evolocumab and alirocumab studies.[43–45]

A meta-analysis of 24 randomized trials (n = 10,159) in various clinical situations (familial hypercholesterolemia; other hypercholesterolemia; statin-intolerant hypercholesterolemia; intensive, non-intensive, or no statin therapy) found that anti-PCSK9 abs reduced all-cause death (odds ratio [OR] 0.45, 95% CI 0.23–0.86), cardiovascular death (OR 0.50, CI 0.23–1.10), and myocardial infarction (OR 0.49, CI 0.26-0.93).[43] No statistical heterogeneity was found among the results of the included trials, suggesting that, as has been seen with statins, the relative anti-PCSK9 abs may be similar across clinical situations and the baseline risk of cardiovascular disease.

Another study after the meta-analysis had similar findings. In two open-label trials of the evolocumab monoclonal antibody combined for analysis, 4465 patients who had completed one of the twelve phase 2 or phase 3 trials of evolocumab were randomly assigned to evolocumab (140 mg injected subcutaneously every two weeks or 420 mg every two weeks). months.) plus standard therapy, or standard therapy alone [44]. Patients in the trial that included those on statin therapy (approximately 70 percent), including high-intensity statin therapy (about 27 percent), as well as patients who were intolerant to statins or who were not on other lipid-lowering therapy, and the median duration of follow-up was 11.1 months. Patients with evolocumab had a lower combined incidence rate (1.0 versus 2.2 percent; HR 0.47, 95% CI 0.28–0.78).

Pharmacology properties

The highlights of the pharmacokinetics and pharmacodynamics of evolocumab and alirocumab are presented in a table (table 1).[13,46-48]

Table 1. Pharmacokinetics and pharmacodynamics of alirocumab and evolocumab.[13,46-48]

	Alirocumab	Evolocumab
Pharmacokinetics		
Absorption	Median Tmax: ▪ Three to seven days	Median Tmax: ▪ Three to four days
	Estimated absolute bioavailability: ▪ 85%	Estimated absolute bioavailability: ▪ 72%
	Cmax: ▪ 1.54 ± 1.02 ng/mL following 150 mg dose	Cmax: ▪ 18.6 ± 7.3 ug/mL following 140 mg dose ▪ 59.0 ± 17.2 ug/mL following 420 mg dose
	AUC: ▪ 129 ± 35.7 mg • day/L following 75 mg dose	AUC: ▪ 188 ± 98.6 day • ug/mL following 140 mg dose ▪ 924 ± 346 day • ug/mL following 420 mg dose
Distribution	0.04 to 0.05 L/kg	3.3 (0.5)* L
Metabolism and elimination	Specific metabolism studies were not conducted, because the antibodies are proteins. The antibodies are expected to degrade to small peptides and individual amino acids.	Specific metabolism studies were not conducted, because the antibodies are proteins. The antibodies are expected to degrade to small peptides and individual amino acids.
	At low concentrations, the elimination is predominately through saturable binding to target (PCSK9), while at higher concentrations the elimination is largely through a non-saturable proteolytic pathway.	At low concentrations, the elimination is predominately through saturable binding to target (PCSK9), while at higher concentrations the elimination is largely through a non-saturable proteolytic pathway.
	Effective half-life of 17 to 20 days. When co-administered with a statin, the half-life is 12 days.	Effective half-life of 11 to 17 days.
Pharmacodynamics		
	Following a single subcutaneous administration of 75 or 150 mg, maximal suppression of free PCSK9 occurred within four to eight hours.	▪ Following single subcutaneous administration of 140 mg or 420 mg, maximum suppression of circulating unbound PCSK9 occurred by four hours. ▪ A mean nadir in LDL-C lowering response occurs by 14 and 21 days following 140 or 420 mg dos, respectively. ▪ Subcutaneous regimens of 140 mg every two weeks and 420 mg once monthly were equivalent in average LDL-C lowering. ▪ LDL-C lowering efficacy was sustained with continued use, in measurements made over 112 weeks.
	Unbound PCSK9 concentrations returned toward baseline when antibody concentrations decreased below the limit of quantitation.	▪ Unbound PCSK9 concentrations returned toward baseline when antibody concentrations decreased below the limit of quantitation. ▪ No rebound in PCSK9 or LDL-C above baseline was observed during the washout of evolocumab.

* Standard deviation

AUC: exposure as measured by the area under the concentration x time curve; Cmax: maximum plasma concentration; PCSK9: proprotein convertase subtilisin/kexin type 9; Tmax: time to maximum plasma concentration.

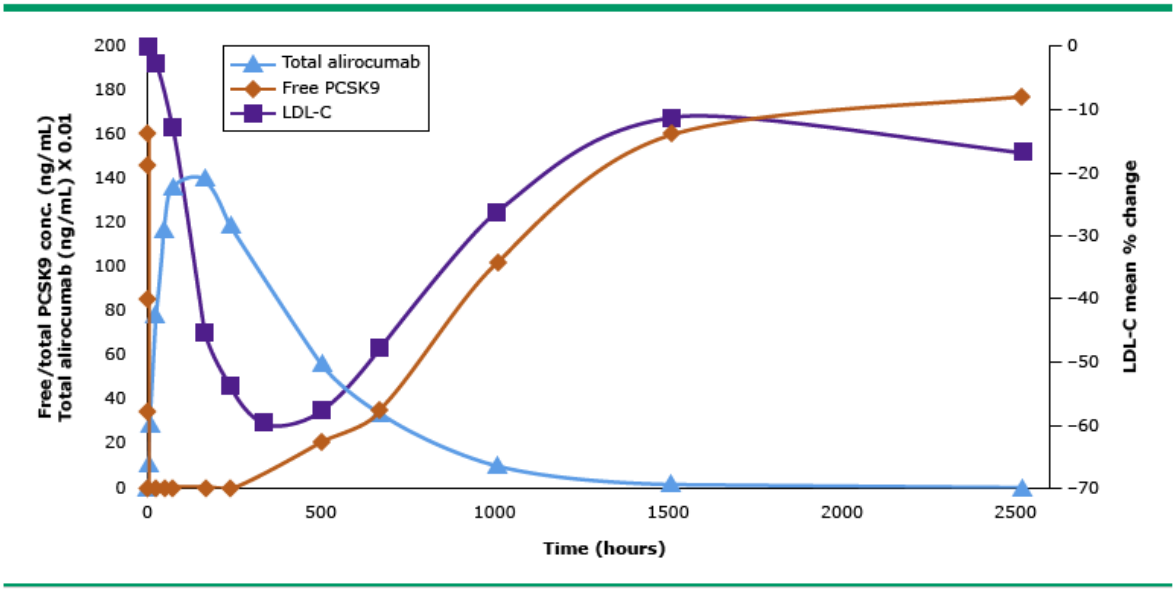

Figure 3. Effect of PCSK9 antibody on free PCSK9 concentration

PCSK9 monoclonal antibodies bind to free PCSK9 (that is, not bound to other proteins) rapidly, so there is no availability until free PCSK9 for two to three weeks after administration. When PCSK9 activity is suppressed, hepatocytes recycle and express most of the LDL-R surface receptors that more efficiently clear LDL-C from plasma. When suppression of free PCSK9 levels fell below 75 to 85 percent, the opposite occurred, and plasma LDL-C increased (figure 3).

Metabolism and clearance

After initial subcutaneous injection of alirocumab or evolocumab, systemic bioavailability was 85 and 72 percent, respectively. The apparent volume of distribution of the two PCSK9 inhibitors was approximately 3.3 liters, indicating a restricted tissue distribution. The onset of PCSK9 enzyme inactivation occurs within four to eight hours after the first subcutaneous injection of the PCSK9 monoclonal antibody.[47,48] Formal metabolic studies were not carried out because the PCSK9 monoclonal antibody is composed of protein and carbohydrates and elimination is expected to occur via saturable binding to the PCSK9 enzyme and unsaturated proteolysis of small peptides and amino acids. The exposure and cleaning properties estimates given in the manufacturer's labeling are based on a population analysis. The effective elimination half-life of the available PCSK9 inhibitors is 11 to 20 days. The half-life and is slightly reduced when given with statins.[47,48] In patients with renal or hepatic impairment, adjustment of the dose of alirocumab or evolocumab is unnecessary. However, no data are available in patients with severe renal or hepatic impairment.

Adverse side effects

PCSK9 inhibitors appear to be well tolerated.[43–45,50] In an analysis of data collected from clinical trials, the overall rate of adverse events with PCSK9 inhibitors was similar to that of placebo.[51] A local injection site reaction which is usually mild (eg, erythema, pain, or bruising) is one of the most frequently reported adverse events occurring in 6 and 7 to 10 percent of patients treated with evolocumab and alirocumab.[47,48] PCSK9 inhibitors do not appear to cause muscle toxicity or elevated liver enzymes.[51] PCSK9 clinical trials have evaluated safety for nearly five years.[41,52-55] Serious side effects appear to be rare.

Dosis

Evolucumab

- In primary hyperlipidemia or secondary prevention of cardiovascular events, the recommended dosage of evolocumab is 140 mg subcutaneously every two weeks or 420 mg once monthly; both doses are clinically equivalent.[35,47,70]
- In homozygous familial hypercholesterolemia (HoFH), evolocumab 420 mg subcutaneously once monthly is the recommended starting dose. The dose may be increased after 12 weeks of treatment to 420 mg subcutaneously once every two weeks if a clinically meaningful response has not been achieved. HoFH patients on lipid apheresis may initiate evolocumab treatment as 140 mg once every two weeks to correspond with their apheresis schedule, ie, directly after apheresis.[49,71-73]

Alirocumab

- In primary hyperlipidemia or secondary prevention of cardiovascular events, the starting dose of alirocumab is 75 mg subcutaneously once every two weeks or 300 mg once every 4 weeks. The maintenance dose is 75 to 150 mg subcutaneously once every two weeks.[11] An increase in maintenance

dosage to 150 mg every two weeks can be initiated if LDL-C lowering is inadequate. LDL-C plasma levels should be measured within 4 to 12 weeks of initiating or changing the dose and every 3 to 12 months thereafter. For patients receiving 300 mg once every 4 weeks, the LDL-C should be measured just prior to the next scheduled dose.

- In patients with heterozygous familial hypercholesterolemia undergoing LDL apheresis, HoFH, or very high-risk with LDL-C levels >50 percent above their minimal acceptable LDL-C goal, we initiate therapy with alirocumab 150 mg once every two weeks. Alirocumab can be administered without regard to the timing of LDL apheresis.

Small interfering RNA (siRNA, inclisiran)

Another interesting alternative for deriving monoclonal antibodies to degrade PCSK9 is the small interfering RNA molecule (siRNA) PCSK9.[74] Theoretically, these molecules offer profound (intra and extracellular) PCSK9 reduction at lower dose frequencies and potentially at lower cost. One siRNA (RNAi) inhibitor of PCSK9 synthesis (incisiran) has undergone phase 1 2 and 3 evaluations all in the context of the ORION trial.[75-78]

- The ORION-9, -10, and -11 phase 3 trials were published in 2020.[77,78] In all studies, the coprimary endpoints were the placebo-corrected percentage change in LDL-C from baseline to day 510 and the time-adjusted percentage change in LDL-C from baseline after day 90 and up to day 540.
- IN ORION-9, 482 adults with heterozygous familial hypercholesterolemia and treated with maximally tolerated doses of statin were randomly assigned to subcutaneous injections of inclisiran or placebo on days 1, 90, 279, and 450.[77] The mean baseline LDL-C was 153 mg/dL. At day 510, inclisiran reduced LDL-C by 47.9 percent (95% CI 42.3-53.5) and the corresponding time-adjusted reduction of 44.3 percent (95% CI 40.1-48.5).
- Results of the ORION-10 and -11 trials were published together.[78] In these two trials, statin-treated patients with cardiovascular disease (CVD) or at high risk were randomly assigned to receive inclisiran or placebo administered by subcutaneous injection on day 1, day 90, and every six months over a period of 540 days.
- In both trials, the mean baseline LDL-C was approximately 105 mg/dL (2.72 mmol/L).
- ORION-10 enrolled 1561 patients with CVD. At day 510, inclisiran reduced LDL-C by 52.3 percent (95% CI 48.8-55.7) and the corresponding time-adjusted reduction of 53.8 percent (95% CI 51.3-56.2).
- ORION-11 enrolled 1617 patients with CVD or at high risk. At day 510, inclisiran reduced LDL-C by 49.9 percent (95% CI 46.6-53.1) and the corresponding time-adjusted reduction of 49.2 percent (95% CI 46.8-51.6).

In a patient-level pooled analysis of these three trials (3660 participants), the placebo-corrected change in LDL-C with inclisiran at day 510 was -50.7 percent (95% CI -52.1 to -48.9 percent).[79] Serious adverse events were similar between the two groups. Injection-site adverse reactions occurred in 5.0 percent of those receiving drug (compared with 0.7 percent in the placebo group), and most were generally mild. In addition, significant reductions in lipoprotein(a), an independent risk factor for CVD, were seen. Inclisiran has been approved for clinical use in Canada, England, and Europe[80] but not in the United States.

Conclusion

Rapid, innovative developments have emerged within the field of therapeutic cholesterol-lowering, especially in PCSK9 inhibition. There are two approved PCSK9 inhibitors, evolocumab, and alirocumab, with potent and equivalent LDL-C reductions of ranges from 45% to 65%. The development of siRNA to inhibit PCSK9 has garnered much attention because of its LDL-C lowering efficacy, as well as its sustained durability after dosing. Several trials are still underway to assess the impact of inclisiran on cardiovascular outcomes and its efficacy in special populations. Other innovative modes of PCSK9 inhibition that are still in the early phases of development include: vaccines, CRISPR editing, and PCSK9 antagonists along various stages of translation, and small-molecule inhibitors that block the PCSK9-LDLR interaction

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