Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9): Lessons Learned from Patients with Hypercholesterolemia

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BACKGROUND: Identification of the proprotein convertase subtilisin/kexin type 9 (PCSK9) as the third gene causing familial hypercholesterolemia (FH) and understanding its complex biology has led to the discovery of a novel class of therapeutic agents.

CONTENT: PCSK9 undergoes autocatalytic cleavage in the endoplasmic reticulum and enters the secretory pathway. The PCSK9 gene is under the regulatory control of sterol receptor binding proteins 1 and 2. Statins increase PCSK9 and this may modulate the response to this class of medications. In plasma, PCSK9 binds to the epidermal growth factor–like domain of the LDL receptor (LDL-R) on the cell and, once incorporated in the late endosomal pathway, directs the LDL-R toward lysosomal degradation rather than recycling to the plasma membrane. Thus, gain-of-function PCSK9 mutations lead to an FH phenotype, whereas loss-of-function mutations are associated with increased LDL-R–mediated endocytosis of LDL particles and lower LDL cholesterol in plasma. Inhibition of PCSK9 is thus an attractive therapeutic target. Presently, this is achieved by using monoclonal antibodies for allosteric inhibition of the PCSK9–LDL-R interaction. Phase 2 and 3 clinical trials in patients with moderate and severe hypercholesterolemia (including FH) show that this approach is safe and highly efficacious to lower LDL-C and lipoprotein(a).

SUMMARY: PCSK9 has other biological roles observed in vitro and in animal studies, including viral entry into the cell, insulin resistance, and hepatic tissue repair. Given the potential number of humans exposed to this novel class of medications, careful evaluation of clinical trial results is warranted.

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Cellular processing of proteins is a complex process that includes posttranscriptional modifications, transport to and from subcellular compartments, and cleavage into mature protein. Proteins that enter the secretory pathway are initially synthetized as biologically inactive precursors requiring proteolytic cleavage by highly specialized and evolutionarily conserved proprotein convertases. In the past 2 and a half decades, 9 members of the proprotein convertase subtilisin/kexin (PCSK) family, PCSK9, were identified in mammals, with broad substrate specificities. The last member of the PCSK family, PCSK9, was identified by Seidah et al. (1). The chromosomal region 1p32, harboring the proprotein convertase subtilisin/kexin type 9 (PCSK9) gene, was identified by Abifadel et al. as a novel locus for autosomal dominant hypercholesterolemia, with a phenotype clinically similar to familial hypercholesterolemia (FH) due to mutations in the low-density lipoprotein receptor (LDLR) gene (2). In an elegant collaboration, Abifadel et al. were able to show that mutations of the PCSK9 gene were causally related to FH3, the third identified genetic cause of autosomal dominant hypercholesterolemia in families of French origin. Strikingly, the FH phenotype is caused by gain-of-function mutations of PCSK9.

Biochemical Role of PCSK9

PCSK9 is located on the small arm of chromosome 1p32 (Fig. 1) in humans and contains 12 exons and 11 introns (3, 4). It codes for a 25-kDa mature protein that is secreted in plasma. PCSK9 is a typical PCSK in that it has a serine esterase–like structure of the bacterial subtilase family but differs in that it shares similarity with proteinase K.

AUTOCATALYTIC CLEAVAGE

The only known substrate of PCSK9 for proteolytic cleavage is itself. Full-length PCSK9 (prepro-PCSK9) is characterized by a 9-leucine spanning the signal pep-
tide domain, which guides the synthesized protein to the endoplasmic reticulum (ER). PCSK9 has a signal peptide, a prosegment, and a catalytic domain but not a P-domain; instead there is a cysteine/histidine-rich domain in the C-terminal domain (Fig. 1A). A hinge region is also present between the catalytic pocket and the cysteine/histidine-rich domain; the function of the hinge region is poorly understood. Once in the ER the signal peptide domain of PCSK9 is cleaved and the 692–amino acid peptide undergoes autocatalytic processing at position VFAQ152–SIP. This autocatalytic processing deprives mature PCSK9 of its proteolytic property, because the prosegment quickly occupies the catalytic site. This step is necessary for exiting the ER compartment, which ensures that mature PCSK9 is properly secreted from the ER (5, 6).

Eukaryotic cells package cargo proteins for transport from the endoplasmic reticulum to the Golgi into COPII-coated vesicles. The COPII component SEC24A is required for the exit of PCSK9 from the ER to the Golgi (7). PCSK9 is produced and secreted mostly by the liver, and to a lesser extent the kidney and intestines, and is transiently expressed in the central nervous system during embryogenesis. The crystal structure of PCSK9 was determined in 2007 (8, 9) (Fig. 1B). Sequence analysis of the catalytic domain indicates that 3 conserved residues—Asp186, His226, and Ser386—comprise the active site, and mutations at these residues render the protein inactive and the protein is degraded intracellularly (9). The mature protein secreted undergoes posttranslational modifications with an added functional group that confers the final 3-dimensional structure (10). In circulation, PCSK9 undergoes a furin (or PC5/6A) enzymatic inactivation at position 218 of the catalytic domain (11, 12). Furin-cleaved PCSK9 is unable to regulate the LDL receptor compared to intact PCSK9 (13). However, the role of furin in modulating the biological function of PCSK9 is of uncertain biological significance.

**Fig. 1.** (A), The PCSK9 gene is located on the short arm of chromosome 1p32 and has 12 exons. The mature form of PCSK9 consists of a signal peptide (SP), a prosegment (PRO), and a catalytic domain and a cysteine/histidine-rich domain (CHRD) in the C-terminal domain. (B), The crystal structure of the interaction between the EGF region of LDL-R and PCSK9 reveals that the surface of interaction is flat. (C) Mutations identified within PCSK9 that cause FH [gain of function (GOF)] or are associated with lower LCL-C [loss of function (LOF)].
CIRCUITING ISOFORMS, MATURE AND FURIN CLEAVED (POTENTIAL ROLE)

Once in the systemic circulation, mature PCSK9 interacts with the epidermal growth factor (EGF) precursor homology domain of LDL-R on the cell surface by protein–protein interactions to the LDL-R, in a region near the PCSK9 catalytic domain (14). In plasma, it is estimated that approximately 40% of circulating PCSK9 is carried on the LDL cholesterol (LDL-C) particle and is postulated to bind apo B100 (15).

REGULATION AND INTRACELLULAR AND EXTRACELLULAR PROCESSING OF LDL-R

The PCSK9 gene mRNA is sterol regulated by the sterol receptor element binding proteins (SREBPs) SREBP-1a and -2. Low intracellular cholesterol upregulates PCSK9 expression, an effect shared by the hydroxymethylglutaryl coenzyme A reductase (HMG CoA R) inhibitor class of medications (statins). Statins upregulate hepatic expression of LDL-R, contributing to LDL particle clearance from plasma. PCSK9 is also upregulated by statins and may modulate—and potentially limit—the physiological response to statins by providing an opposite biological effect. Loss of function in PCSK9 is associated with decreased LDL-C concentration and lowered apolipoprotein B production. Consistent with these findings is the decrease in apo B concentrations in primary hepatocytes after PCSK9 silencing (5, 16). It has been shown that PCSK9 has an effect on hepatic triglyceride-rich lipoproteins by increasing apo B production (17, 18, 19). A similar finding has recently been reported for intestinal cells as well (20), suggesting that PCSK9 plays a role in apo B metabolism.

Hepatic ligand-activated receptors, including the farnesoid X receptor, PPAR (peroxisome proliferator-activated receptor), and HNF1 (hepatocyte nuclear factor 1) regulate PCSK9 transcription (21). The physiological significance of these regulatory pathways is unknown.

The PCSK9–prosegment complex binds to the EGF-like (EGF-A) domain of the LDL-R by protein–protein interactions (22) (Fig. 1A). This interaction of PCSK9 with LDL-R targets LDL-R for lysosomal degradation (23); the key mechanisms for directing the LDL-R/PCSK9 complex to the degradation pathway remain poorly understood (24, 25). The N-terminal region of the PCSK9 prosegment domain (31–52 aa) is required for LDL-R binding (15). Interestingly, evidence exists for a minor, and possibly physiologically relevant, intracellular pathway that targets LDL-R directly to endosomes/lysosomes (26). Thus there are 2 proposed sites where PCSK9 can interact with LDL-R: on the cell surface of hepatocytes (extracellular route, major) (27) and in the trans Golgi network (TGN) (intracellular route, minor) (26, 28) (Fig. 2). This may explain, in part, why circulating concentrations of PCSK9 do not correlate well with LDL-C concentrations (29).

GENETICS AND MENDELIAN RANDOMIZATION

The PCSK9 gene was first identified by virtue of its homology to other genes of the protein convertase superfamily (1). This gene, present on chromosome 1p32, is composed of 12 exons spanning 25 kb and contains a sterol regulatory element (SRE) region. SREBP-1c and SREBP2 have been shown to enhance the expression of this gene, coding for a 3731-bp mRNA. Intracellular cholesterol concentration is the major regulator of PCSK9 transcription via SREBP2, but insulin has also been shown to regulate PCSK9 expression via SREBP-1c (30, 31).

The first mutation of PCSK9 identified was the S127R mutation. Subsequently, several other gain-of-function PCSK9 mutations associated with a higher affinity and increased degradation of the LDL-R were identified, including F216L (32). Of note is the anglosaxon D374Y mutation, which is associated with a 100-fold increase in the affinity of PCSK9 for the LDL-R and an accentuated FH phenotype (33).

Loss-of-function PCSK9 mutations are more common and were first identified (34) in a Japanese cohort. These mutations are associated with a decreased affinity for the LDL-R and a decrease in the degradation of this receptor, thereby causing a reduction in plasma LDL-C concentration. The effect of 3 of these relatively common loss-of-function PCSK9 variants were studied in the ARIC cohort by Cohen et al. in 2006 (Fig. 3). It is remarkable that the R46L PCSK9 variant is associated with a modest 15% reduction in LDL-C concentration but is also associated with a 47% decreased risk in major cardiovascular events (35). The PCSK9 C679X loss-of-function mutation was associated with an approximately 1 mmol/L (39 mg/dL) decrease in LDL-C and a reduction in atherosclerotic vascular disease of 88% (35). This is in striking contrast to the Cholesterol Treatment Trialist’s estimate of a 21% reduction in cardiovascular risk per 1 mmol/L of LDL-C reduction (36). This Mendelian randomization experiment shows that lifetime low LDL-C protects against cardiovascular disease and lends strong confirmation that LDL-derived cholesterol is causal in atherosclerosis. The R46L PCSK9 polymorphism is more frequent in certain populations, such as the French Canadians, possibly via a founder effect (37). Mutations so far identified are shown in Fig. 1C.
Overall, approximately 2%–3% of patients with FH have a mutation in PCSK9; more than two-thirds have mutations in the LDLR gene, and fewer than 10% have mutations in the apolipoprotein (APOB) gene (FH2) (38). Perhaps as many as 20% of patients with FH do not have a causal mutation in known genes. Recently, exome-wide sequencing using next-generation sequencing protocols and bioinformatics has identified the APOE p.Leu167del mutation (FH4) as being associated with FH in 2 reported cases (39, 40). As many as 20% of patients with the FH phenotype may have an accumulation of genetic polymorphisms in genes associated with LDL-C in genome-wide association studies (GWAS), with their cumulative effect causing severe increases in LDL-C (38). GWAS have confirmed that PCSK9 polymorphisms are associated with decreased LDL-C concentrations (41) and cardiovascular risk (42) (rs11591147, rs28362286 and rs6708943). Indeed, in most lipid GWAS, PCSK9 polymorphisms had stronger associations with LDL-C than did other genetic polymorphisms.

Reports of a young woman with compound heterozygosity for loss-of-function PCSK9 mutations revealed no immune-detectable PCKS9 in plasma and very low LDL-C concentrations [14 mg/dL (0.36 mmol/L)]. This college graduate was in apparent good health with no detectable physical or intellectual disorders (43). Another form of severe LDL-C deficiency, apobetalipoproteinemia, is associated with autosomal dominant truncations of the APOB gene, resulting in a lack of formation of LDL particles. While affected individuals are normal into adulthood, cases of cirrhosis and hepatocellular carcinoma have been identified in patients with heterozygous apo B truncations, most likely because
of hepatic steatosis caused by the inability of the liver to excrete triglyceride-rich lipoproteins (44). Thus, severe LDL-C lowering may have a different clinical course, depending on the molecular basis of the disorder.

Clinical Biochemistry of PCSK9

MEASUREMENT TECHNIQUES AND CHALLENGES

The measurement of plasma (or serum) concentrations of PCSK9 presents unusual challenges. No gold standard method yet exists to quantify PCSK9 mass. The determination of PCSK9 mRNA concentration does not necessarily correlate with biologically active PCSK9 in blood (5). ELISAs are commonly used. However, values measured vary over a 10–100-fold range, in the nanogram per milliliter (mg/L) range, depending on the antibody used (45, 46). Furthermore, PCSK9 gets cleaved in plasma by furin (PC5/6) and is likely biologically inactive. Most ELISAs do not recognize the difference between furin-cleaved and not-cleaved forms of PCSK9. Mass spectroscopy is more precise, but the low abundance of the protein in plasma requires an enrichment step. This technique allows the identification of the various forms of PCSK9 circulating in plasma (47, 48). The range of PCSK9 concentrations in plasma varies between 30–230 ng/mL (45) and 10–3000 ng/mL in healthy individuals (46). Women have higher PCSK9 concentrations than men (29, 46, 49). Across the lifespan, PCSK9 concentrations in youth and adolescence vary differently among boys and girls, possibly associated with hormonal changes (49). In women, PCSK9 concentrations are higher in the premenopausal than the postmenopausal period. In contrast, there are no significant differences in men younger or older than 50 years (46). Interestingly, serum PCSK9 is increased in pregnancy at term. However, concentrations are markedly lower in cord blood than in maternal blood (50). Although postmenopausal estrogens have little effect on PCSK9 concentrations, women undergoing in vitro fertilization with high-dose estrogens have been reported to have lower values (51).

MODULATORS OF PCSK9 CONCENTRATION (DIET AND DRUGS)

The Mediterranean diet can lower plasma PCSK9 concentration in patients with metabolic syndrome without changes in body weight (52). As previously discussed, PCSK9 mRNA is under the transcriptional regulation of SREBP-2; like the LDLR gene, PCSK9 is also regulated by intracellular sterol depletion and inhibitors of HMG CoA R inhibitors (statins)—type medications (53). Moreover, it has been shown that PCSK9 transcription can be suppressed by fasting and induced by insulin, likely by activating the liver X receptor and SREBP-1c (30). By acting as a regulatory checkpoint, PCSK9 modulates the cell-surface availability of the LDL-R. The reasons for this are unclear, but the clinical effect might be to limit the therapeutic efficacy of statins. Indeed, it has been well established in clinical practice that increasing the dose of any statin reaches an asymptote of LDL-C reduction at high doses (29, 31). Yet, the clinical significance of this observation is not well understood. In the JUPITER (Justifica-
tion for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin) study, although the percent decrease in LDL-C could not be predicted by PCSK9 concentrations, a significant trend was observed between PCSK9 values and LCL-C lowering, suggesting that PCSK9 concentrations may be a biomarker of efficient inhibition of HMG CoA R (although this remains hypothetical) (29). Interestingly, the NPCL-1 (Niemann-Pick disease like-1) inhibitor, ezetimibe, which lowers LDL-C by blocking intestinal absorption, does not alter PCSK9 concentrations (54).

Animal Models

The PCSK9 gene sequence is conserved in many animal species, including chimpanzee, rhesus monkey, mouse, rat, chicken, and zebrafish. Adenoviral-mediated expression of PCSK9 in mice was associated with a phenotype similar to the LDLR knockout phenotype (55). The effects of PCSK9 are mostly exerted in a paracrine fashion, as shown by earlier parabiosis experiments (27). As expected, transgenic mice expressing PCSK9 are viable, fertile, and develop severe hypercholesterolemia. Pcsk9 transgenic (Tg) mice on an Apoe knockout (KO) background display an increase in plaque size as well as accelerated atherosclerosis when maintained on a regular diet. However, a protective effect was found when crossing Apoe (KO) mice with Pcsk9 (KO) mice (56). Advance calcified atherosclerotic plaque takes place in Pcsk9 (Tg) mice to a lesser degree (and takes longer to occur) than in Ldlr (KO) mice (57). To further investigate the effect of PCSK9 inhibition, inactivation of Pcsk9 by disrupting the proximal promoter and exon 1 led to a viable and fertile mouse with severe hypercholesterolemia (5). A mouse with a total Pcsk9 gene KO is apparently normal, with a 40% drop in total cholesterol and 80% drop in LDL-C. Total and liver-specific KO mice exhibit approximately 42% and approximately 27% decreases in circulating cholesterol, respectively. This observation confirmed that PCSK9 produced in the liver was responsible for more than two-thirds of the phenotype (5).

As mentioned above, PCSK9 may target other receptors. VLDL-R is upregulated in perigonadal depots of Pcsk9 (KO) mice, specifically females. As a consequence, adipocyte tissue becomes hypertrophied after the excessive free fatty acid internalization due to the accumulation of VLDL-R in the adipocytes of these Pcsk9(KO) mice (8). Conversely, plasma triglyceride concentrations were slightly increased in Pcsk9 (KO) males (+35%; not significant) and females (+46%; $P = 0.03$). Furthermore, in vivo studies have shown that PCSK9 deficiency was associated with a 2-fold decrease in postprandial triglyceride values, suggesting improved triglyceride clearance and a role for PCSK9 in triglyceride metabolism (58). PCSK9 is thus essential in fat metabolism because it maintains high circulating cholesterol concentrations via hepatic LDL-R degradation, but at the same time it restricts visceral adipogenesis possibly via adipose VLDL-R degradation (8).

Physiological Actions of PCSK9

LIVER REGENERATION

Conditional hepatic KO expression is associated with increased LDL-R cell-surface expression. Under severe metabolic stress, such as partial hepatectomy, mice with the Pcsk9$^{-/-}$ genotype exhibit delayed liver regeneration and hepatic fibrosis and necrotic lesions (5). This can be prevented by a high-cholesterol diet. Furthermore, lipid accumulation in the Pcsk9$^{-/-}$ mice is markedly reduced under both regular and high-cholesterol diets, suggesting that hepatic PCSK9 deficiency confer resistance to hepatic steatosis (5).

INSULIN RESISTANCE

Adult mice deficient in PCSK9 exhibit impaired glucose tolerance and may be at risk for diabetes mellitus (59). The mechanism is largely due to lipotoxicity of β-cells of the pancreas. This was confirmed in one population study showing that the common PCSK9 R46L loss-of-function mutation yields a significant increase in markers of insulin resistance, such as insulin concentrations, homeostasis model assessment of insulin resistance, and leptin concentrations, in individuals carrying one copy of apo E2 compared to carriers of other apo E3 or E4 isoforms (37). This suggests a possible apo E2/PCSK9 gene interaction that contributes to insulin resistance.

NEUROLOGICAL

The β-site amyloid precursor protein (APP)-cleaving enzyme 1 (BACE1) is a membrane protein acting as the rate-limiting enzyme in the generation of amyloid β-peptide in Alzheimer disease. BACE1 is transiently acetylated on lysine residues in the ER/ER–Golgi intermediate compartment. PCSK9 contributes to the disposal of nonacetylated BACE1. This finding is intriguing, as BACE1 is necessary for the elimination of amyloid β-peptide. It remains to be seen if this observation has a clinical consequence, because PCSK9 does not appear to be expressed in the adult brain (60).

CANCER METASTASIS

Protein convertases, notably furin and PACE4, have been shown to play a role in tumor progression (61). Intrasplicenic injection of B16 melanoma cells to recipient mice with Pcsk9 wild type (WT) and Pcsk9 (KO) was performed to study cancer cell behavior. Pcsk9 (KO) mice had 50% less liver metastasis compared to
WT mice; however when a high-fat diet was fed over 2- and 4-week periods, this apparent protection was lost (62). The clinical significance of this observation remains uncertain because metaanalyses of statin trials have not shown an increase in the incidence of any cancer (36).

**VIRAL INFECTIONS**

The hepatitis C virus (HCV) is associated with LDL particles in infected patients, and cell entry via the LDL-R has been postulated; there is thus a theoretical possibility that PCSK9 inhibition may increase HCV risk. The HCV particles interact with a number of putative HCV receptors, including CD81, SR-BI (scavenger receptor class B type I), and Claudin-1 (63, 64). Many PCSKs have the ability to negatively regulate cell surface receptors, namely LDL-R and CD81, used by viruses to gain access the cell membrane and infect the host. In vitro experiments showed that soluble purified PCSK9 dose dependently inhibits HCV. This is supported by experiments showing that hepatic CD81 expression is increased in Pcsk9 (KO) mice and PCSK9 downregulates CD81 independently of LDL-R. Therefore, it was proposed that the plasma concentration and/or activity of PCSK9 could modulate HCV infectivity in humans (65). Adding to this controversy, statins enhance LDL-R concentration and activity, increase PCSK9, and still reduce HCV infectivity by unknown mechanisms (66). Thus, it remains uncertain whether increasing PCSK9 pharmacologically (using statins, for instance) would enhance viral entry into cells.

**PCSK9 as a Therapeutic Target**

The past decade has witnessed unprecedented translation from basic science research and the discovery of PCSK9 to the completion of phase II and early phase III clinical trials. The transformational nature of this research highlights the potential therapeutic benefits of PCSK9 inhibitors. As previously reviewed (67), several approaches, including 2′-O-methoxymethyl–modified phosphorothioate antisense oligonucleotide and locked nucleic acid antisense oligonucleotides, have been stopped from further clinical trials. Similarly, siRNA (small interfering RNA) have been used in phase I clinical trials but later abandoned. PCSK9-specific inhibitory adnectins (approximately 12 kDa) have been used in phase I clinical trials but abandoned as well.

A small molecule inhibiting the interaction between PCSK9 and the LDL-R would represent an ideal therapeutic agent. However, this interaction occurs between relatively flat protein surfaces and, to date, a small molecule that would block this interaction has proven elusive. Other potential therapeutic avenues include small molecules that block the autoprocessing of PCSK9 in the ER, inhibiting transport from the ER to the Golgi (7).

Thus, the use of allosteric inhibition by monoclonal antibodies (mAb) represents the best option available to date. Several pharmaceutical and biotechnology companies have mAbs in advance phase III trials in patients with severe hypercholesterolemia, and large-scale outcome studies in patients at high cardiovascular risk have been initiated (Table 1).

There are currently 4 PCSK9 mAbs undergoing extensive clinical trials: alirocumab (Sanofi/Regeneron), evolocumab (Amgen), EFJE (Lilly), and bococizumab (Pfizer). The first 3 mAbs are fully human, whereas bococizumab is a humanized mAb.

Pooled results from phase II clinical trials have now been reported for evolocumab and alirocumab. In the OSLER (Open-Label Study of Long-term Evaluation against LDL Cholesterol) and DECARTES (Durable Effect of PCSK9 Antibody CompARed wiTh placEbo Study) trials performed in patients with FH, statin intolerance, or high cardiovascular risk treated optimally with statins, there was a further 50%–60% LDL-C reduction. In both studies, the adverse effect profile was similar in both arms, drug and placebo. It is noteworthy that PCSK9 mAbs decrease lipoprotein(a) concentrations by approximately 30%, an effect unmatched by other classes of lipid-lowering drugs, including niacin (68, 69).

Extensive phase III trials are currently ongoing to test the hypothesis that PCSK9 mAbs given to patients at high cardiovascular risk will decrease major cardiovascular events when added to optimal medical therapy (including high-dose statins and ezetimibe) (Table 1). These outcome trials will enroll nearly 60000 patients at high cardiovascular risk and randomize them to optimal medical care and LDL-C reduction with statins according to current guidelines or optimal medical care with a PCSK9 mAb. These trials are powered to detect a reduction in the primary end point of cardiovascular death, nonfatal myocardial infarction, stroke, and arterial revascularization.

**Areas of Uncertainty**

**WHEN IS IT INDICATED TO MEASURE PCSK9?**

The clinical usefulness of measuring PCSK9 is uncertain. As previously discussed, measurement techniques have yielded different results; total plasma PCSK9 might not reflect the biologically active form of the protein and there is a lack of correlation between PCSK9 concentrations and the response to statin therapy. Thus, the measurement of PCSK9 concentration remains, for the time being, a research tool, with limited clinical applications.
Table 1. Outcome trials of PCSK9 inhibitors (evolocumab, alirocumab, bococizumab).

<table>
<thead>
<tr>
<th>PCSK9 Inhibitor</th>
<th>ODYSSEY* Outcomes (Secondary prevention)</th>
<th>FOURIER (Secondary prevention)</th>
<th>SPIRE1 (Secondary prevention)</th>
<th>SPIRE2 (Primary prevention)</th>
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<td>12000</td>
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<td>History of clinically evident</td>
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<td>• LDL-C ( \geq 70 ) (1.8 mmol/L) (on atorvastatin 40–80 mg or rosuvastatin 20–40 mg)</td>
<td>CVD: MI, stroke or symptomatic PAD and $\geq 1$ major RF or $\geq 2$ minor RFs</td>
<td>• LDL-C $\geq 70$ (1.8 mmol/L) or Non–HDL-C $\geq 100$ (2.6 mmol/L) (on atorvastatin 20 to 80 mg or equivalent)</td>
<td>• LDL-C $\geq 100$ (2.6 mmol/L) or Non–HDL-C $\geq 100$ (2.6 mmol/L) and receiving background LLT</td>
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<tr>
<td></td>
<td>• LDL-C $\geq 70$ (1.8 mmol/L) or Non–HDL-C $\geq 100$ (2.6 mmol/L) (on atorvastatin 20 to 80 mg or equivalent)</td>
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\* ODYSSEY, Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab SAR236553 (REGN727); FOURIER, Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk; SPIRE, Evaluation Of PF-04950615 (RN316), In Reducing The Occurrence Of Major Cardiovascular Events In High Risk Subjects; sc, subcutaneously; Q2W, every 2 weeks; CHD, coronary heart disease; CVD, cardiovascular; MI, myocardial infarction; UA, unstable angina; ACS, acute coronary syndromes; PAD, peripheral arterial disease; RF, risk factor; LLT, lipid lowering therapy.

ARE THERE CLINICAL SITUATIONS IN WHICH PCSK9 INHIBITION SHOULD BE AVOIDED (EXTRAPOLATION FROM ANIMAL STUDIES)?

To date, these agents have proven to be safe with regards to a variety of side effects reported in clinical trials. Several thousand patients have been exposed to PCSK9 mAbs for short periods of time (12 weeks to 12 months) with little sign of acute toxicity. The theoretical concerns about HCV entry into the host in PCSK9-deficient states, the development of diabetes, recovery after hepatic surgery, cancer metastasis, and possible neurocognitive impairment should prompt regulatory agencies to track such rare events in ongoing clinical trials. As with any drugs, PCSK9 inhibitors should not be used during pregnancy.

WILL PCSK9 THERAPY BECOME A STANDARD OF CARE? IN COMBINATION THERAPY? AS MONOTHERAPY?

There is little doubt about the efficacy of PCSK9 mAbs in reducing LDL-C concentration. In patients with severe hypercholesterolemia and familial hypercholesterolemia, adding a PCSK9 mAb will likely become a standard of care in patients whom the LDL-C target has not been reached with optimal use of statins or ezetimibe (with or without bile acid sequestrants). In patients at high cardiovascular risk who have not reached their therapeutic goal (according to national guidelines) or who are statin intolerant, the results of the phase III outcome trials will be pivotal. Finally, the use of PCSK9 mAbs in primary prevention for individuals at high cardiovascular risk, with increased LDL-C concentration not at goal, will represent an economic challenge in light of the high cost of these agents compared with the cost of generic statins.

The data published for alirocumab (70) show that atorvastatin 80 mg or placebo had remarkably little additive effect in terms of LDL-C lowering. Such data may prompt physicians and patients to abandon statin therapy in favor of PCSK9 mAbs. In light of current data on statin safety and efficacy, this course of action is not advised.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures or Potential Conflicts of Interest: Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:

Employment or Leadership: None declared.
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