PCSK9 Inhibitors for LDL Lowering

James P Walsh, M.D., Ph.D.

Division of Endocrinology, Indiana University School of Medicine, Indianapolis, Indiana, and the Endocrinology Section, Roudebush Veterans Affairs Medical Center, 1481 West Tenth Street, Indianapolis, Indiana 46202

Tel: 317-988-3073, Fax: 317-988-2641, email: jpwalsh@iu.edu

Dr. Walsh has no conflicts of interest to disclose.

This is the author's manuscript of the article published in final edited form as:
Walsh, J. P. (2015). PCSK9 inhibitors for LDL lowering. Trends in cardiovascular medicine.
http://dx.doi.org/10.1016/j.tcm.2015.02.001

In 2003, families were described with a rare, autosomal dominant form of familial hypercholesterolemia due to gain of function mutations in proprotein convertase subtilisin/kexin type 9 (PCSK9) (1). Subsequent studies demonstrated that PCSK9 is secreted from liver and accelerates degradation of LDL receptors (2,3). Heterozygosity for PCSK9 null mutations in 2.6% of African-Americans was found to be associated with 28% reduction in LDL cholesterol (LDL-C) and an 88% reduction in CAD risk (4). These findings stimulated intense interest in PCSK9 as a pharmaceutical target. In this issue of *Trends in Cardiovascular Medicine*, Desai and Sabatine thoroughly review recent and ongoing trials of three monoclonal antibodies that disrupt PSCK9 binding to LDL receptors, leading to marked reductions in LDL-C (5). As detailed in their review, evolocumab (AMG145), alirocumab (SAR236553/REGN727), and bococizumab (RN316) given every 2-4 weeks, reduced LDL-C up to 75% in patients on statin therapy as compared to statin therapy alone, up to 51% in statin intolerant patients and other patients not in statin therapy, and up to 55% in patients with heterozygous familial hypercholesterolemia, most of whom were on statin therapy. Evolocumab reduced LDL-C up to 31% in patients with homozygous familial hypercholesterolemia, although there was no effect in LDL receptor-negative patients. Ongoing cardiovascular outcome trials that will rigorously test the ability of these agents to reduce major adverse cardiovascular events in high risk patients are also described.

PCSK9 is secretory serine endoprotease (6). Pro-PCSK9 is autocatalytically cleaved during its maturation in the golgi, but the prodomain remains tightly bound after secretion, precluding further catalytic activity (6). Effects of PCSK9 on LDL-C clearance thus do not depend on its protease activity. Rather, circulating PCSK9 binds the first epidermal growth factor-like repeat domain in the extracellular portion of the LDL receptor (6). Endocytosed LDL receptor is normally recycled back to the plasma membrane, but PCSK9 locks the receptor in an open conformation that targets it to lysosomes for degradation (6). The PCSK9-binding monoclonal antibodies under development disrupt this interaction and prevent LDL receptor degradation. Up to 40% of circulating PCSK9 is bound to LDL particles via a specific interaction

with apoB100 (6,7). Only free, unbound PCKS9 down regulates LDL receptors (6). PCSK9 does not bind VLDL or chylomicrons, suggesting that the interaction site may be unmasked during the intravascular remodeling of apoB100-containing lipoproteins (6,7). There is also a separate, intracellular pathway in which nascent PCSK9 binds LDL receptors and directs them from trans-golgi to lysosomes (8). However the extracellular pathway is the predominant mechanism of PCSK9-mediated LDL receptor degradation *in vivo*. Statins deplete intracellular cholesterol and upregulate LDL receptor transcription through sterol regulatory element-binding protein-2 (SREBP-2) (9). As PCSK9 transcription is also regulated by SREBP-2, PCSK9 levels are increased with statin therapy, which attenuates statins' LDL-C lowering effects (9). Consistent with this, PCSK9 knockout mice exhibit an exaggerated LDL-C response to statin treatment (10).

Beyond its role in reducing LDL receptor levels, little is known about the biology of PCSK9. Its expression is highest in liver, and lower in kidney, intestine, brain, and lung (11). Evolocumab lowers lipoprotein(a) up to 30%, an effect also seen with other PCSK9 inhibitors (5). Statins do not decrease lipoprotein(a) and the LDL receptor is not thought to be involved in lipoprotein(a) clearance (12). So the mechanism of lipoprotein(a) lowering is not clear. PCSK9 inhibitors cause modest decreases in triglycerides (13). Some of this may reflect LDL receptor-dependent clearance of apoB containing remnant particles. However, decreased production of triglyceride-rich lipoproteins and upregulation of VLDL receptors may also contribute to the triglyceride lowering (7,14). VLDL receptor expression is increased in visceral adipose tissue of PCSK9 null mice, leading to an 80% increase in visceral adiposity (15). Intracellular degradation of apolipoprotein receptor 2 (ApoER2) is increased by PCSK9 (14). There is evidence that PCSK9 regulation of ApoER2 modulates neuronal apoptosis (16). PCSK9 has been shown to mediate degradation of LDL receptor-related protein-1 (LRP1) (14). LRP1 is widely expressed and has been implicated in inflammation and atherosclerosis. Other potential PCSK9 targets include the endothelial sodium channel (ENaC) and the hepatic receptor for hepatitis C virus, CD81 (7,14). The decrease in

lipoprotein(a) with PCSK9 inhibitors may contribute to improved cardiovascular outcomes. However the implications of most of these observations for PCSK9 inhibitor therapy are unknown. Published trials of PCSK9 inhibitors have generally found adverse events, other than minor injection site reactions, to be comparable between groups (5). Moreover, the description of a few individuals in apparent normal health with undetectable circulating PCSK9 due to PCSK9 null mutations and LCL-C levels as low as 14 mg/dL offers some reassurance that PCSK9 inhibitors will not have serious on-target side effects (17). Ongoing long term trials of PCSK9 inhibitors and additional basic research on PCSK9 biology will provide important information on long term safety.

A specific concern with antibody-based therapies is immune reactions. These are much less likely with humanized and fully human antibodies, but can still occur (18). Most common are development of anti-drug antibodies, which in some cases can be associated with loss of therapeutic efficacy. For example, 28% of rheumatoid arthritis patients treated with adalimumab, a fully human antibody targeting tumor necrosis factor, developed anti-adalimumab antibodies over a three year period and these antibodies were associated with lower drug levels and decreased of therapeutic efficacy (19). Anti-evolocumab antibodies developed in 2/901 patients in one 52 week trial (13). In neither of these patients were the antibodies associated with loss of efficacy. More serious immune reactions can also occur. One patient on alirocumab developed leukocytoclastic vasculitis that responded to steroids and drug discontinuation (18). Large, current trials of PCSK9 inhibitors should clarify the extent to which immune reactions are a concern.

A recent *post-hoc* analysis of patients treated with alirocumab for a mean of 65 weeks found a 54% reduction of treatment emergent cardiovascular events (20). If ongoing trials demonstrate improved cardiovascular outcomes and good safety, as seems likely, patients with familial hypercholesterolemia and other LDL hyperlipidemias inadequately responsive to maximal statin therapy will be strong

candidates for addition of anti-PCSK9 therapy. As PCSK9 inhibitors have shown favorable tolerability versus ezitimibe in statin-intolerant patients, this group may also benefit (21). As noted by Desai and Sabatine, it is also possible that lowering LCL-C with PCSK9 inhibitors to levels below those recommended in current guidelines may offer additional benefit to very high risk patients (5). However this will need to be demonstrated in clinical trials. A trial of PCSK9 inhibitors in patients with advanced renal disease, a population in which the benefits of statins are unclear, would also be worthwhile (22). Overall, monoclonal antibodies targeting PCSK9 are a promising new therapy for LDL hyperlipidemia, and results of ongoing cardiovascular outcome trials are eagerly awaited.

References

1. Abifadel M, Varret M, Rabès JP, Allard D, Ouguerram K, Devillers M, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. Nat Genet 2003; 34:154-6.
2. Maxwell KN, Fisher EA, Breslow JL. Overexpression of PCSK9 accelerates the degradation of the LDLR in a post-endoplasmic reticulum compartment. Proc Natl Acad Sci USA 2005; 102:2069-74.
3. Lagace TA, Curtis DE, Garuti R, McNutt MC, Park SW, Prather HB, et al. Secreted PCSK9 decreases the numbers of LDL receptors in hepatocytes and in livers of parabiotic mice. J Clin Invest 2006; 116:2995-3005.
4. CohenJC, Boerwinkle E, Mosley TH, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. N Engl J Med 2006; 354:1264-72.
5. Desai NR, Sabatine MS. PCSK9 inhibition for patients with lipid abnormalities. Trends Cardiovasc Med 2015 *This Issue*
6. Lagace TA. PCSK9 and LDLR degradation: regulatory mechanisms in circulation and in cells. Curr Opin Lipidol 2014; 25:387-93.
7. Tavori H, Rashid S, Fazio S. On the function and homeostasis of PCSK9: reciprocal interaction with LDLR and additional lipid effects. Atherosclerosis 2015; 238:264-70.
8. Poirier S, Mayer G, Poupon V, McPherson PS, Desjardins R, Ly K, et al. Dissection of the endogenous cellular pathways of PCSK9-induced low density lipoprotein receptor degradation: evidence for an intracellular route. J Biol Chem 2009; 284:28856-64.
9. Lambert G, Sjouke B, Choque B, Kastelein JJP, Hovingh GK. The PCSK9 decade. J Lipid Res 2012; 53:2515-24.

10. Rashid S, Curtis DE, Garuti R, Anderson NN, Bashmakov Y, Ho YK, et al. Decreased plasma cholesterol and hypersensitivity to statins in mice lacking PCSK9. Proc Natl Acad Sci USA 2005; 102:5374-9.
11. Seidah NG, Benjannet S, Wickham L, Marcinkiewicz J, Jasmin SB, Stifani S, et al. The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC1): Liver regeneration and neuronal differentiation. Proc Nat Acad Sci 2003; 100:928-33.
12. Kostner KM, März W, Kostner GM. When should we measure lipoprotein (a)? Eur Heart J 2013; 34:3268-76.
13. Blom D, Hala T, Bolognese M, Lillestol MJ, Toth PD, burgess L, et al. A 52-week placebo-controlled trial of evolocumab in hyperlipidemia. New Engl J Med 2014; 370:1809-19.
14. Norata GD, Tibolla G, Catapano AL. PCSK9 inhibition for treatment of hypercholesterolemia: promises and emerging challenges. Vascul Pharmacol 2014; 62:103-11.
15. Roubtsova A, Munkonda MN, Awan Z, Marcinkiewicz J, Chamberland A, Lazure C. Circulating proprotein convertase subtilisin/kexin 9 (PCSK9) regulates VLDLR protein and triglyceride accumulation in visceral adipose tissue. Arterioscler Thromb Vasc Biol 2011; 31:785-91.
16. Kysenius K, Muggalla P, Mätlik K, Arumäe U, Huttunen HJ. PCSK9 regulates neuronal apoptosis by adjusting ApoER2 levels and signaling. Cell Mol Life Sci 2012; 69:1903-16.
17. Marais AD, Kim JB, Wasserman SM, Lambert G. PCSK9 inhibition in LDL cholesterol reduction: genetics and therapeutic implications of very low plasma lipoprotein levels. Pharmacol Ther 2015; 145:58-66.
18. Catapano AL Papadopoulos N. The safety of therapeutic monoclonal antibodies: implications for cardiovascular disease and targeting the PCSK9 pathway. Atherosclerosis 2013; 228:18-28.
19. Bartelds GM, Krieckaert CL, Nurmohamed MT, van Schouwenburg PA, Lems WF, Twisk JW, et al. Development of antidrug antibodies against adalimumab and association with disease activity and treatment failure during long term follow up. JAMA 2011; 305:1460-8.

20. Robinson JG, Farnier M, Krempf M, Bergeron J, Luc G, Averna M, et al. Long-term safety, tolerability, and efficacy of alirocumab versus placebo in high cardiovascular risk patients: first results from the ODYSSEY LONG TERM study in 2,341 patients. Presented at the European Society of Cardiology Congress 2014, Barcelona, Spain.
21. Stroes E, Colquhoun D, Sullivan D, Civeira F, Rosenson RS, Watts GF, et al. Anti-PCSK9 antibody effectively lowers cholesterol in patients with statin intolerance. J Am Coll Cardiol 2014; 63:2541-8.
22. Tonelli M, Wanner C, Kidney Disease: Improving Global Outcomes Lipid Guideline Work Group Members. Lipid management in chronic kidney disease: synopsis of the Kidney Disease: Improving Global Outcomes 2013 clinical practice guideline. Ann Intern Med 2014; 160:182-9.