PCSK9: Regulation and Target for Drug Development for Dyslipidemia

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Abstract

Proprotein convertase subtilisin/kexin type-9 (PCSK9) is a secreted zymogen expressed primarily in the liver. PCSK9 circulates in plasma, binds to cell surface low-density lipoprotein (LDL) receptors, is internalized, and then targets the receptors to lysosomal degradation. Studies of naturally occurring *PCSK9* gene variants that caused extreme plasma LDL cholesterol (LDL-C) deviations and altered atherosclerosis risk unleashed a torrent of biological and pharmacological research. Rapid progress in understanding the physiological regulation of PCSK9 was soon translated into commercially available biological inhibitors of PCSK9 that reduced LDL-C levels and likely also cardiovascular outcomes. Here we review the swift evolution of PCSK9 from novel gene to drug target, to animal and human testing, and finally to outcome trials and clinical applications. In addition, we explore how the genetics-guided path to PCSK9 inhibitor development exemplifies a new paradigm in pharmacology. Finally, we consider some potential challenges as PCSK9 inhibition becomes established in the clinic.

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death in developed countries (1). Atherogenic lipoproteins, which are assessed by measurement of low-density lipoprotein cholesterol (LDL-C), non-high-density lipoprotein cholesterol (non-HDL-C), or apolipoprotein B (ApoB) levels, are independent, modifiable risk factors for CVD (2, 3). Statin-based reductions in LDL-C have attenuated CVD event risk successfully, reinforcing the central role of LDL-C-lowering in reducing CVD morbidity and mortality (4). Despite statin therapy, additional or alternative LDL-C-lowering strategies for further risk reduction are needed. For example, even at maximal statin doses, some patients, especially those with familial hypercholesterolemia (FH), cannot attain LDL-C targets (5, 6). Moreover, some patients either are completely statin-intolerant or can tolerate only low doses because of side effects (7).

The circulating liver-derived protein proprotein convertase subtilisin/kexin type-9 (PCSK9) has emerged as a major new drug target in cardiovascular medicine. Landmark discoveries in human genetics established a clear link between PCSK9 function and LDL-C concentrations (8). On the one hand, missense mutations in *PCSK9*, subsequently determined to confer a gain of function (GOF), cause a rare form of autosomal codominant FH (9). On the other hand, loss-of-function (LOF) mutations in *PCSK9* were associated with hypocholesterolemia and significant protection from coronary heart disease (CHD) (10). The discovery of additional *PCSK9* variants has provided further clues to PCSK9 function while reinforcing PCSK9 as a therapeutic target.

Mechanistic studies in cultured cells and mice revealed that secreted PCSK9 binds to the LDL receptor (LDLR) at the hepatocyte cell surface and promotes its lysosomal degradation (11). Molecular mapping of the PCSK9-LDLR binding interface aided the development of therapeutic anti-PCSK9 monoclonal antibodies (mAbs) that effectively block this interaction at the cell surface (12). These treatments reduce plasma LDL-C substantially with an excellent safety profile in both completed and ongoing clinical trials (reviewed in 13).

Here we review the discovery of PCSK9, its molecular and cellular biology, its genetics, and finally its inhibitors. We conclude with the lessons learned from meta-analyses of completed PCSK9 inhibitor trials as well as the anticipated impact of ongoing clinical trials.

STRUCTURAL AND CELLULAR BIOLOGY OF PCSK9

The 22-kb human *PCSK9* gene, located on chromosome 1p32, contains 12 exons and 11 introns and codes for a 692–amino acid proteinase K-like serine protease (**Figure 1**) (14). In rodents and humans, *PCSK9* is expressed primarily in liver with lower expression in the small intestine and kidney (14, 15). PCSK9 is a secreted protein that exhibits an atypical zymogen activation pathway. Following cleavage of its signal peptide in the endoplasmic reticulum (ER), proPCSK9 is unable to leave the ER until it undergoes cotranslational autocatalytic cleavage at Gln_{152} (**Figure 1*b***). The N-terminal prodomain remains tightly associated with the 63-kDa mature protein and chaperones PCSK9 through the secretory pathway (15). The terminal portion of the prodomain blankets the catalytic triad, preventing further proteolytic activity. The crystal structure of PCSK9 revealed the catalytic domain resembles that of other subtilisin-like serine proteases except for the high-affinity binding of the prodomain that shields the active site (16). Thus, PCSK9 has no other substrate than itself. PCSK9 undergoes posttranslational modifications, none of which are required for secretion or function (17).

PCSK9 Targets the Low-Density Lipoprotein Receptor

The best-characterized function of secreted PCSK9 (i.e., the prosegment bound to mature PCSK9) is its role in targeting the LDLR for degradation. The catalytic subunit contains a site

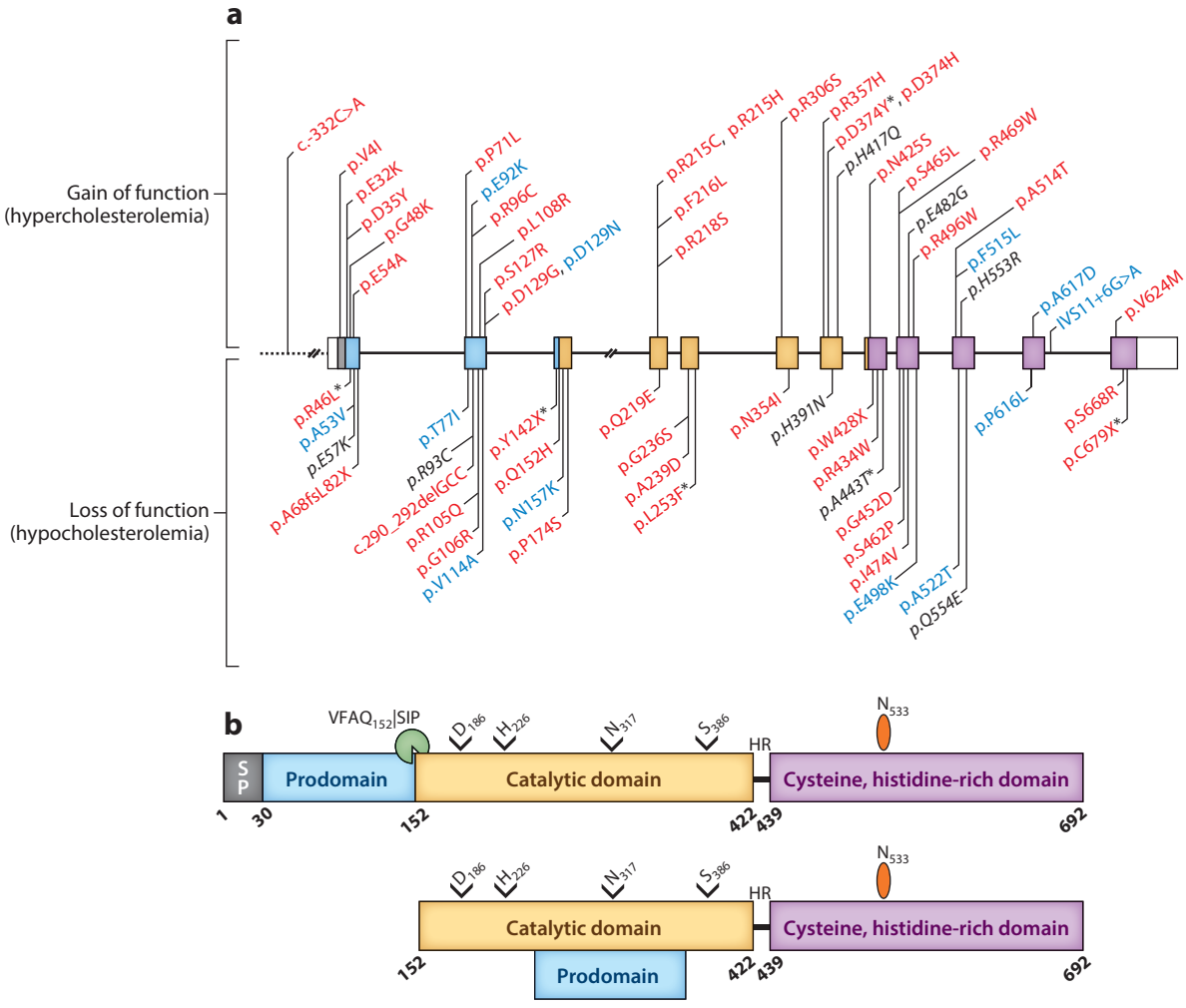


Figure 1

Schematic representation of the proprotein convertase subtilisin/kexin type-9 (*PCSK9*) gene and protein. (*a*) *PCSK9* gain-of-function variants are labeled above the gene transcript, whereas loss-of-function variants are below. The color coding represents the current status for the effect of each variant on low-density lipoprotein cholesterol (LDL-C) phenotypes: causative (*red*), possibly causative (*blue*), and associated (*black*, *italicized*). Asterisks indicate variants that have been shown to associate with both LDL-C and coronary artery disease. All variants are mapped to their appropriate location: upstream of the 5′ untranslated region (UTR) (*dotted line*), in the 5′ and 3′ UTRs (*white boxes*), in the 12 exons (*colored boxes*, with each color mapping to the appropriate protein domain shown in panel *b*), and in the 11 introns (*solid lines*). The diagram is not to scale. (*b*) Domain structure of proPCSK9 and mature secreted PCSK9 after autocatalytic cleavage. In the endoplasmic reticulum, the secretory pathway-targeting signal peptide (SP) (*dark gray*) is removed and PCSK9 undergoes autocatalytic cleavage at Gln_{152}. $VFAQ_{152}SIP$ (*green*) represents the cleavage recognition sequence. The cleaved prodomain (*blue*) reassociates with PCSK9, blanketing the catalytic triad: aspartate (D_{186}), histidine (H_{226}), and serine (S_{386}). This renders PCSK9 catalytically inert. The oxyanion hole, which is considered critical for catalysis, is located between S_{386} and N_{317}. A small, 18-amino acid hinge region (HR) links the catalytic subunit (*gold*) to the C terminus (*purple*). The orange oval at N_{533} depicts the only N-glycosylated site.

that binds the LDLR epidermal growth factor-A (EGF-A) domain at the hepatocyte cell surface, ultimately leading to LDLR degradation (**Figure 1*b***) (18–20). As hepatic LDLRs mediate the majority of plasma LDL clearance, a detailed understanding of PCSK9 structure and function has assumed therapeutic significance.

Normally, LDL bound to the LDLR enters cells via clathrin heavy chain-coated vesicles (**Figure 2**). Following internalization, the acidic pH of endosomes disrupts the allosteric association of LDL with LDLR (21). A recycling vesicle containing the LDLR is formed and returns LDLRs to the cell surface, whereas LDL particles remain within endosomes. Endosomes fuse with lysosomes where LDL is degraded, cholesteryl esters are hydrolyzed, and free cholesterol is distributed to other cellular compartments (21) (**Figure 2**). PCSK9 bound to a cell surface LDLR also enters cells via clathrin-coated vesicles. However, the complex becomes more tightly associated at endosomal acidic pH, preventing the LDLR from entering a recycling vesicle and inhibiting its return to the cell surface. The PCSK9:LDLR complex is escorted to lysosomes for proteolytic degradation (16, 18, 22) (**Figure 2**).

Recent evidence in cultured hepatocytes suggests PCSK9 also enhances intracellular LDLR degradation prior to its secretion. PCSK9 binding to the LDLR within the Golgi network increases trafficking of PCSK9:LDLR from the *trans* Golgi network to lysosomes for degradation (**Figure 2**). This pathway requires clathrin light chains and involves sorting mechanisms distinct from the extracellular LDLR degradation pathway. It is not clear whether the intracellular pathway occurs in all tissues (23, 24).

Other domains of PCSK9 influence its function. Sequences within the C terminus bind the LDLR ligand-binding domain and prevent dissociation of the PCSK9:LDLR complex within acidic endosomes (25). A recent study reported that an N-terminal region of the PCSK9 prodomain binds ApoB on plasma LDL particles, hindering PCSK9:LDLR binding and thereby blunting PCSK9-mediated LDLR degradation (26).

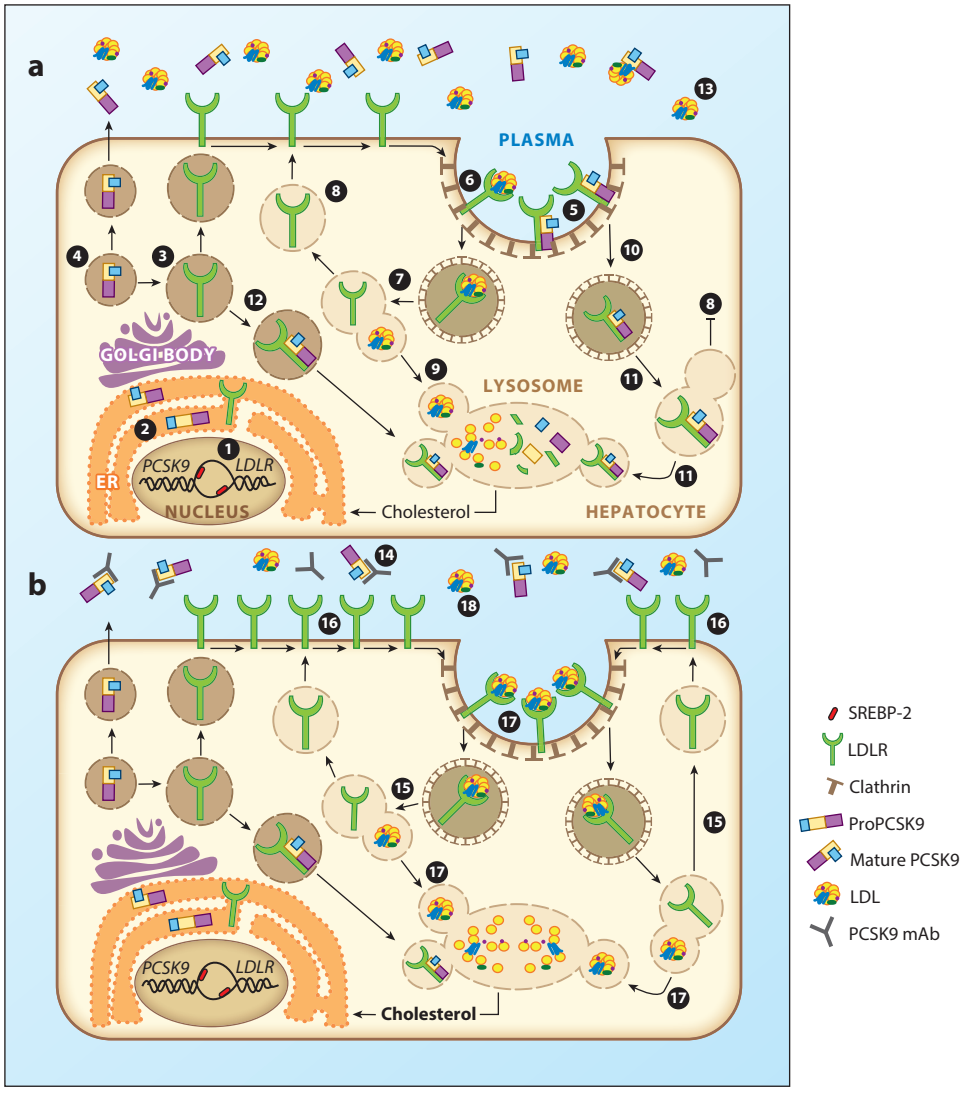
Figure 2

Low-density lipoprotein cholesterol (LDL-C) and LDL receptor (LDLR) metabolism in the presence or absence of proprotein convertase subtilisin/kexin type-9 (PCSK9). (*a*) ❶ Low cellular cholesterol activates sterol regulatory element-binding protein-2 (SREBP-2), which increases gene expression of both *LDLR* and *PCSK9*. ❷ Autocatalytic processing of PCSK9 in the endoplasmic reticulum (ER) releases the prodomain, which remains bound to PCSK9, blocking its catalytic site. ❸ LDLRs are secreted to the cell surface. ❹ Cleaved PCSK9 is secreted into the plasma and ❺ binds the extracellular epidermal growth factor-A domain of the LDLR at the plasma membrane. ❻ Plasma LDL binds the LDLR and is internalized by clathrin-dependent endocytosis. ❼ Within endosomes, the LDLR dissociates from LDL. ❽ A recycling vesicle is formed, and LDLR returns to the cell surface. ❾ LDL-containing endosomes fuse with lysosomes, LDL is degraded, and cholesterol is delivered to cell membranes, including the ER. ❿ PCSK9:LDLR is internalized by clathrin-dependent endocytosis. The association of PCSK9 with LDLR becomes stronger in the acidic endosome. This prevents LDLR recycling back to the plasma membrane (❽), and ⓫ the PCSK9:LDLR complex is directed toward lysosomal degradation. ⓬ An intracellular pathway also exists in which active PCSK9 binds LDLR in the secretory pathway and targets the LDLR for lysosomal degradation. Steps ⓫ and ⓬ result in low LDLR expression at the cell surface and ⓭ increased levels of circulating LDL-C. (*b*) ⓮ Injected anti-PCSK9 monoclonal antibodies (mAbs) enter the circulation, bind plasma PCSK9, and promote PCSK9 degradation, thereby inhibiting the binding of PCSK9 to the LDLR (❺). ⓯ In the absence of PCSK9, internalized endosomal LDLR dissociates from LDL, a recycling vesicle is formed, and LDLR recycles to the cell surface. ⓰ This increases the abundance of active LDLR at the plasma membrane and ⓱ enhances LDL binding, uptake, and degradation, thereby lowering circulating LDL (⓲).

Other PCSK9 Target Proteins

The LDLR is the best characterized PCSK9 target and likely the most physiologically relevant. Cell culture studies revealed PCSK9 promotes degradation of other LDLR-family members, namely the very-low-density lipoprotein receptor (VLDLR), ApoE receptor-2 (ApoER2), and LDLR-related protein 1 (27–29). Although PCSK9 binds the EGF-A-like domains of VLDLR and ApoER2, the degradation pathways differ (27). VLDLR and ApoER2 have been confirmed as PCSK9 target proteins in mice in some investigations (30) but not in others (17). Furthermore, the physiological relevance of PCSK9-mediated degradation of these receptors has been demonstrated neither in *Pcsk9*$^{-/-}$ mice nor in humans who are completely deficient in PCSK9 or carry LOF mutations (31, 32).

CD36, a scavenger receptor with multiple ligands and functions, including free fatty acid uptake, was recently shown to be a PCSK9 target in cultured hepatocytes and adipocytes (30, 33).



The PCSK9 catalytic domain binds the extracellular loop of CD36, mediating its internalization and degradation through a mechanism involving the proteasome (33). Liver and adipose tissue expression of CD36 was increased in *Pcsk9*$^{-/-}$ mice, which was associated with increased liver triglyceride and gonadal fat pad weight. A second strain of *Pcsk9*$^{-/-}$ mice has been reported to be resistant to liver steatosis and does not become overtly obese (34). It remains to be determined if PCSK9 regulates CD36 in humans, although clinical studies using PCSK9 inhibitors have not reported altered liver function or adiposity (35, 36). PCSK9 has also been demonstrated to degrade CD81, the cell surface receptor for hepatitis C virus (HCV), which suggests that reduced PCSK9 function may enhance susceptibility to HCV infection (37). Increased HCV infectivity in humans deficient in PCSK9 or exposed to PCSK9 inhibition has not been reported.

PCSK9 Regulation

PCSK9 is regulated transcriptionally primarily by sterol regulatory element–binding protein-2 (SREBP-2), a membrane-bound transcription factor that regulates cellular cholesterol homeostasis (**Figure 2**) (38, 39). Two main SREBP isoforms are expressed in liver that have distinct yet partially overlapping target genes. SREBP-2 regulates genes involved in cholesterol synthesis and uptake, including *Ldlr* and *Pcsk9*, whereas SREBP-1c primarily regulates genes involved in fatty acid synthesis (40). Proteolytic processing of both SREBPs to active nuclear forms occurs in response to lower ER cholesterol. SREBP-1c is also stimulated by insulin at both transcriptional and protein processing levels (40). In vitro, processed forms of both SREBP-1c and SREBP-2 bind the sterol regulatory element (SRE) within the *PCSK9* proximal promoter (39). In healthy humans, plasma PCSK9 levels decrease with fasting and increase upon refeeding in parallel with plasma markers of hepatic cholesterol synthesis (41, 42), consistent with regulation by SREBP-1c and -2. Statins induce SREBP-2 activity by inhibiting hydroxymethylglutaryl-coenzyme A reductase, the rate-limiting enzyme in cholesterol synthesis, which in turn decreases ER cholesterol, increases SREBP-2 processing, stimulates hepatic *LDLR* expression, and promotes plasma LDL-C clearance. However, SREBP-2-induced *PCSK9* expression under these same conditions likely attenuates statin efficacy by stimulating PCSK9-mediated LDLR degradation (43). PCSK9 may represent a counterregulatory mechanism imbedded into the SREBP pathway to limit LDLR activity (44).

Several studies have demonstrated that increased PCSK9 expression promotes ApoB secretion in HepG2 cells and mouse liver (45, 46). This may occur, in part, through a neutralizing effect on LDLR activity (45) or through a direct interaction of PCSK9 with ApoB100 (46). Whether PCSK9 regulates hepatic ApoB100 secretion in humans has not been reported.

PCSK9 is also regulated by hepatocyte nuclear factor-1α (HNF1α), a transcription factor that cooperates with SREBP-2 in the basal transcription of *PCSK9* (47). The *PCSK9* promoter contains an HNF1α binding site close to the SRE, which is absent from the *LDLR* promoter. Studies in HepG2 cells and dyslipidemic hamsters demonstrated that altering HNF1α activity influences *PCSK9* transcription and thus LDLR protein expression (47, 48). In mice, stimulation of mammalian target of rapamycin complex 1 by insulin initiates a signaling cascade resulting in nuclear exclusion of HNF1α, decreased *Pcsk9* transcription, and ultimately increased hepatic LDLR protein expression (49). Recently, activation of G protein–coupled estrogen receptor 1 (GPER) in HepG2 cells by estrogen or a specific GPER agonist was shown to decrease PCSK9 transcription and protein expression without any effect on *LDLR* mRNA. This increased LDLR protein and enhanced LDL cellular uptake (50). As SREBP-2 was not involved, it is possible that activated GPER attenuates HNF1α activity. This regulation of *PCSK9* transcription through GPER likely explains low LDL-C levels observed in women who carry a GOF variant in *GPER* (50) or in women undergoing high-dose estrogen treatments for in vitro fertilization (51).

PCSK9 ANIMAL MODELS

Transgenic overexpression of human *PCSK9* in the liver of mice increases LDL-C to levels observed in *Ldlr*$^{-/-}$ mice (19, 34, 52). PCSK9's effect on LDL-C is exerted primarily in a paracrine/endocrine fashion, as revealed in parabiotic mice (52). LDLR protein was not detected in the liver of *PCSK9* transgenic mice, whereas in nonhepatic tissues, LDLR appeared to be either unregulated or not accessible to circulating PCSK9. Transgenic *PCSK9* mice on an *Apoe*$^{-/-}$ background and fed a chow diet developed accelerated atherosclerosis with larger lesions compared to *Apoe*$^{-/-}$ mice (53). Liver-specific overexpression of the human *PCSK9* GOF p.D374Y mutant in Yucatan minipigs significantly reduced hepatic LDLR levels, elevated plasma LDL-C, and accelerated atherosclerosis development, demonstrating the role of PCSK9 in LDL-C regulation and, in turn, lesion formation (54). Furthermore, this created a large animal model of human FH, readily amenable for studies of disease pathogenesis and the investigation of new therapeutics, including PCSK9 inhibitors.

The knockout of *Pcsk9* in mice reduced LDL-C markedly, which was associated with increased hepatic LDLR protein and enhanced LDL-C clearance (55). Studies in liver-specific *Pcsk9*$^{-/-}$ mice revealed that liver is the primary source of plasma PCSK9 and loss of liver PCSK9 contributes to the majority of LDL-C lowering (34). Although atherosclerotic lesions were modified minimally in *Pcsk9*$^{-/-}$ mice on an *Apoe*$^{-/-}$ background (53), lesions were attenuated in *APOE**3-Leiden-*CETP* transgenic mice treated with anti-PCSK9 antibodies (56). In *Pcsk9*$^{-/-}$ mice, adipocyte hypertrophy has been reported, possibly owing to upregulation of VLDLR, CD36, or both (33, 34). Evidence for this in other mouse *Pcsk9*$^{-/-}$ strains or in humans carrying *PCSK9* LOF mutations has not been reported (32).

Annexin A2 has been shown to be an extrahepatic inhibitor of PCSK9. In annexin A2–deficient mice, plasma PCSK9 increases and LDLR decreases in extrahepatic tissues, including adrenals and small intestine, but not in liver (57). Thus, high levels of annexin A2 in adrenals appear to be responsible for adrenal LDLR insensitivity to degradation by PCSK9 (57). *Pcsk9*$^{-/-}$ mice display increased transintestinal cholesterol excretion (TICE), an alternate pathway to hepatobiliary cholesterol secretion, suggesting PCSK9 can repress TICE (58). Whether PCSK9 regulates TICE in humans has not been reported. One strain of *Pcsk9*$^{-/-}$ male mice displays impaired glucose tolerance (59). Although the mechanism is unknown, it may be related to increased LDLR expression in pancreatic islet cells resulting from the absence of PCSK9, leading to beta cell lipotoxicity (59). *PCSK9* deficiency or LOF *PCSK9* mutations in humans have not been associated with glucose intolerance or insulin resistance (31, 32). Following partial hepatectomy in *Pcsk9*$^{-/-}$ mice, hepatic regeneration capacity was delayed and necrotic lesions formed (34). Whether this also applies to humans has not been demonstrated.

PHYSIOLOGICAL VARIATION IN PCSK9 IN HUMANS

Population-based studies have shown that plasma PCSK9 is associated with age, sex, and multiple metabolic markers in children and adolescents. In boys, PCSK9 concentrations decreased with age, whereas in girls, levels increased with age. In both sexes, positive associations were reported between plasma PCSK9 and fasting glucose, insulin, markers of insulin resistance, total cholesterol, LDL-C, and triglycerides (60). In the Dallas Heart Study, plasma PCSK9 levels were significantly higher in women than in men (61). Premenopausal women had higher plasma PCSK9 than postmenopausal women, whereas PCSK9 levels were similar in men above or below 50 years of age.

In normal subjects, a Mediterranean diet has been reported to lower circulating PCSK9 and LDL-C (62). In mice and humans, PCSK9 was suppressed by fasting and induced by refeeding,

most likely related to SREBP-1c activation by insulin (41, 42, 63). As discussed above, both *LDLR* and *PCSK9* are upregulated by intracellular sterol depletion as well as by statins. Statin treatment of *Pcsk9*$^{-/-}$ mice results in a greater reduction in LDL-C compared to wild-type mice, and FH patients who carry the *PCSK9* LOF p.R46L variant are more responsive to statin treatment (55, 64). In clinical trials, statins increase circulating PCSK9, although LDL-C levels are reduced significantly (65). These studies imply that the statin-mediated induction of *LDLR* transcription predominates over statin induction of *PCSK9*.

HUMAN GENETICS' ROLE IN DEFINING THE IMPORTANCE OF PCSK9

The rise to prominence of PCSK9 in metabolism and CVD owes much to the field of human genetics and, in particular, the autosomal codominant disorder FH. The heterozygous form of FH (HeFH) affects about 1 in 300 individuals in most populations and is classically associated with extremely elevated LDL-C levels, characteristic physical findings, and accelerated atherosclerotic CVD (66). Genetic causes of HeFH include >1,700 LOF mutations in the *LDLR* gene and >20 different mutations within the receptor-binding domain of the *APOB* gene (66). However, in a few multigenerational HeFH families, no mutations in either *LDLR* or *APOB* were detected (67). Linkage analysis in these families revealed a region on chromosome 1p34.1-p32 that cosegregated with elevated LDL-C (67, 68). This region contained the gene for a newly discovered serine protease called *PCSK9*, originally named neural apoptosis regulatory convertase (15). DNA sequencing revealed a novel missense mutation in *PCSK9*, p.S127R, which cosegregated perfectly with disease status in the family (**Figure 1*a***) (9). Sequencing of *PCSK9* in a similar family identified another rare missense mutation, p.F216L (**Figure 1*a***) (9). These findings indicated that *PCSK9* was the third human locus of FH, sparking a massive research effort to understand the function of PCSK9 in LDL-C metabolism, as summarized above.

Gain-of-Function versus Loss-of-Function Variants in *PCSK9*

Animal model studies suggested that *PCSK9* mutations causing hypercholesterolemia were associated with a GOF because overexpression of human *PCSK9* in transgenic mice was associated with hypercholesterolemia (19). Similarly, *Pcsk9*$^{-/-}$ mice had very low LDL-C, suggesting that *PCSK9* mutations associated with hypocholesterolemia were LOF (55). Population-based DNA sequencing of individuals at the extremes of the plasma LDL-C distribution identified relatively common inactivating nonsense variants that were associated with a 40% reduction in LDL-C (69). The two main variants, p.Y142X and p.C679X, were relatively common in African Americans (3–4%) but were virtually absent from European American samples (**Figure 1*a***). These variants cosegregated with very low LDL-C levels within families, implying LOF (69). Additional studies identified several other rare missense and noncoding *PCSK9* LOF variants that were shown to segregate similarly with low plasma LDL-C levels in families (**Figure 1*a***) (70).

A major advance followed from the analysis of the relationship between these *PCSK9* LOF variants and atherosclerotic CHD in the Atherosclerosis Risk in Communities (ARIC) prospective study. In one of the first and most successful applications of the now widely used Mendelian randomization approach (71), individuals in the ARIC cohort were genotyped for the *PCSK9* LOF variants (10). As expected, ~3% of African Americans were carriers of these variants and had ~40% lower plasma LDL-C compared to noncarriers. Furthermore, these individuals had ~88% reduced incidence of CHD over the 15-year follow-up period (10). A milder LOF variant in *PCSK9*, p.R46L, was found in ~2% of Caucasians and was associated with ~47% reduction

in CHD (**Figure 1*a***) (10). This established a causal relationship between lifelong genetically low LDL-C levels and protection from CHD and, importantly, identified PCSK9 as a potential pharmaceutical target. The use of naturally occurring genetic variants that are associated with favorable human phenotypes as probes for potential drug targets has since become a very popular approach for drug discovery (72).

Human GOF variants in *PCSK9* act through a variety of distinct mechanisms at the molecular level, but ultimately, all lead to increased expression or activity of PCSK9 and increased degradation of the LDLR (11). Similarly, LOF variants are associated with low circulating PCSK9, resulting in enhanced hepatocyte LDLR density. LOF mutations occur primarily in the catalytic domain of the protein but have also been reported in the prodomain, P-domain, and C terminus (**Figure 1*a***) (73). Most of these variants reduce or nullify PCSK9 synthesis or disrupt trafficking, leading to reduced secretion. For instance, the p.Y142X variant results in nonsense-mediated RNA decay; p.Q152H prevents autocatalytic processing, thereby inhibiting secretion; and p.C679X disrupts folding, leading to retention of proPCSK9 within the ER and blunted secretion (**Figure 1*a*,*b***) (31, 74). The result of all *PCSK9* LOF variants is low circulating PCSK9, absent or attenuated interaction with the LDLR, reduced targeting of LDLR for lysosomal degradation, enhanced LDLR recycling and longevity, and lower plasma LDL-C levels (11).

The protection from CHD in carriers of LOF variants was replicated in other populations (75), helping to fuel industry's drive to develop PCSK9 inhibitors. Although GOF variants are individually too rare to be studied in populations, family-based (76) and observational studies in cohorts of heterozygous GOF mutation carriers (77) strongly suggest these individuals have increased risk of premature CHD. Further insights from the human genetics of *PCSK9* include the observations that homozygosity for ultrarare GOF variants is associated with a more severe hypercholesterolemia phenotype (78), whereas rare homozygotes for LOF variants have very low LDL-C and appear healthy (31, 79). The latter observation provides reassurance regarding possible extreme side effects of a novel drug mechanism that inhibits PCSK9 function (72).

INHIBITORS THAT TARGET PCSK9: PRECLINICAL RESEARCH

As discussed above, interest in inhibiting PCSK9 for LDL-C lowering arose from genetic discoveries in families and populations (9, 10) as well as from mechanistic studies in cultured cells and mice (11). Two major strategies have dominated drug development during the last decade: (*a*) inhibition of PCSK9 binding to the LDLR and (*b*) inhibition of hepatic PCSK9 synthesis. Progress over the past 10 years has resulted in two clinically approved anti-PCSK9 mAbs, with other pharmacological PCSK9 inhibitors in the pipeline, including small interfering RNAs (siRNAs), antisense oligonucleotides (ASOs), adnectins, and peptide inhibitors.

STRATEGIES THAT REDUCE PCSK9-MEDIATED LOW-DENSITY LIPOPROTEIN RECEPTOR DEGRADATION

Monoclonal Antibodies: Preclinical Studies

Presently, mAbs are used clinically to treat many cancers and autoimmune diseases. mAbs have high specificity for target antigens and are highly potent with low dosing frequency. The first anti-PCSK9 mAb (mAb1) was discovered in 2009 (12). mAb1 binds the catalytic and prodomain of PCSK9, sterically blocking interaction with the LDLR, thereby neutralizing PCSK9 activity and promoting its clearance. This protects the LDLR from PCSK9-mediated lysosomal degradation and promotes LDLR recycling (**Figure 2*b***). In HepG2 cells overexpressing human *PCSK9*,

mAb1 neutralized elevated PCSK9 and increased LDLR protein 10-fold (**Figure 2*b***). A statin acted additively, increasing LDLR protein 2-fold over mAb1 alone. In C57Bl6 mice, mAb1 treatment elevated hepatic LDLR protein and dose-dependently reduced serum cholesterol (12). The effect was reversible, and the duration of cholesterol lowering increased with increasing dose. Importantly, mAb1-mediated reductions in plasma cholesterol were absent in *Ldlr*$^{-/-}$ mice. In *APOE**3-Leiden-*CETP* transgenic mice, treatment with chimeric mAb1 or alirocumab (another mAb) reduced atherosclerosis (56, 80) and enhanced the atheroprotective effects of atorvastatin (80). In monkeys, single-dose injections of mAb1 or other anti-PCSK9 mAbs (1D05 or 1B20) reduced plasma free PCSK9 by ~70% and LDL-C by ~80% at 10 days, effects that were fully reversed at 21 days (12, 81, 82). Following these preclinical studies, several human mAbs produced in either transgenic mice or humanized mice have undergone clinical trials, are now in clinical use, and are discussed below.

Adnectins (Monobodies)

Adnectins, or monobodies, are a new class of therapeutic proteins designed after the tenth fibronectin type III domain because of its similarity to antibody variability domains (83). Similar to mAbs, adnectins can be selected to have high target specificity, low toxicity, and low immunogenicity, and they are less expensive and easier to manufacture than mAbs (83). The adnectin BMS-962476 (by Bristol-Myers Squibb) was discovered in a screen identifying adnectins that could competitively displace LDLR or the EGF-A domain from binding PCSK9 (84). BMS-962476 has high binding affinity to human PCSK9, moderate affinity for cynomolgus monkey PCSK9, and no binding affinity for murine PCSK9, showing species preference (84). In mice overexpressing human *PCSK9*, BMS-962476 treatment reduced serum free PCSK9 and cholesterol (84). In cynomolgus monkeys, single-dose intravenous (IV) administration of BMS-962476 rapidly reduced plasma PCSK9 to undetectable levels and decreased LDL-C by ~50%, both of which were reversible (84). These results were reiterated in a recently completed Phase I clinical trial (85). BMS-962476 was well tolerated, and adverse events were similar to placebo. Maximal dosing (1 mg/kg) reduced free PCSK9 by >90% and achieved LDL-C lowering up to 48%.

STRATEGIES THAT REDUCE PCSK9 SYNTHESIS AND PROCESSING

Small Interfering RNA: Preclinical Studies

The discovery that some *PCSK9* LOF mutations inhibit its hepatic secretion, together with the profound hypocholesterolemia in *Pcsk9*$^{-/-}$ mice (described above), highlighted PCSK9 synthesis as an attractive therapeutic target (10, 55). One strategy has been to target *PCSK9* mRNA for translational arrest and degradation using short, double-stranded RNA–interfering molecules (siRNAs) (86). siRNAs have been developed to contain modified spacer regions that reduce off-target effects (87). siRNAs are targeted to the liver by IV administration of small lipoid nanoparticles containing the siRNA (86). In rats, a single injection of PCSK9-targeted siRNA reduced liver *Pcsk9* mRNA by >50%, increased hepatic LDLR protein, and reduced circulating cholesterol (by ~60%), effects that were reversed by day 21. Similar results were achieved in cynomolgus monkeys (86). An siRNA [Alnylam Pharmaceuticals (ALN-PCS)] recently completed Phase I clinical trials. Administration of ALN-PCS to healthy volunteers reduced circulating PCSK9 by 70% and LDL-C by 40% from baseline (88).

Antisense Oligonucleotides

ASOs are short nucleic acid sequences that bind and silence mRNA. Recently, the US Food and Drug Administration approved an ASO, mipomersen, targeting *APOB* mRNA in patients with FH. Although mipomersen reduced hepatic lipoprotein production, it increased liver fat content. Therefore, PCSK9 remains a more attractive target for antisense therapy. Second-generation ASOs and locked nucleic acid (LNA) ASO designs, which have enhanced affinity and target specificity, have been tested for PCSK9 inhibition (89, 90). In mice, parenterally administered PCSK9 LNA ASOs reduced liver *Pcsk9* mRNA by 60% and increased hepatic LDLR protein ~3-fold (89, 90). In nonhuman primates, continuous treatment with the LNA ASO SPC5001 reduced serum PCSK9 by 85% and LDL-C by 50%, effects that were reversible upon drug cessation (91). Recently, the development of SPC5001 and BMS-844421 (second-generation ASOs) were both halted during Phase I clinical trials for undisclosed reasons. Currently, there are no ongoing PCSK9 ASO clinical trials.

STRATEGIES IN EARLY PHASES OF DEVELOPMENT

Peptide Mimetics

Peptide fragments of both PCSK9 and the LDLR have been used to provide mechanistic insight into PCSK9-mediated LDLR degradation (92–94). PCSK9 binding to the LDLR can be outcompeted by peptides resembling natural binding partners. Truncated LDLR EGF-A-domain peptides have been designed to bind PCSK9, thereby preventing PCSK9:LDLR interaction and inhibiting LDLR lysosomal degradation (28, 95, 96). In hepatocytes, EGF-A mimetics increased LDLR recycling to the cell surface and increased LDL uptake by 90% (95, 96). No preclinical animal studies testing these peptides have been reported. Although peptide mimetics have advanced the understanding of PCSK9-mediated LDLR degradation significantly, administration and rapid metabolism of peptides in vivo remains a therapeutic challenge.

Small Molecules

Small molecules are compounds that bind target proteins, DNA, or RNA, inhibiting the target's biological function. These molecules have advantages over mAbs and siRNAs because they can be taken orally and are less expensive. They could theoretically act anywhere along the sequence of PCSK9 and affect autocatalytic processing, secretion, and/or LDLR interaction. Two difficulties in developing small-molecule inhibitors are that PCSK9 catalytic activity is not involved in LDLR degradation and the structure of the catalytic triad is similar to many other proteases, precluding specificity. Additionally, PCSK9 is difficult to target with small molecules because there is no surface binding pocket that would facilitate specific binding (97). Therefore, small-molecule development has lagged behind mAbs and siRNA.

PHARMACOKINETICS AND PHARMACODYNAMICS OF PCSK9 INHIBITORS

The pharmacokinetics of PCSK9 inhibition with mAbs have been characterized in healthy volunteers and patients (98, 99). Prior to treatment, free PCSK9 circulates in plasma. Upon administration of a single dose of anti-PCSK9 mAbs, plasma antibodies increase rapidly, reaching peak levels in ~8 days. The mAbs bind free PCSK9 rapidly, which reaches its lowest level by ~2 days. With less free PCSK9, it would be anticipated that fewer hepatic LDLRs are targeted for

lysosomal degradation and more LDLRs are recycled, thereby increasing their cell surface density (**Figure 2*b***). Consequently, more LDL becomes bound to the increased number of LDLRs and is internalized. Indeed, LDL-C levels decrease rapidly, reaching their lowest concentrations by ~15 days. With time, the mAbs are metabolized and plasma concentrations decrease, reaching undetectable levels by ~60 days. Free PCSK9 increases, reaching preinjection concentrations by ~60 days. Free PCSK9 would then be expected to bind the LDLR, target it for lysosomal degradation, reduce hepatocyte LDLR levels, and attenuate plasma LDL uptake (**Figure 2*a***). As anticipated, LDL-C increases slowly from its nadir, reaching preinjection levels by ~60 days at a rate that parallels the rebound in free PCSK9 levels. With repeated doses of anti-PCSK9 mAbs, the lowest, most stable and sustained concentrations of LDL-C were obtained with biweekly injections at intermediate doses (150 mg of alirocumab and 140 mg of evolocumab) (100, 101).

COMPLETED CLINICAL TRIALS OF PCSK9 INHIBITORS

To date, almost 30 Phase II and III randomized clinical trials (RCTs) have been reported, mainly using evolocumab or alirocumab versus various comparators in patients treated for up to 78 weeks. Patient groups studied included those with (*a*) FH, (*b*) hypercholesterolemia with high cardiovascular risk on usual or maximal lipid-lowering therapy, and (*c*) statin intolerance. Hereafter we focus on results of recent meta-analyses of the Phase II and III RCTs of PCSK9 inhibitors (**Table 1**) (102–105).

One meta-analysis summarized efficacy and adverse events grouped according to the PCSK9 inhibitor and showed that for equipotent dosages and dosing intervals, evolocumab and alirocumab had essentially identical LDL-C lowering efficacy and side-effect profiles (104). A second meta-analysis of 20 RCTs reported similar efficacy across both agents in absolute terms (103); because other meta-analyses evaluated biochemical efficacy in relative terms (percent LDL-C reduction), this meta-analysis could not be compared directly, although the overall results were consistent with studies that reported percent changes (**Table 1**). The meta-analyses by Navarese et al. (102) and Lipinski et al. (105) included 24 and 17 RCTs that enrolled a total of 10,159 and 13,083 patients, respectively. They showed very similar results, including LDL-C reductions versus placebo of ~60% when added to baseline lipid-lowering therapy. Both reports showed significant reductions in all-cause mortality of ~55% (102, 105), and one report revealed reductions in myocardial infarction of ~50% (102). Among biochemical variables, plasma lipoprotein (a) was reduced by ~26% in both meta-analyses, whereas HDL-C was increased by ~6%. Serious adverse events were generally neutral in both studies, although Lipinski et al. (105) found a significant 2.3-fold increased risk of neurocognitive adverse events, such as self-reported memory loss. More definitive results will emerge from new studies that evaluate neurocognitive function specifically and objectively through mental status examination and psychometric testing (106). Finally, a focused meta-analysis of 16 RCTs found no effect of PCSK9 inhibitors on C-reactive protein levels (107). We next discuss particular patient demographic groups in whom PCSK9 inhibitor therapy may be considered.

PCSK9 INHIBITION AND HIGH CARDIOVASCULAR RISK

PCSK9 Inhibition Despite Existing Lipid-Lowering Therapy

PCSK9 inhibition decreased LDL-C robustly in patients receiving statin therapy at maximally tolerated doses. These studies are consistent with the concept that both agents lower LDL-C by targeting the LDLR through different but complimentary mechanisms: statins via transcriptional

Table 1 Summary of meta-analyses on Phase II and III randomized clinical trials of PCSK9 inhibitors

Reference	Number of trials	Number of patients	Outcome metric[a]	*P* value
All-cause mortality				
Navarese et al. (102)	24	10,159	0.45 (0.23–0.86)	0.015
Lipinski et al. (105)	13	11,430	0.43 (0.22–0.82)	0.010
Cardiovascular mortality				
Navarese et al. (102)	24	10,159	0.49 (0.23–1.07)	NS (0.070)
Lipinski et al. (105)	12	11,340	0.50 (0.22–1.13)	NS (0.10)
Selected cardiovascular end points				
Navarese et al. (102)	10	5,195	Myocardial infarction 0.49 (0.26–0.93)	0.03
Navarese et al. (102)	6	3,894	Unstable angina pectoris 0.51 (0.05–4.86)	NS (0.56)
Lipinski et al. (105)	12	11,340	Major cardiovascular events 0.67 (0.43–1.04)	NS (0.07)
Selected adverse effects				
Navarese et al. (102)	24	10,159	Elevated serum creatine kinase 0.73 (0.55–0.97)	0.03
Navarese et al. (102)	24	10,159	All serious adverse events 1.01 (0.88–1.16)	NS (0.89)
Li et al. (103)	15	7,464	All serious adverse events 1.07 (0.86–1.34)	NS (0.89)
Lipinski et al. (105)	6	9,581	Adverse neurocognitive effects 2.34 (1.11–4.93)	0.02
Low-density lipoprotein cholesterol				
Navarese et al. (102)	24	10,159	−58.8% (−61.0% to −56.5%) versus placebo	<0.001
Lipinski et al. (105)	17	13,083	−59.7% (−60.5% to −58.6%) versus placebo	<0.001
Li et al. (103)	18	9,136	−65.3 mg/dL (−72.1 to −58.5 mg/dL) versus comparators	<0.00001
Total cholesterol				
Navarese et al. (102)	10	5,357	−39.8% (−40.7% to −37.4%) versus placebo	<0.001
Lipinski et al. (105)	17	13,083	−36.7% (−37.6% to −35.8%) versus placebo	<0.001
Triglycerides				
Li et al. (103)	16	8,696	−12.2 mg/dL (−16.2 to −8.22 mg/dL) versus comparators	<0.00001
High-density lipoprotein cholesterol				
Navarese et al. (102)	14	4,378	+6.14% (+5.31% to +6.97%) versus placebo	<0.001
Lipinski et al. (105)	17	13,083	+6.04% (+5.31% to +6.78%) versus placebo	<0.001
Li et al. (103)	17	9,003	+3.40 mg/dL (+3.12 to +3.68 mg/dL) versus comparators	<0.00001
Apolipoprotein B				
Li et al. (103)	17	9,003	−41.0% (−46.1% to −35.9%) versus comparators	<0.00001
Lipinski et al. (105)	17	13,083	−48.4% (−49.3% to −47.6%) versus placebo	<0.001
Apolipoprotein A1				
Li et al. (103)	13	5,724	+6.75% (+4.64% to +8.86%) versus comparators	<0.00001
Lipinski et al. (105)	17	13,083	+3.83% (+3.00% to +4.66%) versus placebo	<0.001
Lipoprotein (a)				
Navarese et al. (102)	12	6,566	−28.0% (−31.2% to −24.7%) versus comparators	<0.001
Lipinski et al. (105)	17	13,083	−24.3% (−28.4% to −19.7%) versus placebo	Significant
Li et al. (103)	16	8,696	−0.94 (−1.12 to −0.77) standardized mean change	<0.00001

Abbreviation: NS, nonsignificant.

[a]Outcome metric for discrete events is the odds ratio; for continuous traits, either the treatment-related relative change in percent or the treatment-related absolute change in mg/dL is shown.

upregulation and PCSK9 inhibitors through increased LDLR protein density. The completed studies with greatest impact were long-term extensions of the Open-Label Study of Long-Term Evaluation Against LDL-C (OSLER) trials of evolocumab (36) and ODYSSEY LONG TERM for alirocumab (35). Both studies showed LDL-C reductions from ~3.1 mmol/L in the comparator or control arms to ~1.2 mmol/L with active treatment of either evolocumab (150 mg biweekly for 52 weeks) or alirocumab (140 mg biweekly for 78 weeks) (35, 36). Both studies showed an additional 48–53% reduction of CVD events when added to statin therapy. Most adverse events occurred with similar frequency in the two groups; however, the rate of neurocognitive adverse events was higher with active treatment than with placebo. Based on these promising results, national regulators made these agents available for prescription for limited indications in late 2015, despite the absence of large RCTs powered for outcomes. For more details on all other individual Phase II and III studies, the reader is referred to several excellent reviews (108–112). Currently, a single published dose-ranging study with bococizumab has shown absolute LDL-C reductions of up to 1.36 mmol/L (113).

PCSK9 Inhibition in Familial Hypercholesterolemia

The molecular lesion in HeFH is usually within the gene encoding the LDLR, but because heterozygotes inherit one normal copy of the gene, PCSK9 inhibitors can delay degradation of LDLR from the normal allele and increase overall receptor-mediated endocytosis of LDL particles. Both evolocumab and alirocumab have been evaluated in numerous trials conducted specifically in patients with HeFH, namely RUTHERFORD I and II (evolocumab) (114, 115) and ODYSSEY FH (alirocumab) (116). In HeFH patients on statin therapy, alirocumab administered every 2 weeks at doses of 75 and 150 mg reduced LDL-C by 50% and 63%, respectively, from baseline, and more than 60% of patients reached rigorous LDL-C target levels, compared to almost no patients in the placebo groups (116). In statin-treated HeFH patients, evolocumab at doses of 140 mg every 2 weeks and 420 mg every 4 weeks reduced LDL-C by more than 60% from baseline, and most patients reached rigorous LDL-C target levels, compared to almost no placebo-treated patients (115). Evolocumab was also evaluated in patients with homozygous FH (i.e., individuals who inherited two poorly functioning *LDLR* genes) (117). Evolocumab reduced LDL-C by ~30% in a patient subgroup that had at least one mutant *LDLR* allele with residual functionality, whereas individuals with two null or completely nonfunctional alleles did not respond (117). Based on these findings, patients with HeFH who are not at their treatment goals represent a target demographic for both agents, whereas evolocumab is further indicated for very-difficult-to-treat homozygous FH patients with residual LDLR function.

PCSK9 Inhibition in Diabetes

There has been no RCT of PCSK9 inhibitors performed in patients with diabetes, but in Phase II and III trials published to date, ~24% of patients had diabetes [mainly type 2 diabetes (T2D)]; diabetic patients did not respond differently to either alirocumab or evolocumab compared to nondiabetic subjects (118). A recent meta-analysis of diabetic subgroups in evolocumab studies confirms similar atherogenic lipoprotein reductions in T2D patients compared to patients without T2D (119). Although dedicated trials enrolling only diabetic patients are under way, even before results are available, PCSK9 inhibitor therapy may be appropriate in diabetic patients, particularly if there is concurrent genetic hyperlipidemia, persistent high LDL-C despite maximal conventional lipid-lowering therapy with existing macrovascular disease, or very high risk factor burden.

PCSK9 Inhibition in Statin Intolerance

Statin intolerance is a challenging clinical state to both define and manage (120). The Goal Achievement after Utilizing an Anti-PCSK9 Antibody in Statin Intolerant Subjects (GAUSS)-2 trial found that evolocumab reduced LDL-C 53–56% from baseline, significantly greater than the 16–19% reductions achieved with ezetimibe ($P < 0.001$). Muscle-related adverse events occurred in 12% of evolocumab-treated patients and 23% of ezetimibe-treated patients (121). The ODYSSEY ALTERNATIVE study found that alirocumab reduced mean LDL-C by 45.0% versus 14.6% with ezetimibe (mean difference 30.4%) and was associated with significantly fewer skeletal muscle-related events (122). However, unless the patient also meets another indication such as FH or recurrent CVD with recalcitrant and elevated LDL-C, most regulators have shied away from statin intolerance as an indication for PCSK9 inhibitors because statin intolerance is so difficult to define and the size of this patient demographic is potentially enormous.

FUTURE DIRECTIONS AND CHALLENGES

Although PCSK9 inhibitors are currently available for prescription and are used as adjunctive treatments in some niche patient demographic groups, positive outcomes from the large-scale prospective RCTs, namely FOURIER for evolocumab (123), ODYSSEY LONG TERM for alirocumab (124), and SPIRE I and II for bococizumab (125), will be critical in determining the potential breadth of the target demographic and ultimate range of indications in preventive cardiology. Recent analyses suggest these agents may not be maximally cost-beneficial at current prices in the United States but might reflect better economic value at current prices in Canada and Europe (126). Also, the ability of PCSK9 inhibitors to induce regression of atherosclerotic plaques is currently being explored (127). In this context, a sequential approach may prove beneficial, with intense induction therapy using PCSK9 inhibition for the first year or two after the event, followed by maintenance therapy using only a statin thereafter (128). Finally, given the variety of non-LDLR-related targets and mechanisms that have emerged (as described above and in 11), it will be critical to maintain vigilance for possible off-target effects, including neurocognitive adverse events (105, 106) and increased susceptibility to infections (37, 129). Some very low-risk long-term adverse effects of statins, such as the slightly increased risk of T2D mellitus (130), were not apparent in individual short-term clinical trials and did not emerge until decades after statins had been in widespread clinical use. Thus, long-term pharmacovigilance protocols will be essential, especially with potential premature termination of outcomes studies if positive efficacy end points are achieved early.

CONCLUSIONS

In less than 15 years, PCSK9 has evolved from a genetic curiosity to a key regulator of cholesterol metabolism to a prime drug target for which commercially available treatments now exist. Interest extends beyond its fascinating biology: PCSK9 inhibition represents the first major development in lipid management in more than a decade. Furthermore, PCSK9 continues to provide novel scientific insights into lipoprotein metabolism and atherogenesis. If ongoing clinical outcome trials with PCSK9 inhibitors are positive, they will shift the treatment paradigm from one in which LDL-C is not even mentioned in certain clinical practice guidelines toward an assertive focus on LDL-C reduction for high-risk patients. Furthermore, the genetics-directed path to PCSK9 development exemplifies a new paradigm in pharmacology. Although PCSK9 inhibitors in current use have an excellent safety profile to date, vigilance for possible rare adverse effects is

necessary. Finally, the high cost of PCSK9 inhibition sometimes creates a barrier to accessibility that might be mitigated by increased competition in this treatment space.

DISCLOSURE STATEMENT

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LITERATURE CITED

1. Agarwal SK, Avery CL, Ballantyne CM, Catellier D, Nambi V, et al. 2011. Sources of variability in measurements of cardiac troponin T in a community-based sample: the Atherosclerosis Risk in Communities study. *Clin. Chem.* 57:891–97
2. Boekholdt SM, Arsenault BJ, Mora S, Pedersen TR, LaRosa JC, et al. 2012. Association of LDL cholesterol, non-HDL cholesterol, and apolipoprotein B levels with risk of cardiovascular events among patients treated with statins: a meta-analysis. *JAMA* 307:1302–9
3. Sniderman AD, Williams K, Contois JH, Monroe HM, McQueen MJ, et al. 2011. A meta-analysis of low-density lipoprotein cholesterol, non-high-density lipoprotein cholesterol, and apolipoprotein B as markers of cardiovascular risk. *Circ. Cardiovasc. Qual. Outcomes* 4:337–45
4. Genser B, Marz W. 2006. Low density lipoprotein cholesterol, statins and cardiovascular events: a meta-analysis. *Clin. Res. Cardiol.* 95:393–404
5. Avis HJ, Hutten BA, Gagne C, Langslet G, McCrindle BW, et al. 2010. Efficacy and safety of rosuvastatin therapy for children with familial hypercholesterolemia. *J. Am. Coll. Cardiol.* 55:1121–26
6. Cannon CP, Blazing MA, Giugliano RP, McCagg A, White JA, et al. 2015. Ezetimibe added to statin therapy after acute coronary syndromes. *N. Engl. J. Med.* 372:2387–97
7. Needham M, Mastaglia FL. 2014. Statin myotoxicity: a review of genetic susceptibility factors. *Neuromuscul. Disord.* 24:4–15
8. Abifadel M, Rabès JP, Devillers M, Munnich A, Erlich D, et al. 2009. Mutations and polymorphisms in the proprotein convertase subtilisin kexin 9 (*PCSK9*) gene in cholesterol metabolism and disease. *Hum. Mutat.* 30:520–29
9. Abifadel M, Varret M, Rabès JP, Allard D, Ouguerram K, et al. 2003. Mutations in *PCSK9* cause autosomal dominant hypercholesterolemia. *Nat. Genet.* 34:154–56
10. Cohen JC, Boerwinkle E, Mosley TH Jr., Hobbs HH. 2006. Sequence variations in *PCSK9*, low LDL, and protection against coronary heart disease. *N. Engl. J. Med.* 354:1264–72
11. Seidah NG, Awan Z, Chrétien M, Mbikay M. 2014. PCSK9: a key modulator of cardiovascular health. *Circ. Res.* 114:1022–36
12. Chan JC, Piper DE, Cao Q, Liu D, King C, et al. 2009. A proprotein convertase subtilisin/kexin type 9 neutralizing antibody reduces serum cholesterol in mice and nonhuman primates. *PNAS* 106:9820–25
13. Shimada YJ, Cannon CP. 2015. PCSK9 (proprotein convertase subtilisin/kexin type 9) inhibitors: past, present, and the future. *Eur. Heart. J.* 36:2415–24
14. Seidah NG, Prat A. 2012. The biology and therapeutic targeting of the proprotein convertases. *Nat. Rev. Drug Discov.* 11:367–83
15. Seidah NG, Benjannet S, Wickham L, Marcinkiewicz J, Jasmin SB, et al. 2003. The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): liver regeneration and neuronal differentiation. *PNAS* 100:928–33
16. Cunningham D, Danley DE, Geoghegan KF, Griffor MC, Hawkins JL, et al. 2007. Structural and biophysical studies of PCSK9 and its mutants linked to familial hypercholesterolemia. *Nat. Struct. Mol. Biol.* 14:413–19
17. Horton JD, Cohen JC, Hobbs HH. 2009. PCSK9: a convertase that coordinates LDL catabolism. *J. Lipid Res.* 50(Suppl.):S172–97

18. Benjannet S, Rhainds D, Essalmani R, Mayne J, Wickham L, et al. 2004. NARC-1/PCSK9 and its natural mutants: zymogen cleavage and effects on the low density lipoprotein (LDL) receptor and LDL cholesterol. *J. Biol. Chem.* 279:48865–75
19. Maxwell KN, Breslow JL. 2004. Adenoviral-mediated expression of Pcsk9 in mice results in a low-density lipoprotein receptor knockout phenotype. *PNAS* 101:7100–5
20. Park SW, Moon YA, Horton JD. 2004. Post-transcriptional regulation of low density lipoprotein receptor protein by proprotein convertase subtilisin/kexin type 9a in mouse liver. *J. Biol. Chem.* 279:50630–38
21. Brown MS, Goldstein JL. 1986. A receptor-mediated pathway for cholesterol homeostasis. *Science* 232:34–47
22. Holla OL, Cameron J, Berge KE, Ranheim T, Leren TP. 2007. Degradation of the LDL receptors by PCSK9 is not mediated by a secreted protein acted upon by PCSK9 extracellularly. *BMC Cell Biol.* 8:9
23. Poirier S, Mayer G, Poupon V, McPherson PS, Desjardins R, et al. 2009. Dissection of the endogenous cellular pathways of PCSK9-induced low density lipoprotein receptor degradation: evidence for an intracellular route. *J. Biol. Chem.* 284:28856–64
24. Poupon V, Girard M, Legendre-Guillemin V, Thomas S, Bourbonniere L, et al. 2008. Clathrin light chains function in mannose phosphate receptor trafficking via regulation of actin assembly. *PNAS* 105:168–73
25. Saavedra YG, Day R, Seidah NG. 2012. The M2 module of the Cys-His-rich domain (CHRD) of PCSK9 protein is needed for the extracellular low-density lipoprotein receptor (LDLR) degradation pathway. *J. Biol. Chem.* 287:43492–501
26. Kosenko T, Golder M, Leblond G, Weng W, Lagace TA. 2013. Low density lipoprotein binds to proprotein convertase subtilisin/kexin type-9 (PCSK9) in human plasma and inhibits PCSK9-mediated low density lipoprotein receptor degradation. *J. Biol. Chem.* 288:8279–88
27. Poirier S, Mayer G, Benjannet S, Bergeron E, Marcinkiewicz J, et al. 2008. The proprotein convertase PCSK9 induces the degradation of low density lipoprotein receptor (LDLR) and its closest family members VLDLR and ApoER2. *J. Biol. Chem.* 283:2363–72
28. Shan L, Pang L, Zhang R, Murgolo NJ, Lan H, Hedrick JA. 2008. PCSK9 binds to multiple receptors and can be functionally inhibited by an EGF-A peptide. *Biochem. Biophys. Res. Commun.* 375:69–73
29. Canuel M, Sun X, Asselin MC, Paramithiotis E, Prat A, Seidah NG. 2013. Proprotein convertase subtilisin/kexin type 9 (PCSK9) can mediate degradation of the low density lipoprotein receptor-related protein 1 (LRP-1). *PLOS ONE* 8:e64145
30. Roubtsova A, Munkonda MN, Awan Z, Marcinkiewicz J, Chamberland A, et al. 2011. Circulating proprotein convertase subtilisin/kexin 9 (PCSK9) regulates VLDLR protein and triglyceride accumulation in visceral adipose tissue. *Arterioscler. Thromb. Vasc. Biol.* 31:785–91
31. Zhao Z, Tuakli-Wosornu Y, Lagace TA, Kinch L, Grishin NV, et al. 2006. Molecular characterization of loss-of-function mutations in *PCSK9* and identification of a compound heterozygote. *Am. J. Hum. Genet.* 79:514–23
32. Bonnefond A, Yengo L, Le May C, Fumeron F, Marre M, et al. 2015. The loss-of-function *PCSK9* p.R46L genetic variant does not alter glucose homeostasis. *Diabetologia* 58:2051–55
33. Demers A, Samami S, Lauzier B, Des Rosiers C, Sock ET, et al. 2015. PCSK9 induces CD36 degradation and affects long-chain fatty acid uptake and triglyceride metabolism in adipocytes and in mouse liver. *Arterioscler. Thromb. Vasc. Biol.* 35:2517–25
34. Zaid A, Roubtsova A, Essalmani R, Marcinkiewicz J, Chamberland A, et al. 2008. Proprotein convertase subtilisin/kexin type 9 (PCSK9): hepatocyte-specific low-density lipoprotein receptor degradation and critical role in mouse liver regeneration. *Hepatology* 48:646–54
35. Robinson JG, Farnier M, Krempf M, Bergeron J, Luc G, et al. 2015. Efficacy and safety of alirocumab in reducing lipids and cardiovascular events. *N. Engl. J. Med.* 372:1489–99
36. Sabatine MS, Giugliano RP, Wiviott SD, Raal FJ, Blom DJ, et al. 2015. Efficacy and safety of evolocumab in reducing lipids and cardiovascular events. *N. Engl. J. Med.* 372:1500–9
37. Labonté P, Begley S, Guévin C, Asselin MC, Nassoury N, et al. 2009. PCSK9 impedes hepatitis C virus infection in vitro and modulates liver CD81 expression. *Hepatology* 50:17–24

38. Dubuc G, Chamberland A, Wassef H, Davignon J, Seidah NG, et al. 2004. Statins upregulate *PCSK9*, the gene encoding the proprotein convertase neural apoptosis-regulated convertase-1 implicated in familial hypercholesterolemia. *Arterioscler. Thromb. Vasc. Biol.* 24:1454–59
39. Jeong HJ, Lee HS, Kim KS, Kim YK, Yoon D, Park SW. 2008. Sterol-dependent regulation of proprotein convertase subtilisin/kexin type 9 expression by sterol-regulatory element binding protein-2. *J. Lipid Res.* 49:399–409
40. Horton JD, Goldstein JL, Brown MS. 2002. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J. Clin. Investig.* 109:1125–31
41. Browning JD, Horton JD. 2010. Fasting reduces plasma proprotein convertase, subtilisin/kexin type 9 and cholesterol biosynthesis in humans. *J. Lipid Res.* 51:3359–63
42. Persson L, Cao G, Stahle L, Sjoberg BG, Troutt JS, et al. 2010. Circulating proprotein convertase subtilisin kexin type 9 has a diurnal rhythm synchronous with cholesterol synthesis and is reduced by fasting in humans. *Arterioscler. Thromb. Vasc. Biol.* 30:2666–72
43. Horton JD, Cohen JC, Hobbs HH. 2007. Molecular biology of PCSK9: its role in LDL metabolism. *Trends Biochem. Sci.* 32:71–77
44. Lagace TA. 2014. PCSK9 and LDLR degradation: regulatory mechanisms in circulation and in cells. *Curr. Opin. Lipidol.* 25:387–93
45. Lambert G, Jarnoux AL, Pineau T, Pape O, Chetiveaux M, et al. 2006. Fasting induces hyperlipidemia in mice overexpressing proprotein convertase subtilisin kexin type 9: lack of modulation of very-low-density lipoprotein hepatic output by the low-density lipoprotein receptor. *Endocrinology* 147:4985–95
46. Sun H, Samarghandi A, Zhang N, Yao Z, Xiong M, Teng BB. 2012. Proprotein convertase subtilisin/kexin type 9 interacts with apolipoprotein B and prevents its intracellular degradation, irrespective of the low-density lipoprotein receptor. *Arterioscler. Thromb. Vasc. Biol.* 32:1585–95
47. Li H, Dong B, Park SW, Lee HS, Chen W, Liu J. 2009. Hepatocyte nuclear factor 1α plays a critical role in *PCSK9* gene transcription and regulation by the natural hypocholesterolemic compound berberine. *J. Biol. Chem.* 284:28885–95
48. Dong B, Wu M, Li H, Kraemer FB, Adeli K, et al. 2010. Strong induction of PCSK9 gene expression through HNF1α and SREBP2: mechanism for the resistance to LDL-cholesterol lowering effect of statins in dyslipidemic hamsters. *J. Lipid Res.* 51:1486–95
49. Ai D, Chen C, Han S, Ganda A, Murphy AJ, et al. 2012. Regulation of hepatic LDL receptors by mTORC1 and PCSK9 in mice. *J. Clin. Investig.* 122:1262–70
50. Hussain Y, Ding Q, Connelly PW, Brunt JH, Ban MR, et al. 2015. G-protein estrogen receptor as a regulator of low-density lipoprotein cholesterol metabolism: cellular and population genetic studies. *Arterioscler. Thromb. Vasc. Biol.* 35:213–21
51. Persson L, Henriksson P, Westerlund E, Hovatta O, Angelin B, Rudling M. 2012. Endogenous estrogens lower plasma PCSK9 and LDL cholesterol but not Lp(a) or bile acid synthesis in women. *Arterioscler. Thromb. Vasc. Biol.* 32:810–14
52. Lagace TA, Curtis DE, Garuti R, McNutt MC, Park SW, et al. 2006. Secreted PCSK9 decreases the number of LDL receptors in hepatocytes and in livers of parabiotic mice. *J. Clin. Investig.* 116:2995–3005
53. Denis M, Marcinkiewicz J, Zaid A, Gauthier D, Poirier S, et al. 2012. Gene inactivation of proprotein convertase subtilisin/kexin type 9 reduces atherosclerosis in mice. *Circulation* 125:894–901
54. Al-Mashhadi RH, Sørensen CB, Kragh PM, Christoffersen C, Mortensen MB, et al. 2013. Familial hypercholesterolemia and atherosclerosis in cloned minipigs created by DNA transposition of a human *PCSK9* gain-of-function mutant. *Sci. Transl. Med.* 5:166ra1
55. Rashid S, Curtis DE, Garuti R, Anderson NN, Bashmakov Y, et al. 2005. Decreased plasma cholesterol and hypersensitivity to statins in mice lacking *Pcsk9*. *PNAS* 102:5374–79
56. Ason B, van der Hoorn JWA, Chan J, Lee E, Pieterman EJ, et al. 2014. PCSK9 inhibition fails to alter hepatic LDLR, circulating cholesterol, and atherosclerosis in the absence of ApoE. *J. Lipid Res.* 55:2370–79
57. Seidah NG, Poirier S, Denis M, Parker R, Miao B, et al. 2012. Annexin A2 is a natural extrahepatic inhibitor of the PCSK9-induced LDL receptor degradation. *PLOS ONE* 7:e41865

58. Le May C, Berger JM, Lespine A, Pillot B, Prieur X, et al. 2013. Transintestinal cholesterol excretion is an active metabolic process modulated by PCSK9 and statin involving ABCB1. *Arterioscler. Thromb. Vasc. Biol.* 33:1484–93
59. Mbikay M, Sirois F, Mayne J, Wang GS, Chen A, et al. 2010. PCSK9-deficient mice exhibit impaired glucose tolerance and pancreatic islet abnormalities. *FEBS Lett.* 584:701–6
60. Baass A, Dubuc G, Tremblay M, Delvin EE, O'Loughlin J, et al. 2009. Plasma PCSK9 is associated with age, sex, and multiple metabolic markers in a population-based sample of children and adolescents. *Clin. Chem.* 55:1637–45
61. Lakoski SG, Lagace TA, Cohen JC, Horton JD, Hobbs HH. 2009. Genetic and metabolic determinants of plasma PCSK9 levels. *J. Clin. Endocrinol. Metab.* 94:2537–43
62. Richard C, Couture P, Desroches S, Benjannet S, Seidah NG, et al. 2012. Effect of the Mediterranean diet with and without weight loss on surrogate markers of cholesterol homeostasis in men with the metabolic syndrome. *Br. J. Nutr.* 107:705–11
63. Costet P, Cariou B, Lambert G, Lalanne F, Lardeux B, et al. 2006. Hepatic PCSK9 expression is regulated by nutritional status via insulin and sterol regulatory element-binding protein 1c. *J. Biol. Chem.* 281:6211–18
64. Berge KE, Ose L, Leren TP. 2006. Missense mutations in the PCSK9 gene are associated with hypocholesterolemia and possibly increased response to statin therapy. *Arterioscler. Thromb. Vasc. Biol.* 26:1094–100
65. Berthold HK, Seidah NG, Benjannet S, Gouni-Berthold I. 2013. Evidence from a randomized trial that simvastatin, but not ezetimibe, upregulates circulating PCSK9 levels. *PLOS ONE* 8:e60095
66. Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, et al. 2013. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. *Eur. Heart. J.* 34:3478–90
67. Varret M, Rabès JP, Saint-Jore B, Cenarro A, Marinoni JC, et al. 1999. A third major locus for autosomal dominant hypercholesterolemia maps to 1p34.1-p32. *Am. J. Hum. Genet.* 64:1378–87
68. Hunt SC, Hopkins PN, Bulka K, McDermott MT, Thorne TL, et al. 2000. Genetic localization to chromosome 1p32 of the third locus for familial hypercholesterolemia in a Utah kindred. *Arterioscler. Thromb. Vasc. Biol.* 20:1089–93
69. Cohen J, Pertsemlidis A, Kotowski IK, Graham R, Garcia CK, Hobbs HH. 2005. Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in *PCSK9*. *Nat. Genet.* 37:161–65
70. Kotowski IK, Pertsemlidis A, Luke A, Cooper RS, Vega GL, et al. 2006. A spectrum of PCSK9 alleles contributes to plasma levels of low-density lipoprotein cholesterol. *Am. J. Hum. Genet.* 78:410–22
71. Johansen CT, Hegele RA. 2013. Using Mendelian randomization to determine causative factors in cardiovascular disease. *J. Intern. Med.* 273:44–47
72. Stender S, Tybjærg-Hansen A. 2016. Using human genetics to predict the effects and side-effects of drugs. *Curr. Opin. Lipidol.* 27:105–11
73. Abifadel M, Elbitar S, El Khoury P, Ghaleb Y, Chémaly M, et al. 2014. Living the *PCSK9* adventure: from the identification of a new gene in familial hypercholesterolemia towards a potential new class of anticholesterol drugs. *Curr. Atheroscler. Rep.* 16:439
74. Mayne J, Dewpura T, Raymond A, Bernier L, Cousins M, et al. 2011. Novel loss-of-function PCSK9 variant is associated with low plasma LDL cholesterol in a French-Canadian family and with impaired processing and secretion in cell culture. *Clin. Chem.* 57:1415–23
75. Benn M, Nordestgaard BG, Grande P, Schnohr P, Tybjærg-Hansen A. 2010. *PCSK9* R46L, low-density lipoprotein cholesterol levels, and risk of ischemic heart disease: 3 independent studies and meta-analyses. *J. Am. Coll. Cardiol.* 55:2833–42
76. Naoumova RP, Tosi I, Patel D, Neuwirth C, Horswell SD, et al. 2005. Severe hypercholesterolemia in four British families with the D374Y mutation in the PCSK9 gene: long-term follow-up and treatment response. *Arterioscler. Thromb. Vasc. Biol.* 25:2654–60

77. Hopkins PN, Defesche J, Fouchier SW, Bruckert E, Luc G, et al. 2015. Characterization of autosomal dominant hypercholesterolemia caused by *PCSK9* gain of function mutations and its specific treatment with alirocumab, a PCSK9 monoclonal antibody. *Circ. Cardiovasc. Genet.* 8:823–31
78. Alves AC, Etxebarria A, Medeiros AM, Benito-Vicente A, Thedrez A, et al. 2015. Characterization of the first *PCSK9* gain of function homozygote. *J. Am. Coll. Cardiol.* 66:2152–54
79. Hooper AJ, Marais AD, Tanyanyiwa DM, Burnett JR. 2007. The C679X mutation in *PCSK9* is present and lowers blood cholesterol in a Southern African population. *Atherosclerosis* 193:445–48
80. Kühnast S, van der Hoorn JWA, Pieterman EJ, van den Hoek AM, Sasiela WJ, et al. 2014. Alirocumab inhibits atherosclerosis, improves the plaque morphology, and enhances the effects of a statin. *J. Lipid Res.* 55:2103–12
81. Ni YG, Di Marco S, Condra JH, Peterson LB, Wang W, et al. 2011. A PCSK9-binding antibody that structurally mimics the EGF(A) domain of LDL-receptor reduces LDL cholesterol in vivo. *J. Lipid Res.* 52:78–86
82. Zhang L, McCabe T, Condra JH, Ni YG, Peterson LB, et al. 2012. An anti-PCSK9 antibody reduces LDL-cholesterol on top of a statin and suppresses hepatocyte SREBP-regulated genes. *Int. J. Biol. Sci.* 8:310–27
83. Lipovsek D. 2011. Adnectins: engineered target-binding protein therapeutics. *Protein Eng. Des. Sel.* 24:3–9
84. Mitchell T, Chao G, Sitkoff D, Lo F, Monshizadegan H, et al. 2014. Pharmacologic profile of the Adnectin BMS-962476, a small protein biologic alternative to PCSK9 antibodies for low-density lipoprotein lowering. *J. Pharmacol. Exp. Ther.* 350:412–24
85. Stein EA, Kasichayanula S, Turner T, Kranz T, Arumugam U, et al. 2014. LDL cholesterol reduction with BMS-962476, an adnectin inhibitor of PCSK9: results of a single ascending dose study. *J. Am. Coll. Cardiol.* 63:A1372
86. Frank-Kamenetsky M, Grefhorst A, Anderson NN, Racie TS, Bramlage B, et al. 2008. Therapeutic RNAi targeting PCSK9 acutely lowers plasma cholesterol in rodents and LDL cholesterol in nonhuman primates. *PNAS* 105:11915–20
87. Lee HS, Seok H, Lee DH, Ham J, Lee W, et al. 2015. Abasic pivot substitution harnesses target specificity of RNA interference. *Nat. Commun.* 6:10154
88. Fitzgerald K, Frank-Kamenetsky M, Shulga-Morskaya S, Liebow A, Bettencourt BR, et al. 2014. Effect of an RNA interference drug on the synthesis of proprotein convertase subtilisin/kexin type 9 (PCSK9) and the concentration of serum LDL cholesterol in healthy volunteers: a randomised, single-blind, placebo-controlled, Phase 1 trial. *Lancet* 383:60–68
89. Graham MJ, Lemonidis KM, Whipple CP, Subramaniam A, Monia BP, et al. 2007. Antisense inhibition of proprotein convertase subtilisin/kexin type 9 reduces serum LDL in hyperlipidemic mice. *J. Lipid Res.* 48:763–67
90. Gupta N, Fisker N, Asselin MC, Lindholm M, Rosenbohm C, et al. 2010. A locked nucleic acid antisense oligonucleotide (LNA) silences PCSK9 and enhances LDLR expression in vitro and in vivo. *PLOS ONE* 5:e10682
91. Lindholm MW, Elmen J, Fisker N, Hansen HF, Persson R, et al. 2012. PCSK9 LNA antisense oligonucleotides induce sustained reduction of LDL cholesterol in nonhuman primates. *Mol. Ther.* 20:376–81
92. McNutt MC, Kwon HJ, Chen C, Chen JR, Horton JD, Lagace TA. 2009. Antagonism of secreted PCSK9 increases low density lipoprotein receptor expression in HepG2 cells. *J. Biol. Chem.* 284:10561–70
93. Alghamdi RH, O'Reilly P, Lu C, Gomes J, Lagace TA, Basak A. 2015. LDL-R promoting activity of peptides derived from human PCSK9 catalytic domain (153–421): design, synthesis and biochemical evaluation. *Eur. J. Med. Chem.* 92:890–907
94. Du F, Hui Y, Zhang M, Linton MF, Fazio S, Fan D. 2011. Novel domain interaction regulates secretion of proprotein convertase subtilisin/kexin type 9 (PCSK9) protein. *J. Biol. Chem.* 286:43054–61
95. Zhang Y, Eigenbrot C, Zhou L, Shia S, Li W, et al. 2014. Identification of a small peptide that inhibits PCSK9 protein binding to the low density lipoprotein receptor. *J. Biol. Chem.* 289:942–55
96. Schroeder CI, Swedberg JE, Withka JM, Rosengren KJ, Akcan M, et al. 2014. Design and synthesis of truncated EGF-A peptides that restore LDL-R recycling in the presence of PCSK9 in vitro. *Chem. Biol.* 21:284–94

97. Lo Surdo P, Bottomley MJ, Calzetta A, Settembre EC, Cirillo A, et al. 2011. Mechanistic implications for LDL receptor degradation from the PCSK9/LDLR structure at neutral pH. *EMBO Rep.* 12:1300–5
98. Stein EA, Mellis S, Yancopoulos GD, Stahl N, Logan D, et al. 2012. Effect of a monoclonal antibody to PCSK9 on LDL cholesterol. *N. Engl. J. Med.* 366:1108–18
99. Koren MJ, Kereiakes D, Pourfarzib R, Winegar D, Banerjee P, et al. 2015. Effect of PCSK9 inhibition by alirocumab on lipoprotein particle concentrations determined by nuclear magnetic resonance spectroscopy. *J. Am. Heart. Assoc.* 4:e002224
100. McKenney JM, Koren MJ, Kereiakes DJ, Hanotin C, Ferrand AC, Stein EA. 2012. Safety and efficacy of a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 serine protease, SAR236553/REGN727, in patients with primary hypercholesterolemia receiving ongoing stable atorvastatin therapy. *J. Am. Coll. Cardiol.* 59:2344–53
101. Dias CS, Shaywitz AJ, Wasserman SM, Smith BP, Gao B, et al. 2012. Effects of AMG 145 on low-density lipoprotein cholesterol levels: results from 2 randomized, double-blind, placebo-controlled, ascending-dose phase 1 studies in healthy volunteers and hypercholesterolemic subjects on statins. *J. Am. Coll. Cardiol.* 60:1888–98
102. Navarese EP, Kolodziejczak M, Schulze V, Gurbel PA, Tantry U, et al. 2015. Effects of proprotein convertase subtilisin/kexin type 9 antibodies in adults with hypercholesterolemia: a systematic review and meta-analysis. *Ann. Intern. Med.* 163:40–51
103. Li C, Lin L, Zhang W, Zhou L, Wang H, et al. 2015. Efficiency and safety of proprotein convertase subtilisin/kexin 9 monoclonal antibody on hypercholesterolemia: a meta-analysis of 20 randomized controlled trials. *J. Am. Heart. Assoc.* 4:e001937
104. Zhang XL, Zhu QQ, Zhu L, Chen JZ, Chen QH, et al. 2015. Safety and efficacy of anti-PCSK9 antibodies: a meta-analysis of 25 randomized, controlled trials. *BMC Med.* 13:123
105. Lipinski MJ, Benedetto U, Escarcega RO, Biondi-Zoccai G, Lhermusier T, et al. 2016. The impact of proprotein convertase subtilisin-kexin type 9 serine protease inhibitors on lipid levels and outcomes in patients with primary hypercholesterolaemia: a network meta-analysis. *Eur. Heart. J.* 37:536–45
106. Kolodziejczak M, Navarese EP. 2016. Role of PCSK9 antibodies in cardiovascular disease: critical considerations of mortality and neurocognitive findings from the current literature. *Atherosclerosis* 247:189–92
107. Sahebkar A, Di Giosia P, Stamerra CA, Grassi D, Pedone C, et al. 2016. Effect of monoclonal antibodies to PCSK9 on high-sensitivity C-reactive protein levels: a meta-analysis of 16 randomized controlled treatment arms. *Br. J. Clin. Pharmacol.* 81:1175–90
108. Desai NR, Sabatine MS. 2015. PCSK9 inhibition in patients with hypercholesterolemia. *Trends Cardiovasc. Med.* 25:567–74
109. Giugliano RP, Sabatine MS. 2015. Are PCSK9 inhibitors the next breakthrough in the cardiovascular field? *J. Am. Coll. Cardiol.* 65:2638–51
110. Bergeron N, Phan BA, Ding Y, Fong A, Krauss RM. 2015. Proprotein convertase subtilisin/kexin type 9 inhibition: a new therapeutic mechanism for reducing cardiovascular disease risk. *Circulation* 132:1648–66
111. Farnier M. 2015. An evaluation of alirocumab for the treatment of hypercholesterolemia. *Expert Rev. Cardiovasc. Ther.* 13:1307–23
112. Roth EM. 2016. Alirocumab for hyperlipidemia: ODYSSEY Phase III clinical trial results and US FDA approval indications. *Future Cardiol.* 12:115–28
113. Ballantyne CM, Neutel J, Cropp A, Duggan W, Wang EQ, et al. 2015. Results of bococizumab, a monoclonal antibody against proprotein convertase subtilisin/kexin type 9, from a randomized, placebo-controlled, dose-ranging study in statin-treated subjects with hypercholesterolemia. *Am. J. Cardiol.* 115:1212–21
114. Raal F, Scott R, Somaratne R, Bridges I, Li G, et al. 2012. Low-density lipoprotein cholesterol-lowering effects of AMG 145, a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 serine protease in patients with heterozygous familial hypercholesterolemia: the Reduction of LDL-C with PCSK9 Inhibition in Heterozygous Familial Hypercholesterolemia Disorder (RUTHERFORD) randomized trial. *Circulation* 126:2408–17
115. Raal FJ, Stein EA, Dufour R, Turner T, Civeira F, et al. 2015. PCSK9 inhibition with evolocumab (AMG 145) in heterozygous familial hypercholesterolaemia (RUTHERFORD-2): a randomised, double-blind, placebo-controlled trial. *Lancet* 385:331–40

116. Kastelein JJ, Ginsberg HN, Langslet G, Hovingh GK, Ceska R, et al. 2015. ODYSSEY FH I and FH II: 78 week results with alirocumab treatment in 735 patients with heterozygous familial hypercholesterolaemia. *Eur. Heart. J.* 36:2996–3003
117. Raal FJ, Honarpour N, Blom DJ, Hovingh GK, Xu F, et al. 2015. Inhibition of PCSK9 with evolocumab in homozygous familial hypercholesterolaemia (TESLA Part B): a randomised, double-blind, placebo-controlled trial. *Lancet* 385:341–50
118. Hoe E, Hegele RA. 2015. Lipid management in diabetes with a focus on emerging therapies. *Can. J. Diabetes* 39:S183–90
119. Sattar N, Preiss D, Robinson JG, Djedjos CS, Elliott M, et al. 2016. Lipid-lowering efficacy of the PCSK9 inhibitor evolocumab (AMG 145) in patients with type 2 diabetes: a meta-analysis of individual patient data. *Lancet Diabetes Endocrinol*. 4:403–10
120. Mancini GB, Tashakkor AY, Baker S, Bergeron J, Fitchett D, et al. 2013. Diagnosis, prevention, and management of statin adverse effects and intolerance: Canadian Working Group Consensus update. *Can. J. Cardiol.* 29:1553–68
121. Stroes E, Colquhoun D, Sullivan D, Civeira F, Rosenson RS, et al. 2014. Anti-PCSK9 antibody effectively lowers cholesterol in patients with statin intolerance: the GAUSS-2 randomized, placebo-controlled Phase 3 clinical trial of evolocumab. *J. Am. Coll. Cardiol.* 63:2541–48
122. Moriarty PM, Thompson PD, Cannon CP, Guyton JR, Bergeron J, et al. 2015. Efficacy and safety of alirocumab versus ezetimibe in statin-intolerant patients, with a statin rechallenge arm: the ODYSSEY ALTERNATIVE randomized trial. *J. Clin. Lipidol.* 9:758–69
123. Sabatine MS, Giugliano RP, Keech A, Honarpour N, Wang H, et al. 2016. Rationale and design of the further cardiovascular outcomes research with PCSK9 inhibition in subjects with elevated risk trial. *Am. Heart J.* 173:94–101
124. Schwartz GG, Bessac L, Berdan LG, Bhatt DL, Bittner V, et al. 2014. Effect of alirocumab, a monoclonal antibody to PCSK9, on long-term cardiovascular outcomes following acute coronary syndromes: rationale and design of the ODYSSEY outcomes trial. *Am. Heart J.* 168:682–89
125. Lepor NE, Kereiakes DJ. 2015. The PCSK9 inhibitors: a novel therapeutic target enters clinical practice. *Am. Health Drug Benefits* 8:483–89
126. Tice JA, Kazi DS, Pearson SD. 2016. Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors for treatment of high cholesterol levels: effectiveness and value. *JAMA Intern. Med.* 176:107–8
127. Puri R, Nissen SE, Somaratne R, Cho L, Kastelein JJP, et al. 2016. Impact of PCSK9 inhibition on coronary atheroma progression: rationale and design of GLAGOV (Global Assessment of Plaque Regression with a PCSK9 Antibody as Measured by Intravascular Ultrasound). *Am. Heart J.* 176:83–92
128. Robinson JG, Heistad DD, Fox KA. 2015. Atherosclerosis stabilization with PCSK-9 inhibition: an evolving concept for cardiovascular prevention. *Atherosclerosis* 243:593–97
129. Syed GH, Tang H, Khan M, Hassanein T, Liu J, Siddiqui A. 2014. Hepatitis C virus stimulates low-density lipoprotein receptor expression to facilitate viral propagation. *J. Virol.* 88:2519–29
130. Preiss D, Seshasai SR, Welsh P, Murphy SA, Ho JE, et al. 2011. Risk of incident diabetes with intensive-dose compared with moderate-dose statin therapy: a meta-analysis. *JAMA* 305:2556–64