A New Approach to PCSK9 Therapeutics

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A highly durable RNAi therapeutic inhibitor of PCSK9
Fitzgerald et al
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PCSK9 (proprotein convertase subtilisin/kexin type 9) inhibition is an effective therapy to reduce low-density lipoprotein cholesterol and cardiovascular events. A recent study shows that 1 or 2 doses of inclisiran, a long-acting synthetic small-interfering RNA that selectively targets hepatic PCSK9, causes a sustained reduction of plasma low-density lipoprotein cholesterol for ≤6 months. Pending further studies of safety and efficacy, this may represent an important addition to the armamentarium for inhibiting PCSK9.

Genetic studies showed that gain-of-function mutations in PCSK9 (proprotein convertase subtilisin/kexin type 9) lead to a high low-density lipoprotein cholesterol (LDL-C) level and premature coronary heart disease, whereas loss-of-function variants are associated with low LDL-C level and reduced coronary heart disease[1,2] identifying PCSK9 as a therapeutic target. The discovery in transgenic mice that PCSK9 is secreted by hepatocytes and enters the circulation[3] laid the foundation for a therapeutic approach using PCSK9 monoclonal antibodies (mAbs). PCSK9 mAbs act by binding to extracellular PCSK9 and preventing its interaction with hepatic LDL receptors. This led to the successful development and US Food and Drug Administration approval of PCSK9 mAbs for use as monthly injections in adult patients with heterozygous familial hypercholesterolemia, homozygous familial hypercholesterolemia, or clinical atherosclerotic cardiovascular disease that requires additional lowering of LDL-C.[4,5]. The just announced positive clinical outcome of a large phase 3 study, FOURIER (*F*urther Cardiovascular *OU*tcomes *R*esearch with PCSK9 *I*nhibition in Subjects with *E*levated *R*isk), further supports the concept of PCSK9 inhibition.[6] A new study shows that inhibition of PCSK9 production by long-acting RNA interference also causes substantial reduction of LDL-C in humans.[7] A remarkable feature of this RNA interference–based therapy is the sustained reduction of LDL-C for ≤6 months after just 1 or 2 doses.

Inclisiran, the agent used in this study, is a long-acting, subcutaneously delivered, synthetic small-interfering RNA (siRNA) directed against PCSK9. The siRNA approach uses the natural RNA interference pathway by binding to the RNA-induced silencing complex, enabling it to specifically cleave mRNA molecules encoding PCSK9. A single siRNA-bound RNA-induced silencing complex is catalytic and cleaves many transcripts. This characteristic is thought to be particularly important when used in conjunction with statins, which are known to upregulate the production of PCSK9.[8] Inclisiran is conjugated to triantennary *N*-acetylgalactosamine carbohydrates, which bind to asialoglycoprotein receptors on hepatocytes,[7] leading to the uptake of inclisiran and suppression of hepatic PCSK9 production. This phase 1 study was designed so that the participants received either single- or multiple-dose inclisiran versus placebo. In the multiple-dose study, some participants were also given statins in combination with inclisiran. Plasma PCSK9 levels were lowered by inclisiran in a dose-dependent fashion, reaching a peak reduction of ≈74% at a dose of ≥300 mg at day 84 after the single-dose administration and remained significantly lowered (>50% reduction) at day 180. Multiple-dose inclisiran administration caused a peak reduction of ≈83% at 500 mg, with or without statins and lasted up to day 196 after the first dose. LDL-C levels were substantially reduced in both the single-dose and multiple-dose groups, with peak reduction of ≈50% in the single-dose and ≈60% in the multiple-dose regimen at day 84. Most importantly, the marked reduction of LDL-C persisted for ≤180 days after receipt of the first dose, paralleling the reduction of plasma PCSK9 levels. Thus, inclisiran treatment has the potential to provide effective management of hypercholesterolemia with administration every 3 to 6 months, as compared with once or twice monthly regimens for the currently approved antibodies.

The effectiveness and durability of this RNA interference therapeutic approach is based on 4 key elements. First, the *N*-acetylgalactosamine carbohydrates conjugated inclisiran is highly efficient in silencing PCSK9 mRNA once delivered into the cell.[9] Second, inclisiran primarily targets PCSK9 in hepatocytes, the main source of PCSK9.[10] Third, the long-term, sustained reduction of PCSK9 and LDL-C reflects chemical modifications in the synthesis of inclisiran that improve molecular stability. These modifications include a combination of phosphorothioate, 2′-*O*-methyl nucleotide, and 2′-fluoro nucleotide modifications that improve the resistance to attack by various nucleotide-modifying enzymes.[9] Fourth, attaching 3 *N*-acetylgalactosamine carbohydrates molecules to the 3′ terminus of the siRNA by means of a triantennary spacer substantially increases the affinity of the ligand to asialoglycoprotein receptor, a molecule highly expressed in hepatocytes

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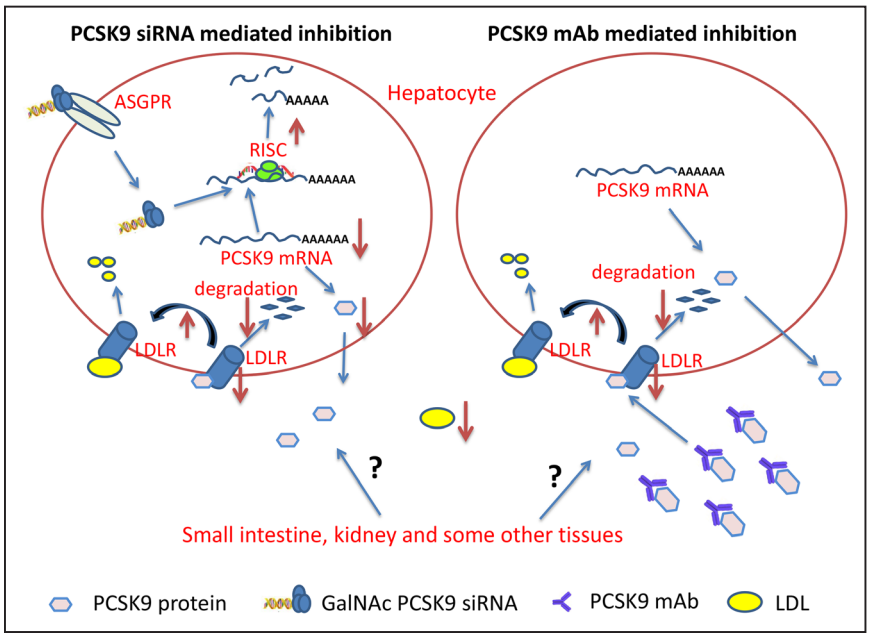

Figure. PCSK9 (proprotein convertase subtilisin/kexin type 9) is primarily produced from hepatocytes and some may come from extrahepatic tissues such as small intestine and kidney. PCSK9 binds low-density lipoprotein receptor (LDLR), promoting its degradation and reducing its recycling. *N*-acetylgalactosamine carbohydrate (GalNAc)–conjugated PCSK9 small-interfering RNAs (siRNAs) are efficiently and selectively delivered into hepatocytes, via asialoglycoprotein receptor (ASGPR), which is highly expressed in hepatocytes. Once in the cell, PCSK9 siRNA efficiently mediates PCSK9 mRNA degradation via the RNA interference mechanism and selectively reduces hepatic but not other tissue PCSK9 production. PCSK9 monoclonal antibodies (mAbs) bind PCSK9 in circulation regardless of its original source and block its binding to LDLR. As a result, hepatic LDLR levels are increased by PCSK9 siRNA or PCSK9 mAbs, and plasma LDL levels are decreased. Thick arrows indicate the direction of the effects.

and greatly improves efficiency and specificity of hepatocyte targeting.[9] An important factor that has limited the use of PCSK9 mAbs is cost (≈$15 000 per annum), which may be partly related to the expense of manufacturing. In this regard, inclisiran has the advantage of a relatively simple manufacturing process that can be readily scaled up. Thus, inclisiran is expected to have potential to improve cost-effectiveness compared with mAbs.

The potential advantage of inclisarin compared with PCKS9 mAb with regard to dosing frequency and cost may be partly offset by somewhat reduced effectiveness of LDL-C lowering. Closer inspection of the data (Figure 2 in the study by Fitzgerald et al[7]) suggests that the reduction in LDL-C with inclisiran therapy might be in the range of ≈40% when adjusted for a small reduction in LDL-C in subjects receiving placebo.[7] This compares to ≈60% placebo-adjusted reduction of LDL-C for multiple-dose PCSK9 mAb administration.[4,5,11,12] Although the inclisarin study is a small phase 1 study and the study populations are different, there are theoretical reasons to think that this may be a real difference. Although liver is the major source for plasma PCSK9[10], at least in animals, other tissues such as the small intestine and kidney do express PCSK9.[10] PCSK9 mAbs target plasma PCSK9 regardless of the original source, whereas inclisiran is expected to primarily target hepatic PCSK9 production. This suggests that theoretical maximum inhibition of PCSK9 and reduction of LDL-C induced by PCSK9 mAbs may be somewhat greater than that resulting from liver-selective inhibition (Figure).

In these small phase 1 studies, the safety and side-effect profile has suggested that inclisiran is safe, with all adverse events being mild or moderate in severity.[7] However, relative to antibody-based therapies in general, therapies using siRNAs are novel, and their long-term safety is unknown. There is also some concern about the long-term safety of PCSK9 inhibition by any approach as a therapy of coronary heart disease. Some studies suggested a low but increased incidence of mild neurocognitive adverse events with the use of PCSK9 mAbs.[5,13] However, the FOURIER study in ≈27 500 patients reportedly showed that evolocumab was noninferior to placebo for its effects on cognitive function.[6] More significantly, large population studies have shown that SNPs in PCSK9 are associated with diabetes mellitus risk,[14] suggesting that like statins,[15] PCSK9 inhibition may cause a small increase in diabetes mellitus risk. Although this has not yet become apparent, it took decades of statin use before the diabetes mellitus side effect was appreciated.[14,15]

The underlying iceberg of residual cardiovascular disease risk in patients taking statins is enormous. The exciting new development of PCSK9 therapeutics and the demonstration of their clinical effectiveness for reducing cardiovascular disease promise to take a large chunk out of residual risk, especially with the development of more effective dosing and cost-effectiveness. In addition to the existing PCSK9 siRNA and mAb approaches, small-molecule inhibitors, vaccines, or antibodies with longer effectiveness may eventually be developed. The lower boundary for cardiovascular disease risk reduction by more effective LDL-C lowering has not yet been reached.

Disclosures

A.R. Tall has served as a consultant to Amgen and Alnylam.

References

1. Abifadel M, Varret M, Rabès JP, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. *Nat Genet*. 2003;34:154–156. doi: 10.1038/ng1161.
2. Cohen JC, Boerwinkle E, Mosley TH Jr, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N Engl J Med*. 2006;354:1264–1272. doi: 10.1056/NEJMoa054013.
3. Lagace TA, Curtis DE, Garuti R, McNutt MC, Park SW, Prather HB, Anderson NN, Ho YK, Hammer RE, Horton JD. Secreted PCSK9 decreases the number of LDL receptors in hepatocytes and in livers of parabiotic mice. *J Clin Invest*. 2006;116:2995–3005. doi: 10.1172/JCI29383.
4. Sabatine MS, Giugliano RP, Wiviott SD, Raal FJ, Blom DJ, Robinson J, Ballantyne CM, Somaratne R, Legg J, Wasserman SM, Scott R, Koren MJ, Stein EA; Open-Label Study of Long-Term Evaluation against LDL Cholesterol (OSLER) Investigators. Efficacy and safety of evolocumab in

reducing lipids and cardiovascular events. *N Engl J Med*. 2015;372:1500–1509. doi: 10.1056/NEJMoa1500858.
5. Robinson JG, Farnier M, Krempf M, et al; ODYSSEY LONG TERM Investigators. Efficacy and safety of alirocumab in reducing lipids and cardiovascular events. *N Engl J Med*. 2015;372:1489–1499. doi: 10.1056/NEJMoa1501031.
6. http://www.amgen.com/media/news-releases/2017/02/amgen-announces-repatha-evolocumab-significantly-reduced-the-risk-of-cardiovascular-events-in-fourier-outcomes-study/.
7. Fitzgerald K, White S, Borodovsky A, Bettencourt BR, Strahs A, Clausen V, Wijngaard P, Horton JD, Taubel J, Brooks A, Fernando C, Kauffman RS, Kallend D, Vaishnaw A, Simon A. A highly durable RNAi therapeutic inhibitor of PCSK9. *N Engl J Med*. 2017;376:41–51. doi: 10.1056/NEJMoa1609243.
8. Rashid S, Curtis DE, Garuti R, Anderson NN, Bashmakov Y, Ho YK, Hammer RE, Moon YA, Horton JD. Decreased plasma cholesterol and hypersensitivity to statins in mice lacking Pcsk9. *Proc Natl Acad Sci USA*. 2005;102:5374–5379. doi: 10.1073/pnas.0501652102.
9. Nair JK, Willoughby JL, Chan A, et al. Multivalent N-acetylgalactosamine-conjugated siRNA localizes in hepatocytes and elicits robust RNAi-mediated gene silencing. *J Am Chem Soc*. 2014;136:16958–16961. doi: 10.1021/ja505986a.
10. Zaid A, Roubtsova A, Essalmani R, Marcinkiewicz J, Chamberland A, Hamelin J, Tremblay M, Jacques H, Jin W, Davignon J, Seidah NG, Prat A. Proprotein convertase subtilisin/kexin type 9 (PCSK9): hepatocyte-specific low-density lipoprotein receptor degradation and critical role in mouse liver regeneration. *Hepatology*. 2008;48:646–654. doi: 10.1002/hep.22354.
11. Stein EA, Mellis S, Yancopoulos GD, Stahl N, Logan D, Smith WB, Lisbon E, Gutierrez M, Webb C, Wu R, Du Y, Kranz T, Gasparino E, Swergold GD. Effect of a monoclonal antibody to PCSK9 on LDL cholesterol. *N Engl J Med*. 2012;366:1108–1118. doi: 10.1056/NEJMoa1105803.
12. Roth EM, McKenney JM, Hanotin C, Asset G, Stein EA. Atorvastatin with or without an antibody to PCSK9 in primary hypercholesterolemia. *N Engl J Med*. 2012;367:1891–1900. doi: 10.1056/NEJMoa1201832.
13. Khan AR, Bavishi C, Riaz H, Farid TA, Khan S, Atlas M, Hirsch G, Ikram S, Bolli R. Increased risk of adverse neurocognitive outcomes with proprotein convertase subtilisin-kexin type 9 inhibitors. *Circ Cardiovasc Qual Outcomes*. 2017;10:e003153. doi: 10.1161/CIRCOUTCOMES.116.003153.
14. Ference BA, Robinson JG, Brook RD, Catapano AL, Chapman MJ, Neff DR, Voros S, Giugliano RP, Davey Smith G, Fazio S, Sabatine MS. Variation in PCSK9 and HMGCR and risk of cardiovascular disease and diabetes. *N Engl J Med*. 2016;375:2144–2153. doi: 10.1056/NEJMoa1604304.
15. Swerdlow DI, Preiss D, Kuchenbaecker KB, et al; DIAGRAM Consortium; MAGIC Consortium; InterAct Consortium. HMG-coenzyme A reductase inhibition, type 2 diabetes, and bodyweight: evidence from genetic analysis and randomised trials. *Lancet*. 2015;385:351–361. doi: 10.1016/S0140-6736(14)61183-1.

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