A New Approach to PCSK9 Therapeutics

Nan Wang, Alan R. Tall

A highly durable RNAi therapeutic inhibitor of PCSK9
Fitzgerald et al

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 identifying PCSK9 as a therapeutic target. The discovery in transgenic mice that PCSK9 is secreted by hepatocytes and enters the circulation 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. The just announced positive clinical outcome of a large phase 3 study, FOURIER (Further Cardiovascular OUcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk), further supports the concept of PCSK9 inhibition. A new study shows that inhibition of PCSK9 production by long-acting RNA interference also causes substantial reduction of LDL-C in humans. 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. Inclisiran is conjugated to triantennary N-acetylglactosamine carbohydrates, which bind to asialoglycoprotein receptors on hepatocytes, 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-acetylglactosamine carbohydrates conjugated inclisiran is highly efficient in silencing PCSK9 mRNA once delivered into the cell. Second, inclisiran primarily targets PCSK9 in hepatocytes, the main source of PCSK9. 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. Fourth, attaching 3 N-acetylglactosamine 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.
Although in these small phase 1 studies, the safety and side-effect profile of inclisarin has been improved, it is expected to have potential to improve cost-effectiveness compared with mAbs.

The potential advantage of inclisarin compared with PCSK9 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) suggests that the reduction in LDL-C with inclisarin therapy might be in the range of ≈40% when adjusted for a small reduction in LDL-C in subjects receiving placebo. 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, 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 inclisarin 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 inclisarin is safe, with all adverse events being mild or moderate in severity. 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.5,11 However, the FOURIER study in ≈27500 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,10 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**

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