COMMENTARY ON CUTTING EDGE SCIENCE

A New Approach to PCSK9 Therapeutics

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Commentary on:
A Highly Durable RNAi Therapeutic Inhibitor of PCSK9
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PCSK9 inhibition is an effective therapy to reduce LDL cholesterol (LDL-C) and cardiovascular events. A recent study shows that one or two doses of inclisiran, a long-acting synthetic small-interfering RNA (siRNA) that selectively targets hepatic PCSK9, causes a sustained reduction of plasma LDL-C for up to 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 proprotein convertase subtilisin/kexin type 9 (PCSK9) lead to a high LDL cholesterol (LDL-C) level and premature coronary heart disease (CHD), whereas loss-of-function variants are associated with low LDL-C level and reduced CHD[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 FDA 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, Further Cardiovascular OUtcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk (FOURIER), further supports the concept of PCSK9 inhibition[6]. A new study shows that inhibition of PCSK9 production by long-acting RNA interference (RNAi) also causes substantial reduction of LDL-C in humans[7]. A remarkable feature of this RNAi-based therapy is the sustained reduction of LDL-C for up to 6 months following just one or two doses.

Inclisiran, the agent used in this study, is a long-acting, subcutaneously delivered, synthetic siRNA directed against PCSK9. The siRNA approach employs the natural RNAi pathway by binding to the RNA-induced silencing complex (RISC), enabling it to specifically cleave messenger RNA (mRNA) molecules encoding PCSK9. A single siRNA-bound RISC is catalytic and cleaves many transcripts. This characteristic is thought to be particularly important when used in conjunction with statins, which are known to up-regulate the production of PCSK9[8]. Inclisiran is conjugated to triantennary *N*-acetylgalactosamine carbohydrates (GalNAc) which bind to asialoglycoprotein receptors (ASGPR) 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 vs 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 or more at day 84 after the single-dose administration, and remained

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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 up to 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 RNAi therapeutic approach is based on four key elements. First, the GalNAc 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 three GalNAc molecules to the 3' terminus of the siRNA by means of a triantennary spacer substantially increases the affinity of the ligand to ASGPR, a molecule highly expressed in hepatocytes, and greatly improves efficiency and specificity of hepatocyte targeting[9]. An important factor that has limited the use of PCSK9 mAbs is cost (approximately $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 to mAbs.

The potential advantage of inclisarin compared to 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 (Fig 2 in ref #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]. While the inclisarin study is a small phase 1 study and the study populations are different, there are theoretical reasons to believe this may be a real difference. While 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, while inclisiran is expected to primarily target hepatic PCSK9 production. This suggests that theoretical maximum inhibition of PCSK9 and reduction of

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LDL-C induced by PCSK9 mAbs may be somewhat greater than that resulting from liver-selective inhibition.

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 employing 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 CHD. 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 non-inferior to placebo for its effects on cognitive function[6]. More significantly, large population studies have shown that SNPs in PCSK9 are associated with diabetes risk[14], suggesting that like statins[15], PCSK9 inhibition may cause a small increase in diabetes risk. While this has not yet become apparent, it took decades of statin use before the diabetes side effect was appreciated[14, 15].

The underlying iceberg of residual CVD risk in patients taking statins is enormous. The exciting new development of PCSK9 therapeutics and the demonstration of their clinical effectiveness for reducing CVD 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 CVD risk reduction by more effective LDL-C lowering has not yet been reached.

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Disclosures

A.T. has served as a consultant to Amgen and Alnylam.

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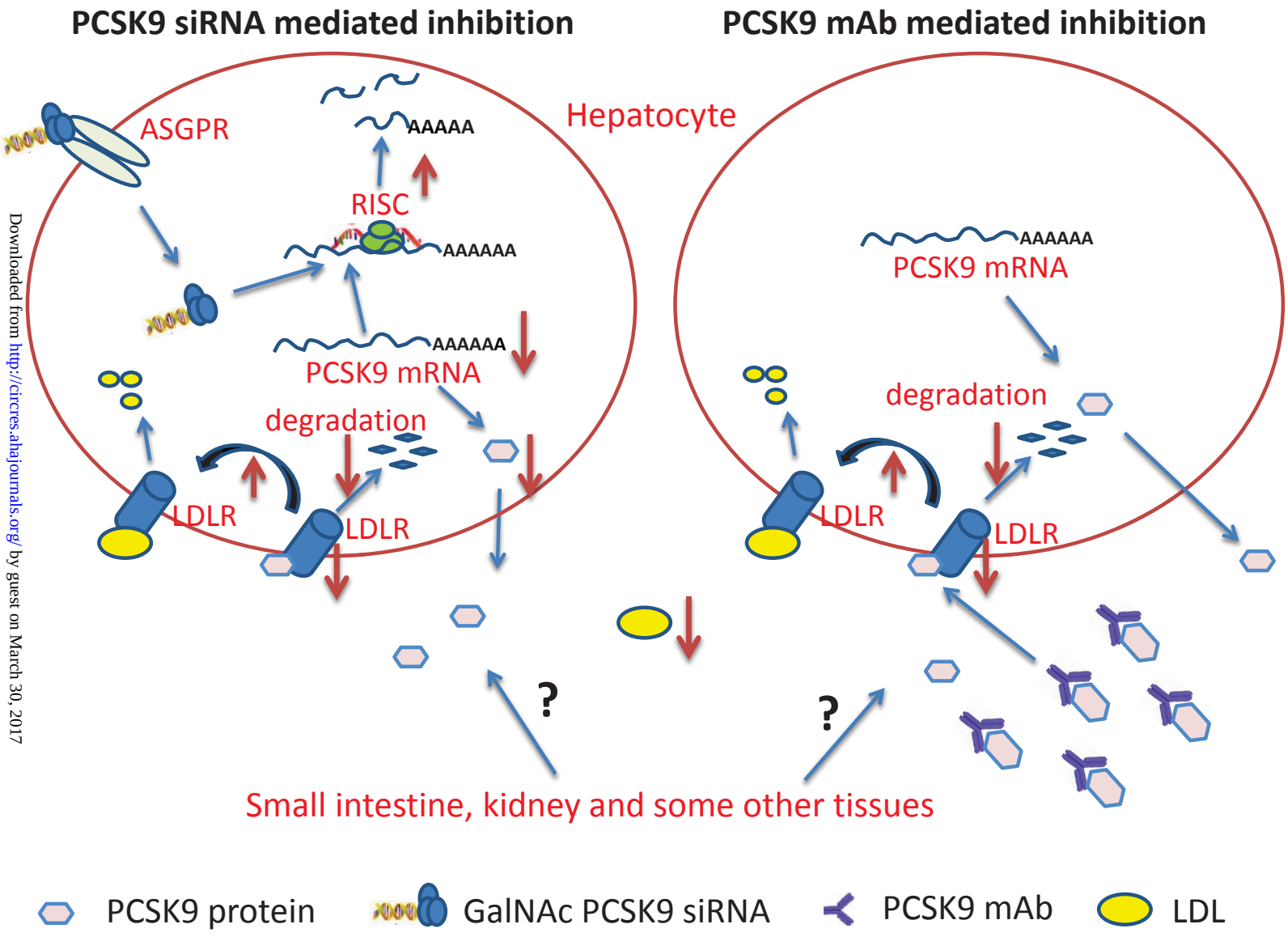
Figure legend

PCSK9 is primarily produced from hepatocytes and some may come from extrahepatic tissues such as small intestine and kidney. PCSK9 binds LDLR, promoting its degradation and reducing its recycling. GalNAc conjugated PCSK9 siRNAs are efficiently and selectively delivered into hepatocytes, via ASGPR which is highly expressed in hepatocytes. Once in the cell, PCSK9 siRNA efficiently mediates PCSK9 mRNA degradation via the RNAi mechanism and selectively reduces hepatic but not other tissue PCSK9 production. PCSK9 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. Red arrows indicate the direction of the effects.



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