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Changes in circulating pro-protein convertase subtilisin/kexin type 9 levels – experimental and clinical approaches with lipid-lowering agents

C Macchi1, M Banach2,3,4, A Corsini1,5, CR Sirtori6, N Ferri7 and M Ruscica1

Abstract
Regulation of pro-protein convertase subtilisin/kexin type 9 (PCSK9) by drugs has led to the development of a still small number of agents with powerful activity on low-density lipoprotein cholesterol levels, associated with a significant reduction of cardiovascular events in patients in secondary prevention. The Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk (FOURIER) and Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab (ODYSSEY OUTCOMES) studies, with the two available PCSK9 antagonists, i.e. evolocumab and alirocumab, both reported a 15% reduction in major adverse cardiovascular events. Regulation of PCSK9 expression is dependent upon a number of factors, partly genetic and partly associated to a complex transcriptional system, mainly controlled by sterol regulatory element binding proteins. PCSK9 is further regulated by concomitant drug treatments, particularly by statins, enhancing PCSK9 secretion but decreasing its stimulatory phosphorylated form (S688). These complex transcriptional mechanisms lead to variable circulating levels making clinical measurements of plasma PCSK9 for cardiovascular risk assessment a debated matter. Determination of total PCSK9 levels may provide a diagnostic tool for explaining an apparent resistance to PCSK9 inhibitors, thus indicating the need for other approaches. Newer agents targeting PCSK9 are in clinical development with a major interest in those with a longer duration of action, e.g. RNA silencing, allowing optimal patient compliance. Interest has been expanded to areas not only limited to low-density lipoprotein cholesterol reduction but also investigating other non-lipid pathways raising cardiovascular risk, in particular inflammation associated to raised high-sensitivity C-reactive protein levels, not significantly affected by the present PCSK9 antagonists.

Keywords
Alirocumab, evolocumab, inclisiran, pro-protein convertase subtilisin/kexin type 9 circulating levels, pro-protein convertase subtilisin/kexin type 9

Introduction
The reduction of low-density lipoprotein cholesterol (LDL-C) has been an extensively evaluated pharmacological target. LDL-C is the modifiable risk factor associated with cardiovascular (CV) risk that has been thoroughly investigated in the last decades, with an outstanding number of therapeutic options.1 Analysis of large primary and secondary prevention patients has generally indicated that a 38.6 mg/dl LDL-C reduction is associated with, roughly, a 22% lowering of CV risk.2 This benefit is consistent also in

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patients starting with LDL-C levels as low as a median of 63 mg/dl and achieving levels as low as a median of 21 mg/dl. The relevance of LDL-C in the initiation and progression of atherosclerosis is also based on ultrasound studies, e.g. those evaluating carotid intima media thickness as a non-invasive tool to assess CV risk; indeed, the rate of atherosclerotic plaque progression is directly linked to the absolute LDL-C level. Very recently, a person’s total atherosclerotic plaque burden has been associated with his/her cumulative LDL exposure evaluated in mg-years by means of the following formula: age × LDL-C, measured in mg/dl.

On this background of epidemiological and pharmacological findings, new approaches have been investigated in order to further reduce LDL-cholesterolaemia and CV risk. In 2006, the Atherosclerosis Risk in Communities (ARIC) epidemiological study demonstrated that ‘loss of function’ mutations of pro-protein convertase subtilisin/kexin type 9 (PCSK9) were associated with lower levels of LDL-C (−28%, −37 mg/dl) and an astonishing 88% risk reduction in myocardial infarction (MI), fatal coronary heart diseases (CHDs) or coronary revascularization, despite a significant prevalence of other non-lipid-related CV-risk factors.

More importantly, a genome-wide association study led to the identification of nine CHD/MI susceptibility loci including PCSK9. This finding, together with the discovery in 2003 of a missense ‘gain of function’ mutation in PCSK9, directly linked to LDL-C, led to the identification of PCSK9 as a new pharmacological target and paved the way for the development of a number of PCSK9 antagonists, e.g. monoclonal antibodies (mAbs) and small interfering RNA (siRNA).

Beyond cholesterol metabolism, a direct role of PCSK9 in atherosclerotic plaque formation has been also identified. The Atherosclerosis Risk in Communities study showed that higher serum PCSK9 levels were linearly associated with a higher necrotic core fraction in coronary atherosclerosis, regardless of serum LDL-C, confirming data from our group in PCSK9 knock-out (KO) mice partially protected from neointimal formation. A significant association between PCSK9 levels and carotid intima–media thickening as well as with arterial stiffness and liver fat accumulation was also shown. PCSK9 expression has been described in different cell types involved in atherosclerosis, e.g. endothelial and smooth muscle cells and macrophages. Circulating platelets were added to the list of PCSK9 targets, together with the description of pro-inflammatory effects on macrophages and the involvement in aortic valve calcification.

The clinical relevance of measuring circulating PCSK9 plasma levels may be hindered by the complexity of PCSK9 biology at the transcriptional and translational levels, leading to concentrations varying over approximately a 100-fold range. Indeed, PCSK9 expression and plasma levels are tightly regulated by nutritional and hormonal status, e.g. hepatic glucagon receptor signaling as well as by a diurnal rhythm. Thus, in order to assess whether PCSK9 levels may contribute to risk prediction, even in patients already on preventive pharmacological therapy, i.e. on statins, it is of major interest to better understand whether drugs affecting lipid metabolism modulate PCSK9 gene expression and protein levels.

The present review article is aimed at covering major biological and clinical aspects of PCSK9 activity and antagonism. Discussion of studies investigating the mechanistic aspects of PCSK9 activity will be followed by detailed evaluation of the impact of lipid-lowering drugs and nutraceuticals on PCSK9 regulation. These different aspects of the present role of PCSK9 in system biology and in atherosclerosis induction/prevention will thus allow a potential forecast of events leading to more effective drug utilization in CV disease prevention, as well indications of possible new approaches to this therapeutic area.

**The biology**

PCSK9 is the ninth member and last-discovered protein belonging to the family of mammalian proprotein convertases (PCs), i.e. endoprotease enzymes. The first seven proteins (PCSK1–PCSK7) are structurally and biochemically similar to each other and to bacterial and yeast proteins - subtilisin and kexin, respectively - from which they probably derive. A further classification is based on the predilection for the substrate cleavage motif which leads to precursor proteolysis; the first seven PCSKs cleave precursors at basic residues, whereas subtilisin kexin isozyme 1/site 1 protease (SKI-1/S1P), the eighth member, does not require basic residues for cleavage and finally PCSK9 cleaves itself at its internal VFAQ sequence.

PCSK9 acts as a protein binding to specific cell surface receptors. PCSK9 possesses the unique feature of being unable to get rid of its prodomain, thus leading to a secreted inactive protease. Produced in the endoplasmic reticulum (ER), PCs navigate through the secretory pathway to their final destination, which is one of the Golgi, the trans-Golgi network, secretory granules, endosomes, the plasma membrane, the extracellular matrix and the cell surface.

Similar to other members of the proprotein convertase family, PCSK9 shares a N-terminal prodomain, a subtilisin-like catalytic domain and a cysteine- and histidine-rich domain. Once in the ER, the signal peptide is cleaved and the zymogen proPCSK9 (aa 31–692, about 74 kDa) undergoes an autocatalytic cleavage of the prodomain (aa 31–152 at VFAQ152 sequence).
this last remains firmly attached to its catalytic pocket by strong noncovalent interactions, thereby preventing any trans-catalytic activity of the enzyme. This phenomenon is a prerequisite for the release of the mature PCSK9 (aa 153–692, about 62 kDa) from the ER to the Golgi, likely because the prodomain serves as a chaperone (Figure 1). Loss-of-function mutations in the prosegment can result in lower PCSK9 circulating levels due to impaired autocalytic processing and secretion. A similar phenotype has been found when misfolded precursors in the ER are present; indeed, they act in a dominant negative manner by strongly decreasing secretion of PCSK9.

Common to other subtilisins, trademarks of the catalytic domain (aa 153–454), are the triad Asp\textsubscript{186}, His\textsubscript{226} and Ser\textsubscript{386} as well as the amino acid sequence 367–380. The catalytic domain is responsible for the binding of PCSK9 to the epidermal growth factor-like repeat homology domain A (EGF-A) of human LDL receptor (LDLR). Finally, the PCSK9 C-terminal domain (aa 455–692) although not required for LDLR binding, plays a pivotal role in targeting LDLRs for subsequent degradation. In circulation, mature PCSK9 undergoes a furin enzymatic inactivation at position 218 of the catalytic domain (RFHR218:QA) resulting in the formation of a 55-kDa-truncated form. This last, representing 15–40% of the total circulating PCSK9, is believed to be inactive. PCSK9 exists in a phosphorylated and non-phosphorylated form and after statin treatment the phosphorylated state is reduced by 25%. Considering that phosphorylation at site S688 could be stimulatory, statin therapy may reduce PCSK9 activity by decreasing the phosphorylated state.

Another unclear aspect of PCSK9 biology remains its ability to circulate bound to lipoproteins (20–40%: one PCSK9 molecule for every 500–1000 LDL particles). Upon lipoprotein-apheresis half of plasma PCSK9 is removed. This has led to the hypothesis that the cellular regulation of cholesterol concentrations is under the tight control of a stochastic extracellular system in which LDLR fate is regulated by the interaction with PCSK9-carrying LDL; this could explain, at least in part, why LDLR recycles hundreds

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**Figure 1.** The mature form of pro-protein convertase subtilisin/kexin type 9 (PCSK9) consists of a signal peptide (SP) (aa: 1–30), a prodomain (aa: 31–152), a catalytic domain (aa: 153–452) and a C-terminal domain (aa: 453–692). Once in the endoplasmic reticulum, the SP is cleaved and autocalysis of the PCSK9 zymogen occurs between Gln152 and Ser153 (VFAQ\textsubscript{152}: SIP). Mature PCSK9 (aa: 153–692, about 60 kDa) undergoes a furin enzymatic inactivation at RFHR218:QA leading to a 55-kDa truncated form. Adapted from Abifadel et al. S1P: site 1 protease.
Transcriptional regulation of PCSK9

Discovered in 2003, 44 PCSK9 is one of the 33 genes regulated by the sterol regulatory element binding protein (SREBP) family of transcription factors. 45 In the same year, Abifadel et al. discovered the association between mutations in the neural apoptosis-regulated convertase-1 (NARC1 or PCSK9) human orthologue and the autosomal dominant form of hypercholesterolaemia. 9

The regulation of PCSK9 by SREBPs is not a matter of surprise, since these transcription factors are finely regulated by intracellular sterols and lipid levels. The SREBP family consists of three different proteins, SREBP1a, SREBP1c and SREBP2. SREBP1a is a potent activator of all SREBP-responsive genes, including those mediating cholesterol, fatty acid and triglyceride (TG) biosynthesis, whereas the roles of SREBP1c and SREBP2 are more selective. 46

It is generally assumed that genes involved in fatty acid metabolism, e.g. fatty-acid-synthase and stearoyl-CoA desaturase-1, are preferentially regulated by SREBP1c, whereas the expression of enzymes involved in cholesterol metabolism (3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) synthase), LDL uptake and intestinal absorption (3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) synthase), LDL uptake and intestinal absorption are preferentially regulated by SREBP1c, whereas the expression of enzymes involved in cholesterol metabolism (3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) synthase), LDL uptake and intestinal absorption are preferentially regulated by SREBP1c. 46

The SREBP cleavage-activating protein (SCAP) contains a sterol-sensing domain (SSD) and, in response to a reduced availability of cholesterol, permits the dissociation of SREBP from the ER-resident membrane protein, INSIG. 47 The conformational change of SCAP exposes a portion of the protein, that signals intracellular synthesis, i.e. statins, PCSK9 promoter activity is induced determining increased transcription and higher circulating levels. 40 Interestingly, the expression of both NPC1L1 and PCSK9 is regulated by a second transcription factor, the hepatocyte nuclear factor 1 (HNF1)z. 51–53 The highly conserved HNF1-binding site has been identified in close proximity to the SRE in the promoter region of PCSK9. 54

A further characterization of the transcriptional control of the PCSK9 gene expression brought the discovery of the histone nuclear factor P (HINFP), also binding PCSK9 promoter sequence. HINFP interacts with a highly conserved HINFP-recognition sequence located between HNF1-binding site and the SRE of PCSK9 promoter. 55 HINFP can exert either a positive or negative impact on target genes through an interaction with the MBD2 protein. 55 [AQ8] In the case of PCSK9, HINFP behaves as a transactivator, by mediating the histone H4 acetylation on the PCSK9 promoter region. 55 Along this line, deficiency of the histone deacetylase sirtuin 6 in the liver leads to elevated PCSK9 expression. 56 Sirtuin 6 is recruited to the PCSK9 promoter by the transcription factor forkhead box protein O3 (FoxO3), leading to deacetylation of histone H3 and repression of PCSK9 transcription by suppression of HNF1 activity. 56 With a similar mechanism, the flavonoid pinostrobin can inhibit PCSK9 expression by up-regulating FoxO3a in hepatic cells. 57 Potent inhibitory effects are also exerted by the epigenetic drug 5-azacytidine, reducing expression of PCSK9 and of additional key genes, i.e. HMG-CoA reductase and fatty acid synthase. 57 Azacytidine activation of the related deacetylase Sirt1 further leads to a marked reduction in PCSK9 secretion by an unknown posttranslational mechanism. 57 These findings confirm a major role of the epigenetic regulation of PCSK9 expression through DNA methylation and histone acetylation.

By using bioinformatics and in vitro functional analysis, microRNAs (miRNA, miR)-191, miR-222 and miR-224 were identified as natural regulators of PCSK9. 58 These small endogenous non-coding RNAs regulate a wide range of molecular pathways by target genes, such as PCSK9, controlling expression at the post-transcriptional level. In particular, miR-122 and miR-33 appear to play a major role in fatty acid and cholesterol metabolism. 58

Pharmacological modulation of PCSK9 levels by drugs affecting lipid metabolism

Statins

Statins are the most commonly prescribed class of lipid-lowering drugs. They act as competitive inhibitors of HMG-CoA reductase, the rate-limiting enzyme in the
cellular cholesterol biosynthetic pathway. The end process is increased expression of LDLRs and uptake of LDL-C from the circulation. Statins have provided outstanding contributions to the primary and secondary prevention of CHD. Analysis of large populations in primary and secondary prevention have generally indicated that a 38.6 mg/dl LDL-C reduction is associated with an approximate 22% lowering of CV risk. A paradoxical effect of statins on PCSK9 expression has been detected. By inhibiting cholesterol biosynthesis, statins favour the nuclear translocation of SREBP2 and, through a direct interaction with the SRE-1 element, raise both LDLR and PCSK9 cellular gene expression, resulting in an elevation of circulating PCSK9 levels.

Beyond the SREBP2-induced activation of PCSK9, statin treatment also stimulates gene and protein expression of hepatocyte nuclear factor 1 (HNF1x), a further transcriptional activator of PCSK9. This results in increased expression and secretion of PCSK9 protein, which binds the LDLR and targets it for lysosomal degradation. Berberine reduces PCSK9 mRNA transcription activity due to a negative interaction with the SREBP2 and HNF1 transcription factors on the PCSK9 promoter. Aloricomab and evolocumab are fully humanised monoclonal antibodies against PCSK9. Inclisiran, a synthetic silencing RNA, targets the 3' UTR of the PCSK9 mRNA. Adapted from Konrad et al.176 IgG: immunoglobulin G; RYR: red yeast rice; SRE: sterol regulatory element.

**Figure 2.** Statin increases the activity/nuclear translocation of sterol regulatory element-binding protein-2 (SREBP2), a transcription factor activating the low-density lipoprotein receptor (LDLR) and pro-protein convertase subtilisin/kexin type 9 (PCSK9) genes. Statin treatment also stimulates gene and protein expression of hepatocyte nuclear factor 1 (HNF1)x, a further transcriptional activator of PCSK9. This results in increased expression and secretion of PCSK9 protein, which binds the LDLR and targets it for lysosomal degradation. Berberine reduces PCSK9 mRNA transcription activity due to a negative interaction with the SREBP2 and HNF1 transcription factors on the PCSK9 promoter. Aloricomab and evolocumab are fully humanised monoclonal antibodies against PCSK9. Inclisiran, a synthetic silencing RNA, targets the 3' UTR of the PCSK9 mRNA. Adapted from Konrad et al.176 IgG: immunoglobulin G; RYR: red yeast rice; SRE: sterol regulatory element.
with a stronger effect (+27%) of atorvastatin 80 mg. In other cohorts, atorvastatin 10 mg/day led to non significant PCSK9 increases, whereas atorvastatin 20 mg raised circulating PCSK9 levels in a range between 30% and 35%. Atorvastatin 40 mg by 34–38%, and atorvastatin 80 mg by 46%. [AQ9] In addition to dose-dependency, the impact on PCSK9 is relatively rapid; 24-hour treatment with atorvastatin 10 and 80 mg raises PCSK9 by 13% and 27%, respectively. [AQ10]

**Rosuvastatin.** In a clinical trial with 60 patients with stable angina, 14-day therapy with rosuvastatin (10 mg) led to a 19% rise in circulating PCSK9 levels (together with a 60% reduction of LDL-C). In a sub-analysis of the Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) study, one-year treatment with rosuvastatin (20 mg) increased PCSK9 by 35% in women and 28% in men vs a similar fall of LDL-C in the two genders (~55% for women and ~53% for men). The largest LDL-C reduction was associated with the largest rise in plasma PCSK9 concentrations, an effect consistent across genders. In a series of 27 hypercholesterolaemic subjects, administration of rosuvastatin for three months resulted in a positive correlation between PCSK9 plasma levels and LDL-C reduction, with a 27% rise of PCSK9 with a dose increase from five to 40 mg. In homozygous familial hypercholesterolaemic (HoFH) patients, LDL-C levels were reduced by 16.5% and in heterozygous familial hypercholesterolaemic (HeFH) patients levels were reduced by 48.5%. Conversely, PCSK9 levels increased by 17% in HoFH patients and by 27% in HeFH patients. Overall, PCSK9 levels are elevated in untreated familial hypercholesterolaemia (FH) patients, particularly HoFH and high-dose statin therapy further increases PCSK9 levels. Thus, the positive correlation between PCSK9 and LDL-C, found in untreated HoFH and HeFH patients, was lost. Finally, the statin-driven stimulatory effect on PCSK9 levels remained consistent when rosuvastatin was administered in combination with ezetimibe.

**Simvastatin.** In a single centre, randomised clinical trial, subjects with fasting LDL-C<190 mg/dl, treated for 14 days with simvastatin (40 mg/day) showed a reduction of LDL-C levels by 41%, and a marked rise of PCSK9 concentrations (+67.8%). The association with ezetimibe lowered LDL-C by 60%, with a comparable effect on PCSK9 to simvastatin monotherapy, i.e. +67.3%. A similar trend was found in a cross-over study, enrolling men with mixed dyslipidaemia, given simvastatin (40 mg/day) for two weeks. Along with LDL-C lowering effect of –24%, simvastatin raised PCSK9 levels significantly in both fed (+29%) and fasted (+16%) states. A different conclusion was instead reached in a double-blind, placebo-controlled, crossover trial involving 215 African- and European-American men. Six-week simvastatin (10 mg/day) treatment did not affect PCSK9 concentrations, despite a 25% drop in LDL-C levels. No significant correlations were found between simvastatin-mediated LDL-C lowering and baseline or on-treatment PCSK9 levels.

Finally, looking at the pleiotropic effects of statins beyond cholesterol-lowering, an interesting effect on PCSK9 expression of the isoprenoid cholesterol precursor geranylgeraniol was shown. Studies in Caco-2 cells indicated that induction of PCSK9 mRNA and protein driven by simvastatin was significantly prevented by the addition of geranylgeraniol. The mechanism involved the suppression of Rac1-GTP levels driven by simvastatin (~35.7%). [AQ10]

**Pravastatin and pitavastatin.** The effect of pravastatin (20 mg/day) and pitavastatin (4 mg/day) treatment on PCSK9 has been evaluated in the Treatment With Statin on Atheroma Regression Evaluated by Intravascular Ultrasound With Virtual Histology (TRUTH) study, i.e. an eight-month prospective, open-labeled, randomised, multicentre trial. Among 164 patients with coronary artery disease (CAD), not on lipid-lowering therapy, assignment to pravastatin or pitavastatin significantly reduced LDL-C by 28% and 41%, respectively, with concomitant rises of total PCSK9, by 39% and 78%, respectively.

**Ezetimibe.** Among other commonly prescribed LDL-C lowering medications, ezetimibe is a cholesterol-absorption inhibitor acting on the absorption of biliary and dietary cholesterol across the brush border membrane of the intestinal enterocyte, by blocking the transport protein NPC1L1. By reducing cholesterol absorption ezetimibe increases liver LDLR expression.

Studies of the effects on PCSK9 levels have provided contrasting findings. The previously quoted trial in 215 African- and European-American with LDL-C between 130–175 mg/dl, showed that six-week treatment with ezetimibe (10 mg/q.d.), simvastatin (10 mg/q.d.) and both in combination did not significantly raise plasma PCSK9 levels. Similar conclusions were reached in healthy and type 2 diabetes (T2D) subjects, for whom ezetimibe alone or in addition to a statin did not affect PCSK9 levels, despite an incremental reduction of LDL-C.

Contrasting findings were provided by Davignon and Dubuc who showed that hypercholesterolaemic
patients on ezetimibe in combination with a statin had higher PCSK9 levels compared with controls and statin-treated subjects. 

These data were confirmed in patients on statins or on combined statin-ezetimibe treatment who presented with roughly 45% and 77% higher PCSK9 levels, respectively, vs controls. The combination regimen was associated with 22% higher plasma PCSK9 levels compared with statin alone.

A meta-analysis showed a non-significant impact of combining statin with ezetimibe vs statin monotherapy in terms of altering PCSK9. Removal from the analysis of the study by Lakoski et al. 74 led to a significant elevation of plasma PCSK9 after ezetimibe. The authors justified this discrepancy, speculating that the lowest intensity of statin therapy (simvastatin 10 mg/day) was used and that African-Americans have a reduced response to statins. More importantly, African-Americans have a higher rate of non-response to ezetimibe, due to frequent NPC1L1 mutations. Finally, if a molecular mechanism is searched, HepG2 and Caco-2 cells treated with ezetimibe 25 μM or 50 μM showed a ~1.5 to two-fold increase of PCSK9 mRNA with no changes in protein secretion. [AQ11]

Overall, if it is indisputable that PCSK9 levels are raised by cholesterol synthesis and absorption inhibitors, treatment with PCSK9-antibodies did not affect the balance between cholesterol synthesis and absorption. [AQ12]

Fibrates

Fibrates are activators of the transcription factor peroxisome proliferator–activated receptor (PPAR) system, mainly PPAR-α. They have shown significant benefit in clinical trials of CV prevention, i.e. reducing occurrence of nonfatal MI in patients with concomitant TG elevation and HDL-C reduction. PPAR-α agonists, i.e. fibrates, are powerful TG-lowering agents. They mainly affect TG catabolism and consequently raise, particularly fibrates, HDL-C levels. Of minor significance, their effect on cholesterol metabolism is characterised by a modest reducing activity of HMG-CoA reductase and an increase of cholesterol 7alpha-hydroxylase (CYP7A1), raising bile acid excretion, in some cases associated with an increased risk of gallstone formation.

Although studies in rodents have clearly showed a decrement of PCSK9 protein content upon fenofibrate treatment, discordant findings have been reported in humans. The effects of bezafibrate and fenofibrate on PCSK9 plasma levels have been assessed in an eight-week open randomised crossover study enrolling 14 dyslipidaemic subjects with impaired glucose tolerance or T2D. Fibrates raised PCSK9 levels by 40% (bezafibrate) and 67% (fenofibrate). In line with these studies, two other studies reported that 12- or 24-week treatments with fibrates raised serum PCSK9 levels by 25% and 17%, respectively. A different conclusion was reached instead in a post-hoc analysis of the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study showing that, in diabetics, six-week treatment with fenofibrate (200 mg/day) modestly, but significantly, reduced plasma PCSK9 concentrations by 8.5%, paralleling a 13% reduction in LDL-C.

Again, also in case of concomitant administration of a statin with fenofibrate, inconclusive evidence has been reported. Six-week treatment with atorvastatin (10 mg) or fenofibrate (160 mg) raised PCSK9 by 14% and 19% respectively; combination therapy enhanced PCSK9 by 19%, thus showing no additive effect. Conversely, in statin-treated T2D patients, fenofibrate (145 mg) as monotherapy led to a 13% drop in PCSK9 levels.

Fibrates and statins share a common pathway regulating PCSK9, i.e. SREBP2 activation. Indeed, like statins, fibrates decrease LDL-C by modest activity on HMG-CoA reductase, thus upregulating SREBP2. This mechanism may be valid when fibrates are administered as monotherapy, whereas they are less likely to further influence cholesterol synthesis and SREBP2 expression when given with background statins. In this condition, PPAR-α activation may counteract PCSK9 induction by statins, repressing PCSK9 promoter activity and furthermore raising furin expression, leading to protein degradation.

Omega-3 fatty acids (omega-3s)

Fatty acids of the n-3 series, i.e. those with multiple double bonds – the first one being in the n-3 position from the terminal methyl group – have provided an important addition to dietary treatment in syndromes characterised by elevated TGs. Omega-3s act as ‘fraudulent fatty acids’, i.e. somewhat similar to drugs with a fatty acid-like structure, particularly fibrates. They do not enter the liver metabolic handling by the classical fatty acetyl coenzyme A (acylCoA) oxidative mechanism with carnitine-mediated transport to mitochondria. Omega-3s rather stimulate the metabolism of fatty acids coming from diet or end products of TG metabolism by the peroxisomal PPAR-α mediated pathway (see review by Botta et al.).

The potential activity of omega-3s on PCSK9 has been evaluated in numerous experimental studies. The transcription factor SREBP1c has been shown to be inhibited by docosahexaenoic acid (DHA), thus reducing expression of PCSK9. In rats, 12-week supplementation with eicosapentaenoic acid (EPA)+DHA reduced liver cholesterol synthesis by downregulating
HMG-CoA reductase and SREBP2. The SREBP involvement was confirmed in a study testing the efficacy of a novel conjugate of EPA with niacin, i.e. CAT-C20, on the prevention of nonalcoholic steatohepatitis. This formulation, designed to be hydrolysed by a fatty acid amidase-releasing EPA, inhibited processing of SREBP1 and SREBP2 to their mature nuclear forms, thus, reducing expression of many SREBP target genes, e.g. PCSK9, HMGCR, and LDLR. CAT-C20, when administered to APOE*3-Leiden mice fed a cholesterol-containing Western diet, lowered PCSK9 along with total cholesterol and TGs.

Clinical studies have examined different aspects of omega-3 activity. In a double-blinded, parallel-group, placebo-controlled intervention study, involving 90 pre- or post-menopausal women, 12-day supplementation with 2.2 g omega-3 polyunsaturated fatty acids (PUFAs) (38.5% EPA, 25.9% DHA and 6.0% DPA) vs control thistle oil led to a PCSK9 drop of 16.1% in premenopausal and 13.1% in postmenopausal women. Neither group showed LDL-C changes.

**Cholesterol ester transfer protein (CETP) inhibitors**

CETP is a hydrophobic glycoprotein mainly secreted by the liver. CETP promotes a net mass transfer of cholesteryl esters (CEs) from the HDL fraction to the potentially proatherogenic non-HDL fractions in exchange for TGs. The discovery of the CETP system has led to the development of agents interfering with CETP, thus elevating HDL-C, decreasing LDL-C and potentially preventing CV disease. However, this pharmacological approach has provided convincing evidence of HDL-C raising activity, but disappointing results in trials of CV prevention.

In mildly hypercholesterolaemic patients, anacetrapib as monotherapy or in combination with a statin reduced LDL by increasing fractional catabolic rate (FCR), most likely reflecting a rise in the number of LDLRs, rather than an involvement of PCSK9. A significant 19% reduction of PCSK9 levels was found only in subjects treated with anacetrapib monotherapy, whereas – given in combination with atorvastatin – anacetrapib had no effect on either the production rate or the FCR of PCSK9. This was confirmed in another study in similar patients.

In an effort to shed light on the mechanism of the side effects observed in the Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events (ILLUMINATE) trial, PCSK9 levels were evaluated in a subset of the study. Atorvastatin treatment was positively associated with PCSK9 levels in a dose-dependent fashion at the end of the run-in period, during which patients were titrated to doses of 10, 20, 40 or 80 mg/day, in order to achieve an LDL-cholesterolaemia ≤100 mg/dl. PCSK9 concentrations were 37 ng/ml higher in patients receiving 80 mg atorvastatin vs those on the lowest 10 mg dose. Conversely, after three months of treatment with either atorvastatin or torcetrapib+atorvastatin PCSK9 levels rose modestly in the torcetrapib group (+3.7%) vs no change with placebo (+0.7%), an effect evident only in patients with T2D.

Overall, in humans, it appears that the impact of CETP inhibition on PCSK9 modulation is mainly explained by the background lipid-lowering therapy.

**Nicotinic acid**

Nicotinic acid (niacin or vitamin B3) is a natural compound exerting a variety of activities on lipids, both cholesterol and TGs, at pharmacological doses, i.e. about 1–2 g/day.

Limited information is available on the activity of niacin on PCSK9 levels. In a study of drug combinations in 70 patients with carotid atherosclerosis, a comparison between simvastatin 20 mg/day alone and simvastatin 20 mg+extended-release (ER) niacin 2 g/day was carried out. PCSK9 levels were raised by simvastatin alone (+13%) whereas they fell with the combination (−13%). This trend was also found in 19 dyslipidaemic patients randomised to atorvastatin 10 mg with the serial addition of fenofibrate 135 mg followed by ER niacin titrated to 2 g/day. Fenofibrate raised PCSK9 by 23% whereas the addition of niacin reduced it by 17%. These findings may provide a demonstration of a possible mechanism whereby niacin lowers LDL-C levels.

**Nutraceuticals**

The role of dietary interventions to lower LDL-C levels is well established with a major impact of low fat and omega-6 and omega-3 fatty acid diets. Recent reports have, however, been focused on the potential of functional foods or food additives with a significant activity on LDL-C levels, promoted for patients with LDL-C elevation.

Consumers have free access to these products and some of them have definitely provided an indication of significant LDL-C lowering activity, in some cases with clear changes in PCSK9 levels.

**Berberine**

Berberine, a natural isoquinoline alkaloid present in several plants such as *Berberis vulgaris*, *Coptis chinensis* and *Berberis aristata*, has a traditional use as an antimicrobial, immunomodulatory and antiarrhoead agent. Berberine administration can reduce total cholesterol (−23.5 mg/dl), LDL-C (−25.1 mg/dl) and TGs...
levels. Somewhat contrasting findings were reported is an effective natural cholesterol-lowering agent.eral monacolins inhibiting HMG-CoA reductase. RYR to moderate hypercholesterolaemia. In statin-intolerant patients, it has been indicated as an alternative to statin therapy for patients with mild an alternative to statin therapy for patients with mild on white rice. RYR contains sev-

Monascus purpureus RYR is a food product made by fermenting the yeast Monascus purpureus on white rice. RYR contains several monacolins inhibiting HMG-CoA reductase. RYR is an effective natural cholesterol-lowering agent. In statin-intolerant patients, it has been indicated as an alternative to statin therapy for patients with mild hypercholesterolaemia. RYR may lead to a rise in PCSK9 levels. Initially, this hypothesis was confirmed in two different studies showing that three-day administration of a purified extract of RYR, Xuezhikang (1200 mg/kg/day), led to an increment in PCSK9 levels in the range of 56–70%

The main mechanisms of the lipid-lowering effects are dependent on increased liver LDLR expression and reduction of PCSK9. The raised LDLR expression does not appear to be dependent on SREBP1 and 2, but consequent to the activation of the extra-signal regulated kinase (ERK). The berberine-associated PCSK9 lowering, from in vitro and in vivo studies, is instead linked to a reduction of hepatic PCSK9 mRNA and protein levels. A study of a combination of berberine and mevastatin showed an increase of LDLR mRNA levels with a suppressed upregulation of PCSK9 mRNA, induced by mevastatin alone. The berberine-mediated drop of PCSK9 thus appears to be directly consequent to a reduction in mRNA transcription activity due to a negative interaction with the SREBP2 and HNF1 transcription factors on the PCSK9 promoter. Berberine can also enhance HNF1a protein ubiquitination and proteasomal degradation. Alternatively, berberine could induce AMP-activated protein kinase (AMPK), in turn activating the PPARγ downregulation of PCSK9 mRNA levels. Somewhat contrasting findings were reported in rats in which berberine raised plasma PCSK9 levels with a concomitant increase of SREBP2 and reduction of HNF1.

In clinical trials, berberine is often administered together with other nutraceuticals or with drugs, with contrasting effects on PCSK9 regulation. In patients with HeFH, treated with statins or a combination of statins and ezetimibe, supplementation with a nutraceutical tablet containing berberine, policosanol and red yeast rice (RYR), further reduced plasma LDL-C levels by 10.6%, possibly mediated by berberine through a modulation of PCSK9 expression. In 23 dyslipidaemic subjects, a combination of berberine (531.2 mg), monacolin K (3.3 mg), and Morus alba extract (200 mg) given for four weeks did not change PCSK9 plasma levels, a neutral effect explained by a rise of PCSK9 levels by monacolin K, with negative modulation by berberine and Morus alba.

PCSK9 clinical evaluation – results from the Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk (FOURIER) and Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab (ODYSSEY OUTCOMES) trials

PCSK9 is at present the most extensively studied drug target for treating dyslipidaemia and several strategies are being explored to reduce plasma levels or to inhibit either PCSK9 protein synthesis or binding to LDLR, by way of mAbs, vaccines, small protein inhibitors (peptides/adnectins), antisense oligonucleotides or siRNA. Among these to date, two fully human mAbs, alirocumab and evolocumab, have been approved in the USA and in the European Union, whereas bococizumab, a humanised mAbs, has been discontinued in November 2016 and will not be given further space in this review article. Alirocumab and evolocumab contain two human heavy chains each covalently linked to a fully human kappa light chain through a disulfide bond. Molecular weights are approximately 146 and 144 kDa, respectively.

Alirocumab is indicated as an adjunct to diet and maximally tolerated statin therapy for the treatment of adults with HeFH or clinical atherosclerotic cardiovascular disease (ASCVD), who require additional lowering of LDL-C; evolocumab is also authorised in patients with HoFH requiring additional lowering of LDL-C, in order to reduce the risk of MI, stroke,
and coronary revascularization in adults with CVD. Overall, in the context of CVD prevention in FH patients, PCSK9 inhibitors are to be considered in patients with severe HeFH without ASCVD with LDL-C ≥200 mg/dl or LDL-C ≥175 mg/dl in the presence of comorbidities, i.e. diabetes mellitus or hypertension. HoFH, excluding those with null LDLR mutations, should receive maximal lipid-lowering therapy including lipoprotein apheresis plus a PCSK9 inhibitor, namely evolocumab. A recent approach, based on the estimation of the number needed to treat (NNT) determined by patients’ absolute risk and estimated relative reduction in risk, has identified the addition of ezetimibe to PCSK9 mAbs to maximally tolerated statin therapy to be cost effective in very high and high-risk patients.

The practical clinical guidance of the European ESC/EAS Task Force advises use of PCSK9 inhibitors in (a) patients with ASCVD with elevated LDL-C levels, despite maximally tolerated statin with or without ezetimibe therapy; (b) patients at very high risk who do not tolerate appropriate doses of at least three statins; (c) in FH subjects without clinically diagnosed ASCVD, at high or very high CV risk despite maximally tolerated statin plus ezetimibe therapy. Absolute bioavailability is 72% for evolocumab and 85% for alirocumab, with distribution volumes of 3.31 and 3.0–3.81, respectively, confirming their limited tissue distribution. Effective plasma half-lives are 11–17 days for evolocumab and 17–20 days for alirocumab, reduced by statin co-administration. Indeed, evolocumab clearance is increased by about 20%, partially due to the statin-driven upregulation of PCSK9. Similarly, alirocumab exposure was reduced by about 40%, 15% and 35% when administered with statins, ezetimibe and fenofibrate, respectively. These drug-drug interactions are not to be rated as clinically meaningful and do not generally require dose adjustment.

**Evolocumab**

The efficacy of evolocumab as a lipid-lowering drug was tested in the clinical development programme, Program to Reduce LDL-C and Cardiovascular Outcomes Following Inhibition of PCSK9 in Different Populations (PROFICIO). The FOURIER study evaluated the efficacy of evolocumab in patients with ASCVD on major adverse cardiovascular events (MACEs), i.e. the composite of CV death, MI, stroke, hospitalization for unstable angina, or coronary revascularization. Evolocumab reduced the primary end-point by 15%, an effect primarily ascribable to MI (–27%), stroke (–21%) and coronary revascularization (–22%) reductions. Over the course of the trial, LDL-C had a median value of 30 mg/dl with 42% of patients reaching <25 mg/dl. Non-HDL was reduced by 52%, similar to apolipoprotein B (apoB) (–49%); Lp(a) levels were reduced by 27%. A post-hoc analysis reported that when baseline Lp(a) levels were ≥37 nmol/l, evolocumab reduced MACEs by 2.5% (NNT = 40). Conversely, if baseline Lp(a) levels were < median, the absolute benefit was reduced to 0.95% (NNT = 105). Similar conclusions were reached in the presence of diabetes, with a 17% RR in diabetics vs 13% in non-diabetics. Overall, the FOURIER trial highlighted how severity and extent of CAD are leading features in order to identify people who benefit the most from LDL-C lowering. Recent MI, multiple prior MIs and residual multivessel coronary disease were independent predictors of CV outcomes, leading to an absolute risk reduction by treatment of over 3% in high risk vs approximately 1% in low-risk groups, respectively. In patients with at least one high-risk feature there was a relative risk reduction in CV death, MI or stroke of 19% during the first year and of 27% beyond the first year. A further sub-analysis showed that in patients with peripheral artery disease (PAD) absolute RR for the primary CV endpoint was 3.5% (NNT = 29) vs 1.6% (NNT = 63) for patients without PAD. Major adverse limb events were further reduced by 42% with evolocumab. This benefit followed a linear correlation with LDL-C reduction. Evolocumab did not raise either the risk of new-onset diabetes or of cognition impairment.

Evolocumab was also the object of assessment of plaque regression, evaluated by IVUS in the GLobal Assessment of Plaque reGression With a PCSK9 antibOdy as Measured by intraVascular Ultrasound (GLAGOV) study. Drug treatment was associated with a small, yet significant, reduction in percentage and total atheroma volume, but with inconsistent changes in coronary fibrous, fibrofatty and necrotic volumes as well as in coronary calcium by virtual histology.

**Alirocumab**

The efficacy of alirocumab has been tested in the ODYSSEY programme including 16 clinical trials, designed to evaluate the antibody together with other lipid-lowering agents, e.g. statins, or as monotherapy across a wide range of patients, including those with high CV risk, intolerant to statins and HeFH. CV outcomes have been studied in the ODYSSEY OUTCOMES trial enrolling a total of 18,924 patients, with an acute coronary syndrome 1–12 months earlier, from 57 countries with a 2.8 year follow-up. In the
Overall, recent data from pooled analysis of the ODYSSEY programme indicate that alirocumab is effective with a similar safety profile in high-risk patients with or without prior coronary revascularization,\textsuperscript{136} in individuals with T2D\textsuperscript{137} and high CV risk with or without mixed dyslipidaemia.\textsuperscript{138}

**Inclisiran**

Among therapeutic strategies aimed at reducing circulating PCSK9 levels, the use of siRNA represents a different strategy for reducing PCSK9 secretion. siRNAs target RNA directly destroying it before the protein is synthesised. Inclisiran, targeting the 3' UTR of the PCSK9 mRNA, is a long-acting, subcutaneously delivered, synthetic siRNA, conjugated to triantennary N-acetylgalactosamine carbohydrates. It binds to asialoglycoprotein receptors on hepatocytes, leading to the uptake of inclisiran and suppression of hepatic PCSK9 production.\textsuperscript{139} The drug is rapidly distributed in plasma with peak concentrations occurring at the end of the infusion, with a roughly dose-proportional increment.\textsuperscript{140}

The first study evaluating intracellular PCSK9 inhibition vs LDL-C reduction was a Phase 1 dose-escalation randomised controlled trial (RCT) enrolling 32 healthy volunteers with LDL-C > 116 mg/dl. PCSK9 and LDL-C were reduced in a dose-dependent manner with maximal 70% and 40% falls when the highest dose of inclisiran (0.40 mg/kg) was administered.\textsuperscript{140} Effects were maintained for at least six months if inclisiran was administered at the dose of 300 mg or more, either in single- or multiple-dose regimens. No serious adverse events were observed.\textsuperscript{141}

These data were corroborated in the ORION-1 study, a Phase 2 dose-ranging trial evaluating the efficacy of inclisiran vs placebo in lowering LDL-C at six months.\textsuperscript{142} Six different doses (three single doses and three two-dose starting regimens administered 90 days apart) were tested. In high CV-risk patients, already at maximally tolerated statin therapy, inclisiran, either as single- or two-dose regimens, dramatically reduced LDL-C, by 42% and 52.6%, respectively. These percentage reductions were in a range similar to those achieved with mAbs with an overall optimal dosage of 300 mg given twice as the starting regimen.\textsuperscript{142} Whether or not there was the presence of diabetes at baseline, inclisiran was equally effective in the management of dyslipidaemias; LDL-lowering was between \( \frac{22.8}{25.2} \) % and \( \frac{25.2}{25.5} \) % in patients without diabetes, and between \( \frac{22.8}{25.2} \) % and \( \frac{25.2}{25.5} \) % in diabetics.\textsuperscript{143} Although Lp(a) was reduced, this did not reach statistical significance.\textsuperscript{144}

Phase 3 trials evaluating the efficacy of inclisiran on HeFH (NCT03397121) or ASCVD (NCT03705234)
patients are ongoing. A CV outcomes trial involving approximately 15,000 subjects with high ASCVD risk is being planned.

**Summary and conclusions**

The pharmacological inhibition of PCSK9 has led to indisputable benefits in terms of LDL-C and CV-risk lowering, but the validity of clinical measurements of plasma PCSK9 for CV-risk prediction is not fully supported by the analysis of RCTs with PCSK9 inhibitors and thus remains an open question. While a number of studies did not report an association between PCSK9 levels and events, different conclusions were reached by others. Specifically, looking at meta-analyses, an association between CV events and PCSK9 levels was found, a conclusion supported by a genome-wide association study of circulating PCSK9 levels showing that genetic reduction of PCSK9 levels by 50% is associated with a similar percentage reduction of CAD risk. Such discrepancies may be based on the following observations: (a) plasma PCSK9 levels differ across study design and clinical outcomes, (b) assay methods for PCSK9 do not discriminate between active and inactive truncated forms and (c) PCSK9 is tightly regulated at the transcriptional level. Besides a diurnal rhythm, nutritional status, environmental pollutants (PM10 particles) and gender differences regulate plasma PCSK9 levels, significantly higher in women than in men, with lower levels in postmenopausal vs premenopausal women. Most recently, evaluation of two cohort studies in diabetics provided discordant results, one study (Non-Insulin Dependent Diabetes, Hypertension, Microalbuminuria or Proteinuria, Cardiovascular Events, and Ramipril (DIABHYCAR)) showing a clear association between PCSK9 levels and incidence of MI, all CV events and stroke, the other (Survie, Diabète de type 2 et Génétique (SURDIAGENE)) reporting no such association, confirming the still inconsistent link between PCSK9 level and CV risk. Finally, in the context of plasma PCSK9 as a possible independent factor beyond the traditional CV-risk biomarkers, in the obese subject PCSK9 appeared to be associated with

<table>
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<tr>
<th>Drug class</th>
<th>Effect on PCSK9</th>
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<tr>
<td>Statins</td>
<td>Atorvastatin</td>
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<td></td>
<td>10 mg, + 19%</td>
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<td>20 mg, + 30–35%</td>
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<td>40 mg, + 34–38%</td>
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<td>80 mg, + 46%</td>
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<td>Rosuvastin</td>
<td>Women, + 35%</td>
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<td></td>
<td>Men, + 28%</td>
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<tr>
<td></td>
<td>HeFH, + 38%</td>
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<td></td>
<td>HoFH, + 21%</td>
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<td>Simvastatin</td>
<td>+16–68%</td>
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<td>Pravastatin</td>
<td>+39%</td>
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<td>Pitavastatin</td>
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<td>Ezetimibe</td>
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<td>Neutral effect</td>
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<td>Fibrates</td>
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<td>Fatty acids</td>
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<td>CETP inhibitors</td>
<td>Anacetrapib</td>
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<td>–19%</td>
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<td>Subgroup of ILLUMINATE (n = 1745): after three months of treatment:</td>
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<tr>
<td></td>
<td>Atorvastatin+torcetrapib</td>
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<td>+3.7% vs run-in with atorvastatin (significant)</td>
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<td>Atorvastatin+placebo</td>
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<td></td>
<td>+0.7% vs run-in with atorvastatin (not significant)</td>
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<tr>
<td>Nicotinic acid</td>
<td>–13%</td>
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<td>Nutraceuticals</td>
<td>Berberine</td>
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<td>Red yeast rice</td>
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CETP: cholesterol ester transfer protein; FIELD: Fenofibrate Intervention and Event Lowering in Diabetes; HeFH: heterozygous familial hypercholesterolaemic; HoFH: homozygous familial hypercholesterolaemic; ILLUMINATE: Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events.
a significant rise of the Framingham Risk Score, a well-established predictor of CV risk.151

The pharmacological regulation of PCSK9 provides a pointer to estimate possible alterations in the overall response to lipid-lowering medications in patients at high CV risk. Prominent in this area has been the discovery that statins, via SREBP2 involvement, upregulate PCSK9 gene expression (Table 1). Although clinical trials confirm that statin therapy raises PCSK9, irrespective of statin type, the magnitude of this effect remains unclear. Notably, the discovery that PCSK9 exists in an active S688-phosphorylated form, reduced by 25% after statin treatment, has led to the speculation that HMG-CoA reductase inhibition may reduce PCSK9 activity by decreasing the active phosphorylated state.37

The pharmacological inhibition of PCSK9 in the clinic, with fully human mAbs against PCSK9, i.e. evolocumab and alirocumab, has provided convincing evidence of a dramatic LDL-C reducing activity, as well as of significant lowering of CV events.154 Interestingly, also in this context, measuring PCSK9 circulating levels has become important. Upon administration of mAbs, total plasma PCSK9 concentrations increase up to 10-fold. This may be consequent either to reduced clearance of the immune complex vs free PCSK9, or to entrapment in the circulation of newly secreted PCSK9 with the antibody or, finally, to the up-regulation of PCSK9 secretion by the liver as a counter-regulatory mechanism.10 These findings have led some authors to hypothesise the use of PCSK9 total concentrations as a diagnostic tool assessing apparent cases of resistance to PCSK9 inhibitors;155 indeed, if PCSK9 elevations are not detected after mAbs administration, this would suggest an altered tissue distribution of the drug. However, when free PCSK9 levels are considered, these are suppressed by 90–100% within one week of administration followed by a 65% fall of LDL-C.156

Before the introduction of PCSK9 inhibitors, there was little to be done to achieve target LDL-C values, except for adding ezetimibe to statin treatment. This left out most of the very high-risk patients, e.g. those with FH, who could not reach LDL-C target values and could not be adequately treated.157 The newly published American College of Cardiology/American Heart Association (AHA/ACC) 2018 cholesterol guidelines recommend – for ASCVD patients who are at very high risk and LDL-C not adequately controlled by statin therapy – a stepped approach of ezetimibe, in addition to the statin. If that combination does not work adequately, i.e. LDL-C levels ≥70 mg/dl, PCSK9 inhibitors could be added.158 It should be noted that a very recent analysis of applicability and cost implications for PCSK9 inhibitors, based on the results of the ODYSSEY OUTCOMES study and on these new guidelines, concludes that only a selective use of PCSK9 antagonists is justified.159

In the context of the interplay between hypercholesterolaemia and the inflammatory burden,160 the clinical CV preventive activity of PCSK9 antagonists may possibly be affected by the poor efficacy of these agents on circulating inflammatory markers, e.g. hsCRP.161,162 In carriers of the PCSK9 R46L loss-of-function mutation, no changes in GlycA,163 a composite NMR biomarker of systemic inflammation correlated with CRP,164 was noted.

This lack of anti-inflammatory efficacy is also detected in FH patients when they are switched from lipoprotein apheresis to PCSK9 mAbs.165 In the large FOURIER trial, patients with elevated hsCRP levels (>3 mg/dl) showed the best preventive response to treatment: those with a lower baseline hsCRP had an absolute risk reduction of 1.8%, vs a reduction of 2.6% for those with the highest baseline levels.166 Open questions still remain on the association of PSCK9 not only with inflammation but also with infections as well; indeed, PCSK9 may play a critical role in the innate immune defense against bacterial and/or viral infections.167

The very recent introduction of a siRNA PCSK9 antagonist, inclisiran, has given the possibility of widely spaced treatments, allowing for optimal compliance. This type of approach, still available to a very limited extent, may provide a very active cholesterol-lowering treatment with minimal effort by the patients.168

Relative to safety concerns on the use of PCSK9 inhibitors, uncertainties persist relative to the potential risk for new-onset diabetes mellitus. While the use of PCSK9 inhibitors led to significant CV-risk reduction, generally similar between individuals with and without diabetes mellitus, with no further worsening of diabetes mellitus,169 data from Mendelian randomization analyses reported that genetic inactivation of PCSK9 is associated with an increased risk of new-onset diabetes mellitus.170–172 We recently showed that mice lacking PCSK9 have an impaired insulin secretion vs wild-type animals, an effect lost in double KO mice for PCSK9 and LDLR.173 The hypothesis that the target responsible for the phenotype of defective glucose homeostasis was the LDLR is supported by the lack of changes of LDLR expression in β cells from mice selectively deficient in liver PCSK9. Overall, pharmacological approaches aimed at targeting liver PCSK9 may not be associated with an increased risk of developing diabetes mellitus.174

**Author contribution**
CM wrote the manuscript. CRS and MR conceived the topic and wrote the manuscript. AC conceived the topic and critically revised the manuscript. NF wrote and critically revised the manuscript.
All gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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