

CORONARY ARTERY DISEASE PREVENTION & THERAPY UPDATE

Novel Hypolipidemic Agents: the Role of PCSK9 Inhibitors

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ABSTRACT

Hyperlipidemia is a major cause of cardiovascular disease despite the availability of first-line cholesterol lowering agents such as statins. Although statin therapy is very efficient to reduce cholesterol, nearly 10-20% of individuals on statins, experience side effects, such myopathy, which hinder the drugs ability to achieve target low-density lipoprotein (LDL) cholesterol (LDL-C) levels. Statin-intolerant patients require more effective therapies for lowering LDL-C.

As proprotein convertase subtilisin kexin type 9 (PCSK9) promotes the degradation of the LDL receptor (LDLR) and prevents it from recycling to the membrane, a new therapeutic approach to lowering LDL-C acts by blocking LDL-receptor degradation by serum PCSK9. Humanized monoclonal antibodies which target PCSK9 and its interaction with the LDL receptor (REGN727/SAR23653, AMG145, and RN316), as well as agents that inhibit PCSK9 synthesis, such as ALN-PCS, are now in clinical trials. The latter is a small interfering RNA (siRNA) that directs sequence-specific messenger RNA for PCSK9 leading to reduced hepatocyte-specific synthesis of PCSK9. Ongoing phase III trials' results are awaited with great interest in order to define these agents' long-term safety, tolerability and efficacy for reducing cardiovascular events.

KEY WORDS: *cholesterol; LDL;
PCSK9; hypolipidemic drugs*

INTRODUCTION

Hypercholesterolemia, particularly high concentration of low-density lipoprotein (LDL) cholesterol (LDL-C), is associated with atherosclerotic vascular disease and adverse events including myocardial infarction, cerebrovascular accidents and transient ischemic attacks and is a leading contributor to cardiovascular-related morbidity and mortality around the world.^{1,2} Statin therapy has been shown to reduce LDL-C levels by up to 63% from baseline and is the first-line pharmacologic treatment for hypercholesterolemia.³ Although statin therapy is very efficient to reduce cholesterol, nearly 10-20% of individuals on statins experience side effects, such as myopathy, which hinder the drugs ability to achieve target LDL levels.⁴ Moreover, single nucleotide polymorphisms have been reported to be linked to resistance to statin therapy.⁵ As a result of these goal-limiting factors, there is an ongoing search for new, non-statin cholesterol lowering agents, such as PCSK9 inhibitors.

ABBREVIATIONS

ADH = autosomal dominant
hypercholesterolemia
CHD = coronary heart disease
FH = familial hypercholesterolemia
GOF = gain-of-function
LDL = low-density lipoprotein
LDL-C = low-density lipoprotein
cholesterol
LDLR = LDL receptor
LOF = loss-of-function
PCSK9 = proprotein convertase subtilisin/
kexin type 9

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THE DISCOVERY OF PCSK9

In 2003, Seidah et al identified the ninth member of the proprotein convertase family, PCSK9.⁶ The human *PCSK9* gene located on chromosome 1p32.3 encodes a 692-amino acid inactive glycoprotein. PCSK9 is expressed in several organs, particularly the liver and also the intestine, intestinal ileum, and colon epithelia and the kidney.⁷ Shortly after that, the identification of two gain-of-function (GOF) mutations in PCSK9, in two French families with a clinical diagnosis of autosomal dominant hypercholesterolemia (ADH) and no detectable mutations in LDL receptor (LDLR) or *apoB100* genes, made evident the involvement of PCSK9 in regulating cholesterol metabolism.⁸ Several other GOF mutations have been reported,⁹ associated with mild to severe hypercholesterolemia and increased risk of coronary heart disease (CHD).¹⁰ Conversely, loss-of-function (LOF) mutations associated with PCSK9 inhibition probably has a role in decreasing LDL-C concentration.

ROLE OF PCSK9

Low-density lipoprotein (LDL) particles are removed from the circulation mainly by hepatic uptake *via* the LDL receptor (LDLR). LDL binds to the LDLR and the LDL/LDLR complex is internalized into clathrin-coated vesicles by endocytosis. Then, LDL is separated from its receptor in the endosomes and the LDLR is recycled for reuse. At the same time, LDL is degraded.

The plasma concentration of LDL-C is regulated by both environmental and genetic factors. In the majority of the cases, familial hypercholesterolemia (FH) is related to mutations in the LDLR itself, and less frequently to mutations in the ligand-binding domain of apolipoprotein (apo)B100, the protein component of LDL that interacts with the LDLR.¹¹ The third gene associated with FH, is related to mutations encoding proprotein convertase subtilisin/kexin type 9 (PCSK9) which plays a central role in regulation of cholesterol homeostasis by enhancing the endosomal and lysosomal degradation of hepatic LDLR, resulting in decreased extraction of LDL-C from the circulation and in increased serum LDL-C concentrations.^{12,13} Furthermore, PCSK9 shortens the half-life of LDLR,¹⁴ a process independent of its catalytic activity.¹ In a subsequent study, real-time PCR analysis showed that increased cholesterol consumption by mice was associated with down-regulation of PCSK9 mRNA.¹⁶ As expected, the presence of mevalonate, a product of HMG-CoA reductase on HMG-CoA, was found to down-regulate PCSK9 mRNA transcription, and conversely, expression of PCSK9 mRNA is up-regulated by HMG-CoA reductase inhibitors.^{15,17}

Gain-of-function mutations in the *PCSK9* gene lead

to autosomal dominant hypercholesterolemia (ADH),⁸ as do mutations in the *LDLR* and *APOB* genes. Conversely, loss-of-function mutations (LOF) of the PCSK9 gene were discovered and linked to low plasma LDL-C levels and a reduction of cardiovascular risk.^{18,19} The first LOF mutations were described in 2005¹⁸ and the effect of lifelong reductions in LDL-C induced by these LOF mutations was examined in the atherosclerosis risk in communities study.¹⁹ LOF mutations Y142X and C679X in African Americans were associated with a 28% reduction in LDL-C and an 88% reduction in the risk of CHD, whereas the R46L mutation in Caucasians was associated with a 15% reduction in LDL-C and a 47% reduction in the risk of CHD.¹⁹ Since PCSK9 is a key regulator for LDLR activity, the PCSK9 inhibition seems an attractive target for the treatment of hypercholesterolemia.⁸⁻¹⁰

PHARMACOLOGICAL STRATEGIES TARGETING PCSK9

So far, several therapeutic strategies have been developed in order to reduce circulating PCSK9 (Table 1). Small-molecule inhibitors of PCSK9 seem to be hard to design and their development has not reached the safety and efficacy stages in preclinical studies, therefore these compounds are not currently included in therapeutic options.

More successful is the gene-silencing approach. PCSK9 can be inhibited by single-stranded antisense DNA-like oligonucleotides or by double-stranded small interference RNA. These treatments successfully increase hepatic LDLR and lower plasma LDL-C in animal models. However, two antisense nucleotides directed at reducing PCSK9 in humans have recently discontinued development. Another approach which has been tested is the PCSK9 peptide sequences which are too short to promote LDLR degradation but long enough to compete with full-length PCSK9. Despite the merits of these approaches, fully human monoclonal antibodies (mAbs) targeting PCSK9 currently appear to lead the way with the publication in 2012 of the results from phase I and II clinical trials.

Three major pharmaceutical teams, Sanofi US (Bridge-water, NJ)/Regeneron Inc. (Tarrytown, NY), Amgen Inc.

TABLE 1.

Investigational approaches targeting PCSK9
Agents that inhibit binding of PCSK9 to the LDL receptor (e.g. monoclonal antibody therapy)
Agents that inhibit PCSK9 synthesis (e.g. small interfering RNA, antisense oligonucleotides)
Potentially, agents that inhibit intracellular processing of PCSK9

(Thousand Oaks, CA), and Pfizer Inc. (New York, NY) have invested extensive research efforts into exploring the potential of blocking LDL-receptor degradation by serum proprotein convertase subtilisin kexin 9 (PCSK9)²⁰ via the humanized monoclonal antibodies designated REGN727/SAR236553 or alirocumab (subcutaneous administration), AMG 145 or evolocumab (subcutaneous administration), and RN316 or bococizumab (intravenous administration) respectively.

In healthy individuals, the Sanofi-Aventis/ Regeneron SAR236553/REGN727 antibody reduced LDL-C by 33–46%, in a dose dependent fashion, when administered subcutaneously.²¹ On top of atorvastatin, in hypercholesterolemic individuals, multiple doses of the Sanofi compound reduced cholesterol levels by 41–58% in patients with familial hypercholesterolemia and by 38–65% in nonfamilial hypercholesterolemia. In a phase II study of patients on a stable dose of atorvastatin (10–40 mg/day), with LDL-C levels at least 100 mg/dl, the administration of SAR236553/REGN727 antibody every 2 weeks resulted in LDL-C reductions of 40–72%.²² In heterozygous familial hypercholesterolemia patients at high cardiovascular risk treated with high to maximal doses of statins with or without ezetimibe, the SAR236553/REGN727 antibody, every 2 weeks, reduced LDL-C by up to 68%.²³ In another study conducted in patients on low-dose atorvastatin (10 mg/day) with LDL-C levels at least 100 mg/dl, the co-administration of 150 mg of the SAR236553/REGN727 antibody every 2 weeks resulted in 66% reduction of LDL-C, whereas an up-titration of atorvastatin, to the maximal dose (80 mg/day) lowered LDL-C by 17%.²⁴

On the other hand, in healthy individuals, the Amgen antibody AMG145 dose-dependently decreased LDL-C by up to 64% in comparison to placebo when injected subcutaneously.²⁵ In patients on stable doses of statins, the administration of AMG145 reduced LDL-C by up to 75%. In familial hypercholesterolemic patients on statins with or without ezetimibe, AMG145 dose-dependently decreased LDL-C levels by up to 55%.²⁶ The Amgen antibody reduced LDL-C by up to 51% in statin-intolerant dyslipidemic patients.²⁷ Administration of 140 mg of the AMG145 antibody every 2 weeks reduced LDL-C by 51% in monotherapy, in patients with hypercholesterolemia,²⁸ and by 66% on top-of-statin treatment vs. placebo.²⁹

Thus, similar doses (140–150 mg) of either SAR236553/REGN727 or AMG145 mAbs infused every 2 weeks appear to display similar efficacies in terms of LDL-C reduction, whether in monotherapy, or on top-of-statin treatment. Noteworthy, both compounds reduced LDL-C down to optimal target levels (≤ 70 mg/dl) in the vast majority of heterozygous familial hypercholesterolemia patients enrolled in those studies,^{23,26} suggesting that PCSK9 inhibition will be athero-protective in familial hypercholesterolemia, as indicated by the positive correlation that exists between elevated plasma PCSK9 levels and the severity of the familial hypercholesterolemia phenotype.³⁰

RN316, from Pfizer Inc, unlike the other two human

monoclonal antibodies is administered intravenously. Gumbiner et al, in a phase II clinical trial, evaluated the efficacy and safety of RN316 in participants with hypercholesterolemia on concurrent high dose statin therapy.³¹ Participants in the first arm of this trial (n=90) had LDL-C ≥ 100 mg/dl and were given either the study drug or placebo while on concurrent high doses of atorvastatin (40 or 80 mg), rosuvastatin (20 or 40 mg), or simvastatin (40 or 80 mg). The second arm (n=45) included participants with LDL-C ≥ 80 mg/dl given study drug or placebo while on atorvastatin 80 mg or rosuvastatin 40 mg. After 12 weeks, pooled results from both arms showed a 56% reduction in LDL-C in the study drug group as compared with a 4% reduction in the placebo group. It is possible that an even greater reduction in the study drug group may have been achieved, since dosing was interrupted at week 4 due to LDL-C dropping below 25 mg/dl in several patients. No study drug related adverse events were reported, and RN316 was well tolerated.³¹

Another therapeutic option available is the use of agents that inhibit PCSK9 synthesis, such as ALN-PCS. This is a small interfering RNA (siRNA) that directs sequence-specific messenger RNA for PCSK9 leading to reduced hepatocyte-specific synthesis of PCSK9.³² A recent placebo-controlled phase I study of healthy individuals with hypercholesterolemia showed that ALN-PCS achieved 40% reduction in LDL-C levels, whereas adverse event proportions were similar in the treatment and placebo groups. No significant increase of liver function enzymes or inflammatory markers was reported.³³

CONCLUSIONS

A significant proportion of high risk patients fail to achieve LDL-C goals recommended by guidelines, despite the intense treatment with currently available lipid lowering agents. Reduction of LDL-C levels through PCSK9 inhibition seems to be a new attractive therapeutic approach. Monoclonal antibodies, such as AMG145 and SAR236553/REGN727, currently appear to lead the way in targeting PCSK9 and to enable most patients at highest risk for adverse cardiovascular events to achieve the most stringent recommended lipid goals. Phase III trials with larger patient populations and over longer periods are currently underway. The results of these trials are awaited with great interest in order to define these agents' long-term safety, tolerability and efficacy for reducing cardiovascular events in humans.

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