The secreted secret of PCSK9

By Kai-Jye Lou, Staff Writer

PCSK9 is a genetically validated target for hypercholesterolemia, but it was unclear whether extracellular blockade of the protein's activity would be sufficient to achieve therapeutic efficacy. Researchers at **Amgen Inc.** have now shown that an antibody against PCSK9 can lower cholesterol in mice and nonhuman primates.¹ The findings bolster the case for using mAbs to soak up PCSK9 in the extracellular space, although competitors **Alnylam Pharmaceuticals Inc.** and **Isis Pharmaceuticals Inc.** think a gene silencing approach that blocks production of the protease could be a better alternative.

Proprotein convertase subtilisin kexin type 9 (PCSK9) is highly expressed in the liver. The protease first emerged as a hypercholesterolemia target in 2003 after a gene-association study showed that individuals with gain-of-function mutations in PCSK9 had higher levels of plasma low-density lipoprotein (LDL) cholesterol than normal individuals.² Conversely, people with loss-of-function mutations had lower plasma LDL levels and a decreased risk of coronary artery disease (CAD) than normal individuals.³

"PCSK9 as a target is very attractive because of the human data that put it on the map," said Daniel Rader, a professor in the Department of Medicine and director of the Clinical and Translational Research Center at the **University of Pennsylvania School of Medicine**. "Humans with loss-of-function mutations in PCSK9 have lower LDL levels and reduced risk for heart disease without any apparent deleterious effects, suggesting that targeting PCSK9 therapeutically would be safe and effective."

Like PCSK9, the LDL receptor also is heavily expressed in the liver. It is responsible for removing the bulk of LDL cholesterol from circulation.

Subsequent functional studies revealed that PCSK9 binds to LDL receptors on the cell surface. Once LDL cholesterol binds to a PCSK9-bound receptor, the entire complex is internalized, at which point PCSK9 directs the receptor to the lysosome for degradation and prevents the receptor from recycling to the cell surface³ (see **Figure 1, "PCSK9-mediated regulation of LDL receptor levels"**).

Because PCSK9 has functional roles both inside and outside of the cell, it was unclear whether antibodies, which predominantly target secreted PCSK9 activity, would be sufficient to achieve clinically meaningful reductions in LDL cholesterol.

Based on the Amgen research, the likely answer is yes. In nonhuman primates, a mAb against PCSK9 significantly lowered baseline circulating

LDL by up to 80% compared with a control antibody ($p=0.0002$). The LDL-lowering effect persisted for about two weeks.

In mice expressing human PCSK9, the mAb caused a dose-dependent decrease in serum cholesterol compared with a control antibody.

The mAb did not significantly affect circulating LDL levels in mice lacking the LDL receptor, confirming that the interaction between PCSK9 and the receptor is necessary for the LDL-modulating effects.

Results were published in the *Proceedings of the National Academy of Sciences*.

In e-mail comments to *SciBX*, Amgen's Simon Jackson and Joyce Chan noted that "the robust LDL cholesterol lowering achieved in our studies, using an antibody that solely targets secreted PCSK9, suggests that secreted, and not intracellular, PCSK9 is the key mediator of LDL receptor function *in vivo*." Jackson is the senior author on the *PNAS* paper and a scientific director at Amgen. Chan is the lead author and a senior scientist at the company.

Jackson and Chan noted that previously published work from other research groups "have not adequately resolved the question of the relative potential contribution of intracellular versus extracellular PCSK9 in terms of LDL receptor regulation."

"The important finding from the Amgen paper is that it shows that if you block the activity of secreted PCSK9, it will show benefit," said Jay Horton, professor of internal medicine at **The University of Texas Southwestern Medical Center**. "By conducting the experiments with an antibody and showing a level of benefit similar to that seen in earlier studies using siRNA or antisense oligonucleotides, the work suggests that the majority of PCSK9's activity is extracellular."

The gene-silencing results were published in 2007 and 2008 by Isis and Alnylam, respectively.

Isis researchers reported that antisense oligonucleotide inhibitors targeting murine PCSK9 significantly decreased circulating LDL in hyperlipidemic mice.⁴

Alnylam showed that RNAi therapeutics targeting PCSK9 significantly lowered LDL in a variety of animal models, including nonhuman primates.⁵ In that study, small interfering RNAs reduced LDL by about 50–60% compared with no treatment. The effect persisted for nearly three weeks.

Golden silence?

Despite the similar preclinical results for antibody and gene-silencing approaches, companies in the latter camp argue there could be added benefits to shutting down PCSK9 production.

Compared to the antibody approach, Kevin Fitzgerald, director of research at Alnylam, thinks blocking PCSK9 production by RNAi "is more akin to" what happens in individuals with loss-of-function mutations in PCSK9. He added that "over time, it may also be important to block the intracellular role of PCSK9 on LDL receptor expression in hepatocytes."

Fitzgerald also noted that the effects of the RNAi approach can persist for over three weeks, which is longer than the effects of Amgen's

Figure 1. PCSK9-mediated regulation of LDL receptor levels. Proprotein convertase subtilisin kexin type 9 (PCSK9) is highly expressed in the liver and is a genetically validated target for hypercholesterolemia. In the *Proceedings of the National Academy of Sciences*, researchers showed that a mAb inhibiting the interaction between PCSK9 and the low-density lipoprotein (LDL) receptor significantly reduced circulating LDL cholesterol levels.¹

[1] PCSK9 is a protease that is synthesized as an enzyme precursor. Following synthesis, PCSK9 undergoes autocatalytic cleavage, which is required for secretion from the cell. Although cleaved, the two domains of PCSK9 remain together. When LDL cholesterol binds to the LDL receptor, they are both internalized into the cell. The process of LDL receptor internalization via binding LDL cholesterol is the mechanism by which LDL cholesterol is removed from circulation.

[2a] If the cholesterol-bound LDL receptor is also bound to PCSK9, the LDL receptor is redirected for lysosomal degradation and prevented from recycling to the cell surface.

[2b] If an internalized LDL receptor is not bound to PCSK9, the receptor is recycled to the cell surface, where it continues to remove LDL cholesterol from circulation.

A number of companies are developing compounds that stifle PCSK9 activity, either by blocking synthesis of the protein or by inhibiting PCSK9 binding to the LDL receptor.

antibody. He added that gene silencing did not lower levels of beneficial high-density lipoprotein (HDL) cholesterol, whereas Amgen's PCSK9-neutralizing mAb significantly decreased circulating HDL compared with no treatment (26% at day 7, $p=0.0178$).

Jackson and Chan countered that "the clinical development path for antibodies, which are already a validated therapeutic, may be more straightforward than that of an RNAi-based approach."

Rader, however, thinks it's probably too early to pick favorites. "All three biological approaches to targeting PCSK9 that are being pursued—antibodies, antisense oligonucleotides and RNAi—have potential merit," he told *SciBX*.

Small molecule PCSK9 inhibitors are notably absent from the discussion for two main reasons. First, the binding of PCSK9 to LDL receptors occurs via a protein-protein interaction. Also, PCSK9-mediated reductions in LDL receptor levels are independent of the PCSK9's proteolytic activity.⁶⁻⁸ These features make PCSK9 difficult to effectively target with a small molecule inhibitor.

Inhabiting statin island

Regardless of how PCSK9 is targeted, researchers contacted by *SciBX* think the target itself is a safe one to hit and that inhibitors could work well with marketed cholesterol-lowering drugs such as statins.

"Right now, I don't think there is a reason to suspect any major safety issues with PCSK9 inhibition based on the human genetic data that is available," said Rader. Indeed, the human genetic studies did not identify any deleterious effects resulting from loss-of-function mutations in PCSK9.^{9,10}

"We don't think the target itself is going to cause intolerable toxicities because humans with loss-of-function mutations in PCSK9 are healthy," said Brett Monia, VP of drug discovery and corporate development at Isis.

"What it will come down to is the magnitude of cholesterol lowering that is achievable with a compound and whether this is sufficient to make the drug viable. Also, one will need to evaluate the compound's nontarget effects and any tolerability issues arising from these effects."

Inhibiting PCSK9 could also mitigate an unwanted side effect of statins. Horton noted that high doses of statins "can upregulate

PCSK9. So a PCSK9 inhibitor could work additively or synergistically with statin drugs to produce further reductions in LDL cholesterol."

Indeed, Chan and Jackson said the cell-based data in their *PNAS* paper support the argument for combination therapy. In human hepatoma cell culture, the combination of the anti-PCSK9 mAb and a statin produced larger increases in LDL receptor levels than either compound alone. Higher LDL receptor levels are associated with lower levels of circulating LDL cholesterol.

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—Jay Horton,

The University of Texas
Southwestern Medical Center

Chan and Jackson think PCSK9-based treatments also could work as standalone therapies for lowering LDL. This could help patients intolerant of marketed drugs or unable to achieve their target cholesterol levels with marketed drugs.

“There is a large population of individuals that cannot get their cholesterol levels down to sufficiently low levels to reduce their risk for coronary heart disease and heart attacks,” said Monia.

Rader did note that none of the current strategies for targeting PCSK9 are oral.

“These therapies would have to be delivered via an intravenous or subcutaneous route, and obviously this will not be as easy as giving a pill. It would be a new approach for cholesterol-lowering treatments,” he said.

Alnylam’s ALN-PCS, an siRNA targeting PCSK9 mRNA encapsulated in a liposomal nanoparticle formulation, is one of the company’s three IND candidates for 2009.

BMS-PCSK9Rx from Isis and partner **Bristol-Myers Squibb Co.** is an antisense molecule targeting PCSK9 mRNA. The compound is expected to enter Phase I testing this year.

Amgen’s PCSK9 antibody is in preclinical development. The company did not disclose when a candidate is expected to enter the clinic.

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REFERENCES

1. Chan, J.C.Y. *et al. Proc. Natl. Acad. Sci. USA*; published online May 11, 2009; doi:10.1073/pnas.0903849106
Contact: Simon M. Jackson, Amgen Inc., South San Francisco, Calif.
e-mail: simon.jackson@amgen.com
2. Abifadel, M. *et al. Nat. Genet.* **34**, 154–156 (2003)
3. Peterson, A.S. *et al. J. Lipid Res.* **49**, 1152–1156 (2008)
4. Graham, M.J. *et al. J. Lipid Res.* **48**, 763–767 (2007)
5. Frank-Kamenetsky, M. *et al. Proc. Natl. Acad. Sci. USA* **105**, 11915–11920 (2008)
6. Li, J. *et al. Biochem. J.* **406**, 203–207 (2007)
7. McNutt, M.C. *et al. J. Biol. Chem.* **282**, 20799–20803 (2007)
8. Grefhorst, A. *et al. J. Lipid Res.* **49**, 1303–1311 (2008)
9. Zhao, Z. *et al. Am. J. Hum. Genet.* **79**, 514–523 (2006)
10. Hooper, A.J. *et al. Atherosclerosis* **193**, 445–448 (2007)

COMPANIES AND INSTITUTIONS MENTIONED

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