Living the PCSK9 Adventure: from the Identification of a New Gene in Familial Hypercholesterolemia Towards a Potential New Class of Anticholesterol Drugs

Marianne Abifadel · Sandy Elbitar · Petra El Khoury · Youmna Ghaleb · Méloïde Chémaly · Marie-Line Moussalli · Jean-Pierre Rabès · Mathilde Varret · Catherine Boileau

Abstract A decade after our discovery of the involvement of proprotein convertase subtilisin/kexin type 9 (PCSK9) in cholesterol metabolism through the identification of the first mutations leading to hypercholesterolemia, PCSK9 has become one of the most promising targets in cholesterol and cardiovascular diseases. This challenging work in the genetics of hypercholesterolemia paved the way for a plethora of studies around the world allowing the characterization of PCSK9, its expression, its impact on reducing the abundance of LDL receptor, and the identification of loss-of-function mutations in hypocholesterolemia. We highlight the different steps of this adventure and review the published clinical trials especially those with the anti-PCSK9 antibodies evolocumab (AMG 145) and alirocumab (SAR236553/REGN727), which are in phase III trials. The promising results in lowering LDL cholesterol levels raise hope that the PCSK9 adventure will lead, after the large and long-term ongoing phase III studies evaluating efficacy and safety, to a new anticholesterol pharmacological class.

Keywords Hypercholesterolemia · LDLR · PCSK9 · Gain of function mutations · Loss of function · Evolocumab · Alirocumab

Introduction

Autosomal dominant hypercholesterolemia (ADH) is a heterogeneous genetic disorder characterized by a selective increase of LDL cholesterol (LDL-C) levels in plasma, giving rise to tendon and skin xanthomas, arcus cornea, and vascular deposits, leading to progressive and premature atherosclerosis, coronary heart disease (CHD), and death. The first two genes implicated in the disease are the gene that encodes the LDL receptor (LDLR at 19p13.3; OMIM 606945, 143890) [1] and the apolipoprotein B (apoB) gene (APOB at 2p23–p24; OMIM 107730, 144010), encoding the ligand of the LDL receptor [2]. The existence of a greater level of genetic heterogeneity in ADH and the involvement of a third locus named HCHOLA3 (formerly FH3; OMIM 603776) were reported by our team. In 2003, we discovered [3] that PCSK9 was the third gene implicated in ADH. This pioneering work revealed a new major
player in cholesterol homeostasis and was the first step of the adventure involving proprotein convertase subtilisin/kexin type 9 (PCSK9) as a promising therapeutic target in lowering LDL-C levels and reducing the risk of cardiovascular diseases. In this review we will follow the PCSK9 adventure from the involvement of its mutations and variants in cholesterol disease and CHD to the several clinical trials that have been launched.

**Discovery of the Involvement of PCSK9 in Cholesterol Metabolism**

Through the French Research Network for ADH (Réseau National de Recherche sur les Hypercholestérolémies Familiales), families with hypercholesterolemia were recruited from several regions of France [3]. After the exclusion of the LDLR and APOB genes, a positional cloning strategy was used to identify the genetic region linked to the disease. Using this classic genetic approach, HCHOLA3 was mapped to 1p34.1–p32 in a French multiplex family (HC2) [4]. A year later Hunt et al. [5] confirmed this localization in an ADH family originating from Utah. Segregation analysis, genetic mapping, and sequencing studies performed helped in excluding several genes, and in refining the boundaries of the region through the identification, by Abifadel et al., of a new French multiplex family, HC92, linked to the same HCHOLA3 locus. Extensive sequencing studies of several candidate genes expressed in the liver allowed us the detection, on the 13 September 2002 in the HC2 and HC92 families, of a common mutation, p.S127R, in the PCSK9 gene and another mutation, p.F216L, in a third French family with ADH [3]. The PCSK9 gene, the ninth member of the proprotein convertase subfamily, had been characterized in 2003 by Seidah et al. [6], who identified it from a patented database in a BLAST search to find proteins related to a recently identified proprotein convertase called SKI-1 (site-1 protease). PCSK9 was formerly designated as neural apoptosis regulated convertase 1 (NARC1) as it was discovered in 2001 by Millenium Pharmaceuticals through the cloning of complementary DNA upregulated after apoptosis induced by serum deprivation in primary cerebellar neurons. It was also designated as LP251, which was identified by Eli Lilly and Co. in 2002 via the cloning of secretory proteins [6]. The mammalian serine proprotein convertase family is responsible for the proteolytic maturation of secretory proteins, including neuropeptides, prohormones, cytokines, growth factors, receptors, serum, and cell surface proteins [6, 7].

**PCSK9 Protein: Structure and Function**

PCSK9 complementary DNA (NM_174936.2) spans 3,617 bp over 12 exons that encode the 692 amino acid protein PCSK9 (NP_777596.2). It is particularly expressed in the liver, gut, kidney, and nervous system [6, 8]. The detailed structure and processing of PCSK9 are given in Fig. 1 [6, 9–19]. The 60-kDa mature form and the furin-cleaved form of PCSK9 are present in the circulation [12, 17].

**PCSK9 Mutation in Hypercholesterolemia**

The p.S127R mutation in a highly conserved region between species in exon 2 was found in the first two French families studied: HC2 and HC92. The second mutation, p.F216L, in a conserved region in exon 4, was identified in a French family in which the proband died from myocardial infarction at the age of 49 years with a total cholesterol level of 441 mg/dl and an LDL-C level of 356 mg/dl [3, 20, 21]. These two mutations allowed us to identify for the first time the involvement of PCSK9 in ADH and cholesterol metabolism [3]. The third mutation, p.D374Y, was reported in 2004 in the hypercholesterolemic Utah kindred [22] previously linked to the 1p32 region [5]. The same mutation was found in three Norwegian families [23] and in three English families, with 12 affected patients having severe hypercholesterolemia and a family history of premature CHD [24].

Other mutations adjacent to these mutations were also reported: p.D374H in Portuguese patients with severe hypercholesterolemia [25]; p.R218S, which we identified in a French family whose proband at the age of 45 years had an LDL-C level of 293 mg/dl and presented with tendinous xanthoma and arcus corneae [26]; p.R215H in two families from Norway [27]; and p.D129G in a family originating from New Zealand [28]. A novel missense mutation of the PCSK9 gene, p.R306S, was found in a Chinese population [29]. More recently, we identified two gain-of-function mutations of PCSK9 in French families: (1) p.L108R, in a black family originating from Mauritius whose proband at the age of 41 years had an LDL-C level of 302 mg/dl and tendon xanthomas; (2) p.D35Y in a family’s proband who had an LDL-C level of 234 mg/dl at the age of 55 years [30]. The PCSK9 mutations inducing ADH are very rare, but well documented (familial segregation analysis, in vitro mutagenesis, etc.). The clinical findings that have been reported in PCSK9 heterozygote carriers are those related to hypercholesterolemia: tendon xanthomas, CHD, premature myocardial infarction, and stroke. Most enzymopathies are recessively inherited, and thus the dominance of the ADH trait associated with PCSK9 was in favor of a gain-of-function mechanism [3]. This was confirmed by cellular and animal models showing that these gain-of-function mutations decreased the number of LDL receptors at the cell surface, leading to hypercholesterolemia [17, 31, 32].

In vitro studies showed that the two gain-of-function mutations p.S127R and p.D374Y resulted in a 23 % decreased
Fig. 1 Structure, processing of proprotein convertase subtilisin/kexin type 9 (PCSK9), and impact of PCSK9 main variants and mutations. The PCSK9 structure is characterized by a signal sequence (amino acids 1–30), a prodomain (amino acids 31–152), and a catalytic domain, followed by a 243 amino acid cysteine-rich and histidine-rich C-terminal region. PCSK9 is synthesized as an inactive proenzyme and contains a triad of residues (Asp-186, His-226, and Ser-386) that are required for catalytic activity. The approximately 74-kDa precursor form of PCSK9 undergoes intramolecular autocatalytic cleavage in the endoplasmic reticulum (ER), which produces an approximately 60-kDa catalytic fragment. Autocatalytic cleavage of the zymogen in the ER is essential for transport from this compartment and for secretion. The PCSK9 crystal structure shows that the cleaved prodomain of approximately 14 kDa remains associated with the catalytic domain, blocking the PCSK9 active site, which could explain why no other proteolytic activity has been reported for PCSK9. The 60-kDa mature and secreted form is cleaved at the motif RFHR↓218 into an approximately 53-kDa inactivated or less efficient fragment by other proprotein convertases, particularly furin and/or proprotein convertase C5/6A (PC5/6A). PCSK9 degrades LDL receptor (LDLR) independently of its catalytic activity by involving mainly extracellular and possibly intracellular pathways. PCSK9 might work in a post-ER compartment, where it might target LDLR for degradation in lysosomes. The binding site for the LDLR EGF-A domain resides on the surface of PCSK9 that is formed primarily by residues 367–381. Key interactions with EGF-A are made by Arg-194 and Asp-238 of PCSK9. Several gain-of-function mutations are reported: The p.S127R variant interferes with autocatalytic cleavage, which is crucial for secretion from the cell. The p.D374Y variant binds LDLR 25 times more tightly than does wild-type PCSK9 at neutral pH, remains in a high-affinity complex at acidic pH, and is approximately tenfold more active in reducing LDLR levels than the wild-type protein. The p.R218S, p.F216L, and p.D374Y mutations result in total (p.R218S) or partial loss of the furin/PC5/6A processing of PCSK9, which increases the stability of PCSK9. Loss-of-function mutations are also represented: no protein was detected with the p.Y142X mutation, probably owing to nonsense-mediated messenger RNA decay. Some mutants associated with hypercholesterolemia either remain in the ER (p.C679X and the p.G106R mutations) or do not sort to endosomes (p.L253F and p.Q554E), resulting in loss of function (Benjannet et al. [9, 12], Lagace et al. [17], Cunningham et al. [10], McNutt et al. [11], Piper et al. [15], Nassoury et al. [19], Zhang et al. [14], Kwon et al. [16], Poirier et al. [13]).
level of cell surface LDL receptors and a 38% decreased level of internalization of LDL compared with wild-type PCSK9 [33]. It was shown more recently that the p.L108R mutant exhibited a marked approximately twofold enhanced degrading activity towards LDL receptor, resulting in a clear and significant gain-of-function in this assay [30]. The mechanisms of action of the gain-of-function mutations are depicted in Fig. 1.

**PCSK9 and Hypcholesterolemia**

Two years after our first report of the involvement of PCSK9 in cholesterol metabolism and disease, two nonsense mutations in PCSK9, p.Y142X and p.C679X, were identified in subjects with low plasma levels of LDL-C (below 58 mg/dl) from the Dallas Heart Study, a multiethnic population of Dallas County, Texas, USA [34]. Subjects with nonsense mutations had significantly lower plasma levels of total cholesterol and LDL-C, but not all of them were hypcholesterolemic [34]. In the USA, one in every 50 African Americans has a nonsense mutation in PCSK9. In the Atherosclerosis Risk in Communities (ARIC) study, comprising 3,363 black and 9,523 white participants aged 45–64 years from four American communities [35], the nonsense mutations occurred in 2.6% of the black subjects examined and were associated with a 28% reduction in mean LDL-C level and an 88% reduction in the risk of CHD. These mutations were found at this same high frequency in a Nigerian population [36], in 3.7% of African women from Zimbabwe and associated with a 27% reduction in LDL-C levels [37], but were very rare in Americans of European origin (less than 0.1%) [36]. However, another variant, p.R46L, was found in 3.2% of the white subjects examined in the ARIC study and was associated with a 15% reduction in LDL-C levels and a 47% reduction in the risk of CHD [34, 35, 38]. The p.Q152H mutation of PCSK9 was identified in a French Canadian, with mean decreases in circulating PCSK9 and LDL-C concentrations of 79% and 48%, respectively, compared with unrelated noncarriers [39]. The p.G106R mutation segregated with low LDL-C levels in a Norwegian family [18]. The impacts of these variants on CHD have been studied and are reported in Fig. 1.

A woman originating from Zimbabwe, homozygous for p.C679X, was reported [37] with a very low LDL-C level (15 mg/dl). Furthermore, Zhao et al. [40] reported a compound heterozygote for the p.Y142X mutation and an in-frame 3-bp deletion (c.290_292delGCC) that deletes an arginine at codon 97. She had no immunodetectable circulating PCSK9. This 32-year-old African American woman with an LDL-C level of only 14 mg/dl was apparently healthy, fertile, and normotensive, with grossly normal hepatic, neuronal, and renal function test results [40]. A 49-year-old Caucasian man with a heterozygous double PCSK9 mutation, undetectable circulating PCSK9, and profound familial hypobetalipoproteinemia (FHBL) (LDL-C level 16 mg/dl) was also reported. A monoallelic PCSK9 double-mutant R104C/V114A cosegregated with FHBL, with no mutation found at other FHBL-causing loci [41]. Two nonsense mutants, p.A68fsL82X and p.W428X, have been identified in Sicilian and Japanese hypcholesterolemic patients [42, 43], respectively. One proband heterozygous for a novel single nucleotide deletion in exon 1 (c.202delG), which causes a frameshift in messenger RNA (mRNA), leading to a premature stop codon (A68fsL82X), was a 34-year-old white overweight male (body mass index 30 kg/m2) who had been referred to the clinic for fatty liver. This loss-of-function mutation was also identified in two healthy blood donors who had no clinical or laboratory signs of liver disease; the results of other routine laboratory tests were normal [42]. In the Dallas Heart Study, no significant difference in the median content of hepatic triglycerides or in the prevalence of hepatic steatosis between the subjects with and without an LDL-lowering mutation in PCSK9 was observed in either ethnic group [36]. Hypcholesterolemia due to a deficiency of PCSK9 appears to be benign, in contrast to other Mendelian forms of severe hypcholesterolemia such as abetalipoproteinemia (OMIM 200100) and homozygous hypobetalipoproteinemia (OMIM 107730), which are both associated with malnutrition, hepatic steatosis, steatorrhea, and manifestation of fat-soluble vitamin deficiency [40].

**PCSK9 in CHD and Large Population Studies**

PCSK9 variants have variable frequencies in different populations, and their impact on cholesterol levels and CHD was analyzed in African [37], American [35], and European [18, 44] populations and in different studies (ARIC [35], PROSPER [45], LCAS [46], TEXGEN [46], PLIC [47]) by evaluating either the protection of the loss-of-functions variants or the severity of coronary atherosclerosis associated with gain-of-functions polymorphisms (mainly p.E670G). These studies, their objectives, their results, and their conclusions are summarized in Table 1 [35, 45–59]. They showed that genotype is a better predictor of lifelong exposure to LDL-C than LDL-C measured in adult life. But the impact on LDL may not be the only effect of PCSK9 on atherogenesis [60]. It is noteworthy that several genome-wide association studies identified an association of the PCSK9 locus and of some PCSK9 variants with the variability of LDL-C levels or early-onset myocardial infarction [61].

**Genotype–Phenotype Correlation**

PCSK9 polymorphisms account for cholesterol variability not only in normolipemic subjects but also among familial hypercholesterolemia (FH) patients sharing the same mutation of
Table 1  Major studies of the impact of PCSK9 variants in different populations and diseases, specifically coronary heart diseases (CHD)

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<td>LCAS (Chen et al. [46])</td>
<td>High-risk population: 372 subjects aged 35–75 years; LDL-C 115–190 mg/dl; coronary lesions causing 30–75 % of diameter stenosis. Replication study population (TexGen): 319 subjects who had plasma LDL-C levels below 130 mg/dl</td>
<td>Determine the effects of PCSK9 variants on plasma LDL-C levels, severity of coronary atherosclerosis, and response to statin therapy</td>
<td>Carriers of E670G SNP had higher LDL-C levels than did non carriers (152 mg/dl vs 143 mg/dl). E670G exerts a dose effect (GG &gt; EG &gt; EE) accounting for 3.5 % of plasma LDL-C level variability. Plasma total cholesterol, apoB, and Lp(a) levels were also associated with the E670G variant.</td>
<td>E670G a common SNP, an important determinant of plasma LDL-C concentration, is associated with the severity of coronary atherosclerosis in the LCAS population</td>
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<td>ARIC (Cohen et al. [35])</td>
<td>3,363 blacks, 9,524 whites, age 45–64 years, 4 American communities</td>
<td>Compare the incidence of CHD over a 15-year interval according to the presence or absence of PCSK9 sequence variations (Y142X, C679X, and R46L)</td>
<td>Frequent in blacks (2.6 %), the Y142X and C679X mutations in PCSK9 were associated with a 28 % reduction in LDL-C levels and 88 % reduction in the risk of CHD. R46L was found in 3.2 % of white subjects and was associated with a 15 % reduction in LDL-C levels and a 47 % reduction in the risk of CHD.</td>
<td>Moderate lifelong reduction in LDL-C levels is associated with a substantial reduction in the incidence of coronary events</td>
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<td>European study, University Hospital Hamburg, Germany (Evans and Beil [48])</td>
<td>506 patients attending the lipid clinic</td>
<td>Study the incidence of the E670G SNP in PCSK9</td>
<td>The frequency in men with polygenic hypercholesterolemia, 0.11, was significantly higher than in men with LDL-C levels below the 50th percentile, 0.03, p=0.01. In women there was no difference in the allele frequencies between the 2 groups.</td>
<td>This observation explains the discrepancy in the results between the Dallas Heart Study and the LCAS, as the majority of the probands in the LCAS were men, but does not explain the lack of association in healthy men in the UK</td>
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<td>Bogalusa Heart Study (Hallman et al. [49])</td>
<td>478 African Americans, 1,086 white Americans, age 4–38 years</td>
<td>Analyze the relation between R46L, Y142X, and C679X PCSK9 variants and serum LDL-C levels (in childhood and adulthood)</td>
<td>Whites carriers of the R46L allele (n=27) and African-Americans carriers of the Y142X or C679X allele (n=12) had, respectively, 12 % and 15 % lower serum LDL-C levels than did noncarriers at their first examination (mean age approximately 9.0±3.0 years)</td>
<td>These PCSK9 variants are associated with significantly lower LDL-C levels starting in childhood</td>
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<td>Belgium Stroke Study (Abboud et al. [50])</td>
<td>237 subjects (age 45–60 years) with small-vessel occlusion and large-vessel atherosclerosis stroke, 326 ethnicity-matched controls (older than 60 years) without a history of stroke.</td>
<td>Find a potential link between PCSK9 and the risk of ischemic stroke or intracranial atherosclerosis</td>
<td>The E670G allele was commoner in large-vessel atherosclerosis patients than in controls (10.8 % vs 4.3 %). It was not related to the risk of small-vessel occlusion in the Belgium population.</td>
<td>PCSK9 is associated with the risk of the large-vessel atherosclerosis stroke subtype, and this risk is mediated by the severity of intracranial atherosclerosis</td>
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<td>The Tampere Coronary Study: 604 Caucasian Finnish autopsy cases of people who had died suddenly out of hospital (64.3 % men and 35.7 % women)</td>
<td>In multivariate analysis, the minor allele (G) appeared as a significant predictor of large-vessel atherosclerosis</td>
<td>In autopsy subjects, the G-allele carriers had severer atherosclerosis in the large intracranial cerebral arteries (EE=4.71 vs G+=5.97)</td>
<td>The PCSK9 R46L allele is associated with a protective plasma lipid profile risk for CHD. Its low frequency means that it does not make a major contribution to determining the population CHD risk in the UK</td>
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<td>Second Northwick Park Heart Study (Scartezini et al. [51])</td>
<td>Determine the relative frequency of PCSK9 variants (R46L, I474V, and E670G) and their association with plasma lipid levels and CHD</td>
<td>R46L allele frequency was significantly lower in FH patients (0.003) than in healthy UK adults (0.010). Unlike FH patients, healthy UK adult carriers of R46L had a significant lower mean LDL-C level [4.01 mmol/l for RR compared with 3.62 mmol/l for RL] and a lower but nonsignificant risk of CHD [HR, 0.46 (95 % CI, 0.11–1.84); p=0.27]. I474V and E670G were not associated with any significant effects on lipid levels or CHD risk</td>
<td>R46L significantly lowers LDL-C levels, but does not greatly reduce CHD risk in an elderly population with a high prevalence of cardiovascular disease</td>
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<td>PROSPER (Poliseck et al. [45])</td>
<td>Examine the effect of 2 PCSK9 SNPs (R46L and E670G) in elderly participants, of whom 43 % had a history of vascular disease and who were randomized to receive either pravastatin or placebo for follow-up for 3.2 years</td>
<td>3.5 % were carriers of R46L, and these subjects had significantly (p&lt;0.001) lower levels of LDL-C (mean, −10 %). No difference in LDL-C-lowering response to pravastatin, and a nonsignificant 19 % unadjusted and a 9 % adjusted decreased risk of vascular disease at the baseline for the R46L carriers. No significant result with the carriers (6 %) of E670G.</td>
<td>The PCSK9 loss-of-function allele provides protection against MI in humans and is a valid target for pharmacologic therapy</td>
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<td>Myocardial Infarction Genetics Consortium (Kathiresan [52])</td>
<td>Test the hypothesis that R46L is associated with the risk of early-onset myocardial infarction</td>
<td>The minor L allele (2.4 % frequency in controls) of R46L was associated with a reduced risk of MI (meta-analysis OR, 0.40; 95 % CI, 0.26–0.61; p=0.00002). This association remained significant after further adjustment for traditional risk factors, including treated hyperlipidemia</td>
<td>The PCSK9 loss-of-function allele provides protection against MI in humans and is a valid target for pharmacologic therapy</td>
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<td>ARIC (Folsom et al. [53])</td>
<td>Determine whether specific PCSK9 (blacks Y142X, C679X; whites R46L) variants are associated with reduced prevalence and incidence of PAD</td>
<td>Carriers of the PCSK9 variants that lower LDL-C levels had a lower prevalence of PAD compared with noncarriers (2.3 % vs 4.6 %). Among the cohort free of baseline PAD (n=13,015), 895 incident PAD events occurred through to 1998. In contrast with the cross-sectional findings, there was no association between PCSK9 variants and incident PAD.</td>
<td>This study provides mixed evidence that variation in PCSK9 may contribute to genetic risk of PAD</td>
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<td>Coronary Artery Risk Development in Young Adults (Huang et al. [54])</td>
<td>1,750 African Americans and 1,828 whites, age 18–30 years</td>
<td>Association of 6 genetics variants of PCSK9 with LDL-C and IMT over 20 years, from young adulthood to middle age</td>
<td>White carriers of the R46L variant, blacks with 3 genetic variants (L255F, C679X, Y142X) and black carriers of A443T at the age of 18 years had significantly lower LDL-C levels than did noncarriers (84.4, 81.5, and 95.5 mg/dl respectively). The increase in LDL-C levels with age was similar to that in noncarriers. The 3 genetic variants and the A443T variant in black men were associated with lower carotid IMT and lower prevalence of coronary calcification measured at ages 38–50 years. carriers of genetic variants of PCSK9 have lower LDL-C levels than noncarriers from the age 18 years to the age of 50 years. Such long-term reduction in LDL-C levels is associated with reduced atherosclerosis burden in black men.</td>
<td>Carriers of genetic variants of PCSK9 have lower LDL-C levels than noncarriers from the age 18 years to the age of 50 years. Such long-term reduction in LDL-C levels is associated with reduced atherosclerosis burden in black men.</td>
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<td>PLIC study (Norata et al. [47])</td>
<td>1,541 middle-aged Caucasian subjects; 1,351 subjects were enrolled to confirm the results for the PLIC population</td>
<td>Study the effects of E670G and I474V on the IMT of the common carotid artery and the possible relation to apoE polymorphisms</td>
<td>E670G was associated with significantly increased levels of plasma total cholesterol (4.9 % increase), LDL-C (7.2 % increase), and apoB (7.0 % increase). IMT was significantly increased in 670G carriers compared with individuals homozygous for the E allele (0.640±0.102 mm vs 0.652±0.092 mm, p&lt;0.05). The presence of the 670G allele was also significantly associated with a greater progression of IMT compared with 670EE subjects during the 7 years of follow-up. I474V SNP does not play a major role. Plasma total cholesterol, LDL-C, and apoB levels and IMT significantly increased from apoE2;PCSK9-670EE carriers to apoE4–PCSK9-670G carriers. Carriers of PCSK9 G670 and the apoE4 allele showed increased plasma LDL-C levels and IMT progression in the general population.</td>
<td>Carriers of PCSK9 G670 and the apoE4 allele showed increased plasma LDL-C levels and IMT progression in the general population.</td>
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<td>3 independent studies and meta-analyses: CCHS, CGPS, and CIHDS (Benn et al. [55])</td>
<td>CCHS: 10,032 subjects (age 20–80 years). CGPS: 26,013 subjects. CIHDS: 4,654 patients and 5,000 unmatched controls without IHD. Meta-analyses of present and previous studies (n=66,698)</td>
<td>Examine the association of R46L with LDL-C levels, and risk of IHD, MI, and death</td>
<td>Combining the 3 studies into 1 large study comprising 8,830 patients and 36,869 control subjects evidenced that R46L allele carriers had a 12 % reduction in LDL-C levels and a 28 % reduction in the risk of IHD compared with noncarriers (OR, 0.70; 95 % CI, 0.58–0.86; p=0.001). Meta-analyses confirmed those results. The observed 12 % reduction in LDL-C levels theoretically predicted an only 5 % reduction in the risk of IHD. The reduction in risk of IHD was larger than predicted by the observed reduction in LDL-C levels alone.</td>
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<td>Italian population (Guella et al. [56])</td>
<td>1,880 Italian patients with early onset of MI (mean age=39); 1,880 healthy matched control subjects; a control older population (n=1,056, +15 years)</td>
<td>Association of the PCSK9 R46L allele with premature MI and plasma lipid levels in the Italian population</td>
<td>LDL-C levels were significantly lower in R46L carriers than in noncarriers (mean, 116.2 mg/dl vs 137.4±47.3 mg/dl). The frequency of the R46L allele was higher in controls than in patients (1.42 % vs 0.80 %).</td>
<td>Highlights the importance of exposure to higher cholesterol concentrations in the older population to reveal the protective effect of the PCSK9 variant against MI.</td>
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<td>POLCA, OLIVIA, CORONA, and 60YO (Chernogubova et al. [57])</td>
<td>4 population-based studies of middle-aged subjects from the greater Stockholm area: POLCA, 624 subjects; OLIVIA, 591 subjects; CORONOA, 719 subjects; 60YO, 3,788 subjects. All subjects were free from CHD</td>
<td>Uncover genetic factors that contribute to the interindividual variation in level of circulating PCSK9 by analyzing its level in 4 cohorts of healthy, middle-aged Swedish subjects</td>
<td>As expected, the minor allele of the PCSK9 R46L variant was in all cohorts associated with reduced PCSK9 levels and decreased plasma LDL-C concentrations. The major finding is a common polymorphism (rs2479415, minor allele frequency 43.9 %), located approximately 6 kb upstream from PCSK9, which is independently associated with increased circulating PCSK9 levels and increased plasma LDL-C concentrations. There was no evidence that cholesterol-lowering variants of PCSK9 were associated with increased risk of total cancer in blacks (HR, 0.66; 95 % CI, 0.31–1.39) or whites (HR, 0.77; 95 % CI, 0.54–1.09)</td>
<td>No common variants have a major influence on PCSK9 levels, which exhibit a considerable interindividual variation. A novel, common polymorphism (rs2479415) was identified as an independent determinant of circulating PCSK9 levels</td>
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<td>PROSPEER 2013 (Postmus et al. [58])</td>
<td>5,804 subjects with preexisting vascular disease or at increased risk were randomly assigned to receive pravastatin or placebo</td>
<td>Assess the association between the PCSK9 SNP rs11591147 (R46L), cognitive performance, activities of daily living, and noncardiovascular clinical events</td>
<td>No association between rs11591147 (R46L) and cognitive performance, functional status, or nonvascular clinical events was observed either at the baseline or during follow-up (all p&gt;0.1).</td>
<td>It is unlikely that medication lowering LDL-C levels via inhibiting PCSK9 will affect cognitive performance, functional status, or risk of noncardiovascular clinical events</td>
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<td>ARIC (Folsom et al. [59])</td>
<td>7,082 men and 8,710 women aged 45–64 years were recruited; whites and African Americans</td>
<td>Examine whether PCSK9 variants (Y142X, C679X, and R46L) linked to lifetime low LDL-C concentrations are associated with cancer risk</td>
<td>There was no evidence that cholesterol-lowering variants of PCSK9 were associated with increased risk of total cancer in blacks (HR, 0.66; 95 % CI, 0.31–1.39) or whites (HR, 0.77; 95 % CI, 0.54–1.09)</td>
<td>With use of a Mendelian randomization design, there was no evidence that variants of PCSK9 that lower LDL-C levels increase the risk of total cancer</td>
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\[ apoB \text{ apolipoprotein B, } apoE \text{ apolipoprotein E, } ARIC \text{ Atherosclerosis Risk in Communities, } CCHS \text{ Copenhagen City Heart Study, } CGPS \text{ Copenhagen General Population Study, } CI \text{ confidence interval, } CIHDS \text{ Copenhagen Ischemic Heart Disease Study, } FH \text{ familial hypercholesterolemia, } HR \text{ hazard ratio, } IHD \text{ ischemic heart disease, } IMT \text{ intima–media thickness, } LCAS \text{ Lipoprotein Coronary Atherosclerosis Study, } LDL-C \text{ LDL cholesterol, } Lp(a) \text{ lipoprotein (a), } MI \text{ myocardial infarction, } OR \text{ odds ratio, } PAD \text{ peripheral artery disease, } PCSK9 \text{ proprotein convertase subtilisin/kexin type 9, } PROSPEER \text{ Prospective Study of Pravastatin in the Elderly at Risk, SNP single-nucleotide polymorphism} \]
and gain-of-function mutations of LDLR is also associated with a reduction of LDL-C levels in FH. It is noteworthy that the p.R496Q variant in PCSK9 was identified in a subject homozygous for apolipoprotein E2 who presented with type III hyperlipoproteinemia.

We identified PCSK9 p.L21tri (p.L15_L16ins2L) mutation in two French-Canadian families with familial combined hypercholesterolemia (FCHL) and in one French-Canadian woman and her father with hypercholesterolemia (FCHL) and in one French-Canadian woman and her father with hypercholesterolemia (FCHL) and in one French-Canadian family. Our report of the involvement of the L11 variant of PCSK9 in FCHL was the first report of the involvement of PCSK9 in this disease. This was confirmed by Brouwers et al. [65], who showed that PCSK9 levels were higher in FCHL patients than in normolipidemic relatives and spouses. They also reported that PCSK9 levels were related to markers of cholesterol synthesis in FCHL.

**PCSK9 and ApoB**

In vivo kinetics of apoB100-containing lipoproteins studied in two subjects carrying the p.S127R mutation in PCSK9 showed that PCSK9 mutation increased the production rate of apoB100 by threefold compared with controls or LDLR-mutated subjects, which is related to direct overproduction of VLDL (threefold), intermediate-density lipoprotein (threefold), and LDL (fivefold) [67]. Expression of the PCSK9 p.D374Y variant increases secretion of apoB100-containing lipoproteins from the cells by twofold to fourfold probably by reducing the degradation of nascent protein [24]. This also suggests that the variants of PCSK9 found in FH influence the secretion of apoB-containing lipoproteins. The same team produced transgenic mice expressing the p.D374Y variant of the human PCSK9 gene at physiological levels and showed that the phenotype closely matched that found in heterozygous p.D374Y patients and that reduced LDL receptor activity is not the sole cause of their hypercholesterolemia. The p.D374Y mice secreted more triglyceride-rich lipoproteins into the circulation than did wild-type mice [68]. Recently Sun et al. [69] studied the impact of PCSK9 overexpression (approximately 400-fold above the baseline) on apoB synthesis and secretion in mouse models. They demonstrated that endogenous PCSK9 interacted with apoB in hepatocytes. The physical interaction of PCSK9 with apoB acts to shunt apoB away from autophagosomes and degradation. In turn, most of the apoB would be destined for assembly and secretion as VLDL from hepatocytes. This observation is consistent with increased apoB production on overexpression of PCSK9. They thus proposed a new role for PCSK9 that involves shuttling between apoB and LDL receptor.

**PCSK9 Expression**

PCSK9 expression seems regulated by nutritional and hormonal status. PCSK9 is upregulated and increased by overexpression of sterol responsive element binding protein 2 (SREBP-2), cholesterol depletions, inflammation, administration of insulin, and statin therapy [71]. PCSK9 is down-regulated by the suppression of SREBP-2, cholesterol feeding, and berberine but also by glucagon [72], ethinylestradiol [72], chenodeoxycholic acid, and farnesoid X receptor agonist [73]. It is now established that several antihyperlipidemic drugs such as statins, fibrates, and ezetimibe induce an increase of PCSK9 levels. This might attenuate their cholesterol-lowering effect by reducing LDL receptor abundance at the cell surface. In 2004 Dubuc et al. [71] showed for the first time that the expression of PCSK9 mRNA was strongly induced by statins in a dose-dependent manner and that human, mouse, and rat PCSK9 promoters contain two typical conserved motifs for cholesterol regulation: a sterol regulatory element and an Sp1 site. Cellular and animal studies by several teams showed that statins increase SREBP-2 levels and lead to an increase of LDL receptor levels but also of the levels of PCSK9, which decreases the abundance of LDL receptor on the cell surface, limiting the hypocholesterolemic action of statins. Several studies in humans showed that different statin (atorvastatin, simvastatin, rosvastatin, etc.) treatments caused an increase in serum PCSK9 levels. The increase of PCSK9 levels caused by atorvastatin was 47 % for 80 mg versus 14 % for 10 mg. These data suggest that the explanation for why increasing doses of statins fail to achieve proportional LDL-C lowering is that statins increase PCSK9 levels in a dose-dependent fashion, and that the increased PCSK9 levels largely negate further statin-induced increases in hepatic LDL receptor levels [74]. Thus, it was suggested that a combination of a statin with a PCSK9 inhibitor could overcome this effect and enhance reduction of cholesterol levels. An initial proof-of-concept was provided by statin administration to Pcsk9−/− mice that produced an exaggerated increase in LDL receptors levels in liver and enhanced LDL clearance from plasma [75]. This has been confirmed in nonhuman primate models and humans.

Furthermore, when added to statin therapy, ezetimibe leads to a further increase of PCSK9 levels (77 % vs 45 % with statins alone) [76, 77]. Several studies have investigated the impact of fibrates on the circulating levels of PCSK9, but the results are conflicting [78–80]. This might be due to the use of different analytical techniques to measure circulating PCSK9 levels. However, there is more evidence currently that fibrates...
increases serum PCSK9 levels and that these increases are highly correlated with fenofibrate-induced changes in LDL-C levels [81].

**PCSK9 Levels in Blood**

PCSK9 is present in human plasma, but the factors that contribute to differences in plasma concentrations are not very well known. Several teams have developed an enzyme-linked immunosorbent assay (ELISA) to measure PCSK9 in plasma. Plasma levels of PCSK9 vary at least 100-fold [82]. Serum PCSK9 levels measured by ELISA seem to be directly correlated with serum LDL-C and total cholesterol levels [83]. In hypercholesterolemic subjects, PCSK9 levels were higher than in control subjects, and increased in proportion to the dose of statin, combined or not combined with ezetimibe [71]. Plasma PCSK9 levels are positively associated with LDL-C levels in FH patients, and might contribute to the phenotypic severity in this disorder [84]. Serum PCSK9 levels display a diurnal rhythm that closely parallels that of cholesterol synthesis [85]. PCSK9 concentrations were lower with a polyunsaturated fatty acid diet [86], a Mediterranean diet [87], administration of estrogens [88], and administration of growth hormone [88]. The PCSK9 level was found to be associated with the γ-glutamyl transferase level in diabetic patients [89] and with carotid intima–media thickness in hypertensive patients [90]. The plasma level of lipoprotein-associated phospholipase A2 is inversely correlated with PCSK9 levels [91]. The plasma level of PCSK9 was increased at the baseline in proteinuric subjects, predicted lipoprotein responses to proteinuria reduction, but remained unchanged after proteinuria reduction [92]. At physiological levels observed in human obesity, it was shown that resistin increases cellular expression of PCSK9, which enhances intracellular LDL receptor lysosomal degradation [93]. Nevertheless, no positive association of plasma PCSK9 with resistin was found in lean and moderately obese individuals [94].

**Therapeutic Strategies to Reduce PCSK9 Levels or Inhibit PCSK9**

Several strategies to inhibit PCSK9 or lower PCSK9 levels have been investigated. Specific inhibition of PCSK9 via a classic pharmaceutical approach such as orally active molecules targeting PCSK9 seems difficult. Strategies known to target proteins not accessible to small molecules have been tested. Gene silencing by RNA interference and specific antibodies or competing peptides targeting PCSK9 have been developed. The details of these molecules or antibodies, and the results obtained in cellular models or animal models (mice or monkeys) and the related patents were reviewed in a previous article [95]. Clinical studies have been launched by several pharmaceutical companies. The details of these studies, their results, and the adverse reactions are given in Table 2 [96, 97•, 98–108, 109••, 110, 111, 112••, 113–115]. The first strategies based on gene silencing that targets PCSK9 intracellular and extracellular functions consisted in a subcutaneous administration of antisense oligonucleotide (ASO) targeting PCSK9 or small interfering RNA (siRNA). ASO studies have been conducted mainly with a second-generation ASO produced by Isis Pharmaceuticals, or with a 13-mer locked nucleic acid (LNA) ASO or a 14-mer LNA-ASO specific for a human PCSK9 sequence from Santaris Pharma. They showed in cellular, mouse, and monkey models a significant reduction of hepatic Pcsk9 mRNA expression and of total cholesterol and LDL-C levels. These ASOs were well tolerated in animals. The most frequent adverse event with this approach was injection-site erythema that seems to resolve spontaneously. To determine whether injection of these compounds results in toxic effects in humans, a clinical trial has been launched by Bristol-Myers Squibb using BMS-844421 (BMS-PCSK9Rx), which is an ASO developed by Isis. Nevertheless, the clinical study has been discontinued and no data are available. The clinical trial launched by Santaris Pharma to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of SPC5001 (a 14-mer LNA-ASO specific for a human PCSK9) has also been discontinued and no data are available either (Table 2). PCSK9 gene silencing in mice and monkeys has also been achieved using siRNA. Active, cross-species siRNAs capable of targeting murine, rat, nonhuman primate, and human PCSK9 have been developed by Frank-Kamenetsky and coworkers [97•, 116]. Delivery of the PCSK9 siRNA to the liver was facilitated by a lipidoid nanoparticle, minimizing toxicity. A phase I clinical trial was conducted by Alnylam Pharmaceuticals to determine the safety, tolerability, pharmacokinetics, and pharmacodynamics of a single dose of ALN-PCS02. The results are given in Table 2.

Other molecules that are currently being studied are adnectins (BMS-962476) [96], which are in phase I trials (Table 2), and small molecule inhibitors (SX-PCSK9, detailed at http://www.serometrix.com/pipeline.html).

Several antibodies or competing peptides targeting PCSK9 have been developed and studied in cellular and animal models (mice and monkeys). Clinical studies are being performed by pharmaceutical companies: LGT209 by Novartis is in a phase II study, LY3015014 by Eli Lilly is in a phase II study, RG7652 (MPSK3169A) [98] by Genentech (Roche) is in a phase II study, and RN316 (bococizumab) by Pfizer has undergone phase I studies and is now in phase II [99] and phase III studies. Available published results of these studies are reported in Table 2.
Table 2 Major clinical trials targeting PCSK9: details of the published studies using RNA interference or anti-PCSK9 monoclonal antibodies to lower PCSK9 levels or inhibit PCSK9, the results, and adverse reactions

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<tr>
<td><strong>Adnectins: BMS-962476, Bristol-Myers Squibb, phase I</strong></td>
<td>Maximal dose related reductions of LDL-C levels up to 48 % occurred between days 4 and 14. Doses above 0.3 mg/kg reduced free PCSK9 levels by more than 90 %</td>
<td>2 serious adverse events were considered unrelated to the study drug, and none resulted in study discontinuation. BMS-962476 was well tolerated, and the adverse effects were similar to those with placebo</td>
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<td>BMS962476 is an anti-human PCSK9 adnectin-based protein therapeutic formatted with 40-kDa branched poly(ethylene glycol) being developed to prevent PCSK9–LDL receptor binding and reduce LDL-C levels. A randomized, double-blind, placebo-controlled, sequential panel, partial overlapping single ascending dose study. 64 healthy subjects on a diet or statins and LDL-C levels above 130 mg/dl or above 100 mg/dl, respectively. At each dose 8 subjects were randomized 3:1 to receive single subcutaneous or intravenous dose of BMS-962476 or placebo. Treatment began in diet-only subjects with 0.01 mg/kg subcutaneously and on the basis of tolerability escalated was sequentially to 0.03, 0.1, and 0.3 mg/kg subcutaneously, followed by 0.3 and 1.0 mg/kg intravenously. Subjects taking statins received subcutaneous doses of 0.1 and 0.3 mg/kg. Study duration, 43 days. Stein et al. [96]; ClinicalTrials.gov identifier NCT01587365</td>
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<td><strong>RNA interference: ALN-PCS02, Alnylam Pharmaceuticals, phase I</strong></td>
<td>The 0.400 mg/kg group showed a mean 70 % reduction in circulating PCSK9 plasma protein levels (( p &lt; 0.0001 )) and a mean 40 % reduction in LDL-C levels from the baseline relative to placebo (( p &lt; 0.0001 ))</td>
<td>The proportions of patients affected by treatment-emergent adverse events were similar in the ALN-PCS02 and placebo groups (79 % vs 88 %). Safe and well tolerated. A mild, macular, erythematous rash occurred with equal frequency in participants given ALN-PCS02 and those given placebo. No clinically significant dose-dependent changes in any laboratory indices, including liver function tests, creatine phosphokinase, C-reactive protein, and hematological measures</td>
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<td>Randomized, single-blind, placebo-controlled, single phase I dose-escalation study. 32 healthy adult volunteers (aged 18–65 years) with serum LDL-C level of 3.00 mmol/l or higher. 1 dose of intravenously administered ALN-PCS02 (with doses ranging from 0.015 to 0.400 mg/kg) or placebo. Study duration, 180 days. Fitzgerald et al. [97], ClinicalTrials.gov identifier NCT01437059</td>
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<td><strong>Specific antibodies or competing peptides</strong></td>
<td>LDL-C concentration reduction of 90 mg/dl (60 %) from the baseline, with a dose-dependent effect that appeared to saturate at the highest doses. RG7652 had similar LDL-C-lowering effects and kinetics when added to atorvastatin therapy</td>
<td>No dose-limiting safety effects were identified. No subjects discontinued taking the study drug because of adverse events. 37 adverse events, all mild, were attributed to the study drug: 27 in 14 RG7652 subjects; 10 in 6 placebo subjects</td>
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<td><strong>RG7652 (MPSK3169A), Genentech (Roche), phase I</strong></td>
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<td>Fully human IgG1 monoclonal antibody directed against PCSK9. 80 healthy volunteers (aged 19–64 years) with elevated serum LDL-C concentrations. Single and multiple doses of RG7652 and placebo given subcutaneously. Subcutaneous administration of 6 ascending single doses and 4 multiple doses of RG7652 and placebo. 2 multiple-dose cohorts had atorvastatin therapy (40 mg daily) prior to administration of the study drug. Tingley et al. [98]</td>
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<td><strong>Bococizumab (RN316/PF-04950615), Pfizer, phase II</strong></td>
<td>Bococizumab significantly reduced LDL-C levels across all doses.</td>
<td>Adverse events were similar across placebo and bococizumab groups</td>
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<td>A 24-week, multicenter, randomized, double-blind, placebo-controlled trial.</td>
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<td>Statin-treated subjects aged 18 years or older with a diagnosis of hypercholesterolemia and LDL-C level of 80 mg/dl or higher</td>
<td>Bococizumab at 150 mg every 14 days significantly reduced LDL-C levels by 53 mg/dl versus placebo at week 12, inclusive of the protocol-directed dose reductions in 33 % of subjects. Up to 44 % of subjects in the bococizumab groups had their dose reduced. Modeling predicted greater LDL-C level reduction in the absence of bococizumab dose reduction</td>
<td>Few subjects discontinued treatment owing to treatment-related adverse events. The every 14 days regimen is being evaluated in larger trials</td>
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<td>Subjects were randomized to receive placebo or bococizumab (50, 100, or 150 mg) subcutaneously every 14 days; or placebo or bococizumab (200 or 300 mg) every 28 days. Doses were reduced if LDL-C readings were 25 mg/dl or lower. Ballantyne et al. [99], ClinicalTrials.gov identifier NCT01592240</td>
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<td>AMG 145 (evolocumab), Amgen, phase I</td>
<td>Phase Ia: up to 64 % reduction of LDL-C levels after 1 dose of AMG 145 of 21 mg or greater compared with placebo (p&lt;0.0001); up to 55 % reduction of apoB levels (p&lt;0.0001); reduction of free PCSK9 levels. Phase Ib: up to 81 % reduction of LDL-C levels compared with placebo (p&lt;0.001) at nadir; up to 59 % reduction of apoB levels (p&lt;0.001); reduction of mean serum levels of Lp(a) by 27 % (35 mg weekly for 6 weeks, p&lt;0.033) to 50 % (heterozygous FH cohort 140 mg every 2 weeks for3 weeks, p&lt;0.001) versus placebo; reduction of free PCSK9 levels Rapid and sustained dose-dependent reduction in LDL-C levels of 43 % and 55 % with the AMG 145 doses of 350 and 420 mg, respectively (measured by ultracentrifugation), compared with 1 % increase in the placebo group, Reductions in total cholesterol, non-HDL-C, and apoB levels were consistent with those seen for LDL-C.</td>
<td>The overall incidence of treatment-emergent adverse events was similar between the AMG 145 and placebo groups. No serious adverse events or adverse events leading to discontinuation occurred during either study. No clinically important effects of AMG 145 were observed on selected laboratory parameters, electrocardiograms, or vital signs. No neutralizing antibodies to AMG 145 were detected during either study.</td>
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<td>Interventional, randomized, double-blind, placebo-controlled, ascending multiple dose trial.</td>
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<td>1 case of myositis was reported as an adverse event in each of 3 phase Ia subjects (7 %, AMG 145). 1 event was reported as treatment-related; it was mild, occurred 21 days after the dose concurrent with elevation of the creatine kinase level to more than 2.5 times to 5 times the upper limit of normal, and resolved 23 days after the dose</td>
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<td>Phase Ia: 56 healthy adults were randomized to receive 1 dose of AMG 145 at 7, 21, 70, 210, or 420 mg subcutaneously or 21 or 420 mg intravenously, or matching placebo. Phase Ib: 57 subjects with hypercholesterolemia (aged 18–70 years): 40 subjects taking low- to moderate-dose statins received multiple subcutaneous doses of AMG 145 (14 or 35 mg weekly for 6 weeks, 140 or 280 mg every 2 weeks for 3 weeks, and 420 mg every 4 weeks for 2 weeks) or matching placebo; 11 subjects taking high-dose statins and 6 subjects with heterozygous FH were randomized to receive subcutaneously AMG 145 (140 mg) or placebo every 2 weeks for 3 weeks. Dias et al. [100], ClinicalTrials.gov identifier NCT01335322</td>
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<td>AMG 145 (evolocumab), Amgen, phase II</td>
<td>Reduction of LDL-C with PCSK9 Inhibition in Heterozygous Familial Hypercholesterolemia Disorder Study (RUTHERFORD) is a multicenter, double-blind, placebo-controlled randomized trial. 168 patients aged 18–75 years with heterozygous FH and LDL-C levels of 2.6 mmol/l (100 mg/dl) or greater despite statin therapy with or without ezetimibe, and levels of triglycerides of 400 mg/dl or lower. Blinded subcutaneous injections every 4 weeks (weeks 0, 4, and 8) of 350 or 420 mg of AMG 145 or placebo. Duration, 12 weeks. Raal et al. [101], ClinicalTrials.gov identifier NCT01357551</td>
<td>No clinically significant safety findings. The most commonly reported adverse events were nasopharyngitis, injection-site pain, headache, and skin burning sensation. 3 patients (1 AMG 145 350 mg, 1 AMG 145 420 mg, and 1 placebo) experienced adverse events that led to discontinuation of the investigational product. Of these, diarrhea, nausea, and groin pain in a patient receiving AMG 145 at 420 mg were considered possibly treatment-related by the investigator; the other patient receiving AMG (at 350 mg) discontinued use of the investigational product because of weight gain. 1 patient (2 %) in the AMG 145 420-mg group experienced an elevation in creatine kinase level of more than 10 times the upper limit of normal at week 8. Serious treatment-emergent adverse events included atrial fibrillation in 1 patient and appendicitis and postoperative wound infection in another; none of these were considered treatment-related</td>
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<td>Rapid and sustained dose-dependent reduction in LDL-C levels of 43 % and 55 % with the AMG 145 doses of 350 and 420 mg, respectively (measured by ultracentrifugation), compared with 1 % increase in the placebo group, Reductions in total cholesterol, non-HDL-C, and apoB levels were consistent with those seen for LDL-C. Modest, but statistically significant, dose-dependent reductions of 15 % and 20 % in the levels of triglycerides. Modest, statistically significant increase of approximately 7 % in HDL-C levels. Changes in apoA1 were not significant. Levels of PCSK9 were reduced by 41 % from the baseline with both doses at week 12. Significant reductions in Lp(a) levels of 23 % and 32 % compared with placebo</td>
<td>No clinically significant safety findings. The most commonly reported adverse events were nasopharyngitis, injection-site pain, headache, and skin burning sensation. 3 patients (1 AMG 145 350 mg, 1 AMG 145 420 mg, and 1 placebo) experienced adverse events that led to discontinuation of the investigational product. Of these, diarrhea, nausea, and groin pain in a patient receiving AMG 145 at 420 mg were considered possibly treatment-related by the investigator; the other patient receiving AMG (at 350 mg) discontinued use of the investigational product because of weight gain. 1 patient (2 %) in the AMG 145 420-mg group experienced an elevation in creatine kinase level of more than 10 times the upper limit of normal at week 8. Serious treatment-emergent adverse events included atrial fibrillation in 1 patient and appendicitis and postoperative wound infection in another; none of these were considered treatment-related</td>
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<td>Monoclonal Antibody Against PCSK9 to Reduce Elevated LDL-C in Subjects Currently Not Receiving Drug Therapy for Easing Lipid Levels (MENDEL), the first and largest reported</td>
<td>AMG 145 significantly reduced LDL-C concentrations in all dose groups. At week 12, the changes from the baseline were as follows: with AMG 145 every 2 weeks,</td>
<td>Treatment-emergent adverse events occurred in 136 (50 %) of 271 patients in the AMG 145 groups, 41 (46 %) of 90 patients in the placebo groups, and 26 (58 %) of 45 patients</td>
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<td>multicenter anti-PCSK9 monotherapy trial, is a randomized, placebo- and ezetimibe-controlled, double blind, dose-ranging study. 406 hypercholesterolemic subjects (aged 18–75 years) with LDL-C levels between 2.6 and 4.9 mmol/l, levels of triglycerides of 4.5 mmol/l or less, and 10-year Framingham risk score for coronary heart disease of up to 10 %. 9 treatment groups: subcutaneously administered placebo or AMG 145 at 70, 105, or 140 mg every 2 weeks; subcutaneously administered placebo or AMG 145 at 280, 350, or 420 mg every 4 weeks; orally administered ezetimibe at 10 mg/day. Koren et al. [102], ClinicalTrials.gov identifier NCT013757777</td>
<td>Goal Achievement after Utilizing an Anti-PCSK9 Antibody in Statin Intolerant Subjects (GAUSS) is a randomized, double-blind, multicenter, placebo- and ezetimibe-controlled study. 160 statin-intolerant hypercholesterolemic subjects (aged 18–75 years). Subcutaneous administration of AMG 145 (280, 350, or 420 mg) every 4 weeks, AMG 145 (420 mg) every 4 weeks plus ezetimibe (10 mg daily), and ezetimibe (10 mg daily) plus placebo (every 4 weeks) Duration, 12 weeks Sullivan et al. [103], ClinicalTrials.gov identifier NCT01375764</td>
<td>LDL-C Assessment with PCSK9 Monoclonal Antibody Inhibition Combined with Statin Therapy–Thrombolysis in Myocardial Infarction 57 (LAPLACE–TIMI 57) is a multinational, randomized, double-blind, dose-ranging, placebo-controlled study. 631 patients (aged 18–80 years) with LDL-C levels above 2.2 mmol/l taking a stable dose of statin (with or without ezetimibe). Subcutaneous injections of AMG 145 (70, 105, or 140 mg) or matching placebo every 2 weeks, or subcutaneous injections of AMG 145 (280, 350, or 420 mg) or matching placebo every 4 weeks. Duration, 12 weeks. Giugliano et al. [104], Kohli et al. [105], ClinicalTrials.gov identifier: NCT01380730</td>
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| | −41.0 % for 70 mg, −43.9 % for 105 mg, and −50.9 % for 140 mg; with AMG 145 every 4 weeks, −39.0 % for 280 mg, −43.2 % for 350 mg, and −48.0 % for 420 mg; with placebo every 2 weeks, −3.7 %; with placebo every 4 weeks, 4.5 %; and with ezetimibe −14.7 %. Significant differences versus placebo were noted for nearly all comparisons of total cholesterol, non-HDL-C, VLDL cholesterol, apoB, apoA1, and Lp(a) concentrations, and ratios of total cholesterol to HDL-C and apoB to apoA1 concentrations. AMG 145 also led to significant reductions in the levels of free PCSK9 (all groups), increases in HDL-C levels (significant for 105 mg every 2 weeks and 140 mg every 2 weeks), and slight but nonsignificant reductions in the concentrations of triglycerides. At week 12, the mean changes in LDL-C levels were −67 mg/dl (−41 %) for the 280-mg group, −70 mg/dl (−43 %) for the 350-mg group, −91 mg/dl (−51 %) for the 420-mg group, and −110 mg/dl (−63 %) for the 420-mg/ezetimibe group compared with −14 mg/dl (−15 %) for the placebo/ezetimibe group (p < 0.001) (cholesterol measured after ultracentrifugation). The maximal reduction in LDL-C level was evident within 2 weeks of commencement of AMG 145 therapy, with or without ezetimibe, and the effect was maintained throughout the 12 weeks of the study. Reduction of total cholesterol, non-HDL-C, and apoB levels, and the total cholesterol/HDL-C and apo B/apoA1 ratios. Modest increase of HDL-C levels from 6 % to 12 % compared with 1 % with placebo/ezetimibe. Increase of apoA1 levels Lp(a) level was reduced by 20–26 % with AMG 145 and 29 % with AMG 145/ezetimibe. Mean free PCSK9 levels at week 12 declined by up to 48.4 % from pretreatment levels with AMG 145 monotherapy and by 1.5 % with ezetimibe monotherapy. Reductions in LDL-C concentrations versus placebo at 12 weeks for the groups receiving AMG 145 every 2 weeks from 41.8 % to 66.1 % and for the groups receiving AMG 145 every 4 weeks from 41.8 % to 50.3 %. Significant reductions from the baseline at week 12 compared with placebo in non-HDL-C and apoB concentrations, and total cholesterol/HDL-C and apoB/apoA1 ratios. Significant reduction in PCSK9 concentrations compared with placebo: from 46.3 % to 72.5 %. 5 of the AMG 145 dose regimens increased HDL-C concentration by 4.5–8.1 % compared with placebo. Administration of AMG 145 was well tolerated, with no significant safety findings reported. 4 serious adverse events were reported with AMG 145 (coronary artery disease, acute pancreatitis, hip fracture, syncope). None were considered treatment-related. Myalgia was the commonest treatment-emergent adverse event during the study, occurring in 5 patients (15.6 %) in the 280-mg group (n = 32); 1 patient (3.2 %) in the 350-mg group (n = 31), 1 patient (3.1 %) in the 420-mg group (n = 32), 6 patients (20.0 %) receiving 420-mg AMG 145/ezetimibe, and 1 patient (3.1 %) receiving placebo/ezetimibe | | | |

| | in the ezetimibe group; no deaths or serious treatment-related adverse events were reported. Overall, the most frequently reported events in the AMG 145, placebo, and ezetimibe groups were upper respiratory tract infection (6 %, 8 %, and 11 %, respectively), nasopharyngitis (4 %, 3 %, and 9 %, respectively), back pain (3 %, 4 %, and 2 %, respectively), and diarrhea (4 %, 3 %, and 2 %, respectively). Injection site reactions were reported in 15 patients (6 %) in the AMG 145 groups | No treatment-related serious adverse events occurred. The frequencies of treatment-related adverse events were similar in the AMG 145 and placebo groups [39 (8 %) of 474 vs 11 (7 %) of 155]; none of these events were severe or life-threatening. Adverse events were reported in 55 % of patients given the study drug, with a higher frequency in those given AMG 145 (58 %) than in those given placebo (46 %). The most commonly reported adverse events in the AMG 145 group were nasopharyngitis, cough, and nausea, none of which were significantly different between AMG 145 and placebo. Overall, 11 (2 %) of 629 patients reported injection-site reactions (e.g., pruritus,
Open-Label Study of Long-Term Evaluation Against LDL-C (OSLER) is a multicenter, controlled, open-label extension study. Of 1,359 randomized and dosed patients in the 4 AMG 145 phase II parent studies, 1,104 were randomized to receive standard of care treatment plus subcutaneous injection of AMG 145 (420 mg) every 4 weeks or standard of care treatment alone. Koren et al. [106], Mearns [107], ClinicalTrials.gov identifier NCT01439880

AMG 145 (evolocumab), Amgen, phase II/phase III

Trial Evaluating PCSK9 Antibody in Subjects with LDL Receptor Abnormalities (TESLA) is a 2-part, phase II/phase III study.

Part A is an open-label, single-arm, multicenter pilot study: 8 patients with LDL-receptor-negative or LDL-receptor-defective homozygous FH receiving stable drug therapy (aged 12–80 years); subcutaneous administration of AMG 145 (420 mg) every 4 weeks for 12 weeks or more, followed by AMG 145 (420 mg) every 2 weeks for an additional 12 weeks.

Part B: double-blind, randomized, placebo-controlled, multicenter study.

Stein et al. [108], ClinicalTrials.gov identifier NCT01588496

AMG 145 (evolocumab), Amgen, phase III

Durable Effect of PCSK9 Antibody Compared with Placebo Study (DESCARTES) is a randomized, double-blind, placebo-controlled, phase III trial.

901 patients with hyperlipidemia were stratified according to the risk categories outlined by the Adult Treatment Panel III of the National Cholesterol Education Program. On the basis of this classification, patients were initially given a background lipid-lowering therapy with diet alone or diet plus atorvastatin at a dosage of 10 mg daily, atorvastatin at a dosage of 80 mg daily, or atorvastatin at a dosage of 80 mg daily plus ezetimibe at a dosage of 10 mg daily, for a run-in period of 4–12 weeks. Patients with an LDL-C level of 75 mg/dl (1.9 mmol/l) or higher were then randomly assigned in a 2:1 ratio to receive either AMG 145 (420 mg) or placebo every 4 weeks.

Reduction of LDL-C levels at 12 weeks was consistent with levels at 52 weeks, showing long-term efficacy. Compared with placebo, the mean LDL-C concentration reductions from the baseline at week 52 were 56 % for the group with only diet modifications, 62 % for the group receiving atorvastatin at 10 mg, 57 % for the group receiving atorvastatin at 80 mg, and 49 % for the group receiving atorvastatin at 80 mg plus ezetimibe at 10 mg.

There were significant reductions from the baseline in the levels of apoB, non-HDL-C, Lp(a), and triglycerides. There were increase of 5.4 % in the HDL-C level ($P<0.001$) and of 3.0 % in the apoA1 level ($P<0.001$). It may be that patients who have already been treated with high-dose statins or combination treatments have a reduced ability to further reduce LDL-C levels.

The overall incidence of adverse events occurring during treatment was similar in the AMG 145 group and the placebo group. The commonest adverse events in the AMG 145 group were nasopharyngitis, upper respiratory tract infection, influenza, and back pain. Elevations of creatine kinase levels to more than 5 times the upper limit of the normal range occurred in 7 patients (1.2 %) in the AMG 145 group and in 1 patient (0.3 %) in the placebo group, with myalgia reported by 24 patients (4.0 %) and 9 patients (3.0 %), respectively; elevations of aminotransferase levels to more than 3 times the upper limit of the normal range occurred in 5 patients (0.8 %) and 3 patients (1.0 %), respectively.

Part A study (pilot study):

LDL-C concentration was reduced by 16.5 % in the 4-week treatment and by 13.9 % in the 2-week treatment. No reduction was seen in the 2 LDL-receptor-negative patients. Over the treatment periods, the mean±SD LDL-C concentration reductions in the 6 LDL-receptor-defective patients were 19.3 % and 26.3 % with 4- and 2-week dosing, respectively ($P=0.0313$ for both values), ranging from 4 % to 48 % with 2-week dosing.

The level of apoB was reduced by 14.9 % in the 4-week treatment and by 12.5 % in the 2-week treatment. The level of Lp(a) was reduced by 11.7 % in the 4-week treatment and by 18.6 % in the 2-week treatment.

The mean reductions in free PCSK9 levels at week 12 after every-4-week and every-2-week treatment were 69.5 % and 80.4 %, respectively, with 4- and 2-week dosing, respectively ($P=0.0313$ for both values), ranging from 49 % to 100 % with 2-week dosing.

Part B study (pilot study):

At 52 weeks, there was a 52.3 % reduction of LDL-C levels, a 42 % reduction in apoB levels, 1/3 reduction of Lp(a) levels, a 9 % reduction of the levels of triglycerides, a 9 % increase of HDL-C levels, and a 4-5 % increase of apoA1 levels.

In total, 73.1 % of controls and 81.4 % of the AMG 145-treated patients experienced adverse events over the 52 weeks; the investigators classified 5.6 % of all adverse events as being possibly related to AMG 145. There were injection-site reactions in 5.2 % of the AMG 145 group.

Adverse events

Common effects (2 %) of 155 were in the placebo group and 8 (2 %) of 474 were in the AMG 145 groups ($P=0.81$); 11 adjudicated clinical cardiovascular events were reported in 8 patients. There were no imbalances in cardiovascular events between groups.

3 of 155 patients reported adverse events, all of which were considered not serious and unrelated to treatment by the investigators. Antibodies to AMG 145 were not detected during treatment.

No patients had creatine kinase concentration elevations more than 5 times the upper limit of normal or concentrations of liver enzymes (alanine aminotransferase or aspartate aminotransferase) more than 3 times the upper limit of normal.
Table 2 (continued)

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<th>Characteristics/subjects/administration</th>
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<tr>
<td>Blom et al. [109], ClinicalTrials.gov identifier NCT01516879</td>
<td>Subcutaneous administration of AMG 145 (140 mg) 40 healthy volunteers (aged 18–65 years) with fasting LDL-C levels greater than or equal to 100 mg/dl and below 190 mg/dl and Framingham risk scores of 10% or lower were randomized (1:1:2:2) to receive placebo orally and placebo subcutaneously biweekly; placebo orally and placebo subcutaneously monthly; ezetimibe and placebo subcutaneously biweekly; ezetimibe and placebo subcutaneously monthly; placebo and AMG 145 (140 mg) orally biweekly; or placebo and AMG 145 (420 mg) orally monthly. Study duration, 12 weeks.</td>
<td>AMG 145 treatment reduced LDL-C levels from baseline, on average, by 55–57% more than placebo and 38–40% more than ezetimibe (P &lt;0.001 for all comparisons). AMG 145 significantly decreased apoB, Lp(a), and non-HDL-C levels, and total cholesterol/HDL-C and apoB/apoA1 ratios. Significant HDL-C concentration increases were observed with AMG 145. The levels of triglycerides and VLDL cholesterol were significantly lowered with monthly administration of AMG 145 versus placebo or ezetimibe and in some comparisons in the biweekly group</td>
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<td>GAUSS-2 is a multicenter, global, randomized, double-blind, ezetimibe-controlled study. 307 patients with high cholesterol levels who could not tolerate effective doses of at least 2 different statins due to muscle-related side effects. Subcutaneous administration of AMG 145 (140 mg) biweekly or AMG 145 (420 mg) monthly both with daily oral administration of placebo; or subcutaneous administration of placebo biweekly or monthly both with daily oral administration of ezetimibe (10 mg). Study duration, 12 weeks. Stroes et al. [111], ClinicalTrials.gov identifier NCT01763905</td>
<td>AMG 145 reduced LDL-C levels from the baseline by 53–56%, corresponding to treatment differences versus ezetimibe of 37% to 39% (p&lt;0.001)</td>
<td>Treatment-emergent adverse events and laboratory abnormalities were comparable across treatment groups. Muscle adverse events occurred in 12% of AMG 145-treated patients and 23% of ezetimibe-treated patients</td>
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<td>2 trials which also resulted in a significant LDL-C concentration reduction but have not been published yet are as follows: LAPLACE-2: 1,896 patients receiving AMG 145 subcutaneously (140 mg every 2 weeks or 420 mg monthly) in combination with different daily doses of statin therapy for 12 weeks (ClinicalTrials.gov identifier NCT01763866, January 2014)</td>
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<td>RUTHERFORD-2: 329 heterozygotes FH patients receiving a stable dose of a statin and other lipid-lowering therapies receiving subcutaneous administration of AMG 145 or placebo every 2 weeks or once monthly for 12 weeks (ClinicalTrials.gov identifier NCT01763918, January 2014) Other studies are being conducted on larger numbers of patients. GLAGOV: 950 subjects undergoing coronary catheterization given AMG 145 for 78 weeks (ClinicalTrials.gov identifier NCT01813422, January 2016) OSLER-2: 3,515 subjects with hyperlipidemia given AMG 145 for 104 weeks (ClinicalTrials.gov identifier NCT01854918, January 2017) FOURIER: 22,500 patients with clinically evident cardiovascular disease given AMG 145 every 2 weeks or once monthly with an effective statin for 5 years (ClinicalTrials.gov identifier NCT01764633, February 2018) GAUSS-3: 500 statin-intolerant subjects. The study is divided into 3 parts (A, B, C). Part A is a double-blind, placebo-controlled, 2-period crossover rechallenge of atorvastatin (20 mg). Part B is a 24-week double-blind comparison of AMG 145 and ezetimibe. Part C is a 2-year open-label AMG 145 extension. (ClinicalTrials.gov identifier NCT01984424, April 2018) TAUSSIG: 310 subjects with severe FH given 2 different subcutaneous doses of AMG 145 every 2 or 4 weeks for 5 years (ClinicalTrials.gov identifier NCT01624142, January 2020)</td>
<td>Well tolerated. A few injection-site reactions, which were mild. Single-dose studies: 2 subjects in the single-dose studies had serious adverse events: a 33-year-old man receiving placebo intravenously, who had abdominal pain and rectal bleeding on study day 83, and a 19-year-old man with a history of appendectomy receiving 50 mg of</td>
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<td>REGN727 (SAR236553) (alirocumab), Regeneron Pharmaceuticals and Sanofi-Aventis, phase I</td>
<td>Randomized, double-blind, placebo-controlled, single ascending dose study 40 healthy volunteers (aged 18–65 years) with LDL-C level above 100 mg/dl (2.59 mmol/l) Intravenous administration of single dose of REGN727 versus placebo for 106 days: after safety assessment with 0.3 mg/kg, the dose of REGN727 was increased sequentially to 1.0, 3.0, 6.0, and 12.0 mg/kg.</td>
<td>Up to 65% reduction of LDL-C levels (28.1–65.4%) which was dose-dependent, with higher doses producing prolonged reductions that were sustained up to day 64. 25–35% reduction of total cholesterol levels and 25–40% reduction of the levels of triglycerides Little or no reduction of HDL-C levels</td>
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<td>Stein et al. [112], Crunkhorn [113], ClinicalTrials.gov identifier NCT01026597</td>
<td>Randomized, double-blind, placebo-controlled, single ascending dose study. 32 healthy volunteers (aged 18–65 years) with LDL-C levels above 100 mg/dl (2.59 mmol/l). 4 sequential dose groups receiving (different ascending doses) of subcutaneously administered REGN727 (50, 100, 150, 250, and mg) or placebo. Study duration, 106 days. Stein et al. [112], ClinicalTrials.gov identifier NCT01074372</td>
<td>Up to 46 % reduction of LDL-C levels (32.5–45.7 %) which was dose dependent, with higher doses producing prolonged reductions that were sustained up to day 64. 25–35 % reduction of total cholesterol and 25–40 % reduction of the levels of triglycerides. Little or no reduction of HDL-C levels. For REGN727 plus atorvastatin versus placebo, LDL-C levels were significantly reduced (p&lt;0.001 for all comparisons): for 50 mg, LDL-C concentration was reduced to 77.5 mg/dl (2.00 mmol/l), a difference from the baseline of -39.2 %; for 100 mg, LDL-C concentration was reduced to 61.3 mg/dl (1.59 mmol/l), -53.7 %; for 150 mg, LDL-C concentration was reduced to 53.8 mg/dl (1.39 mmol/l), -61 %, as compared with placebo. Up to 18 % increase of HDL-C levels and 13 % increase of apoA1 levels. Reduction of Lp(a) levels. Degree and duration of LDL-C concentration decrease corresponded to the reduction of levels of free PCSK9 in plasma.</td>
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<td>Stein et al. [113], Crunkhorn [114], ClinicalTrials.gov identifier NCT01161082</td>
<td>Randomized, double-blind, placebo-controlled, fixed-dose, multicenter study (at 20 sites in the USA) 92 patients (aged 18–75 years) with primary hypercholesterolemia and LDL-C levels of 100 mg/dl or higher after treatment with 10 mg of atorvastatin for at least 7 weeks Subcutaneous administration of 1 ml of REGN727 or placebo every 2 weeks plus 80 mg of atorvastatin daily, or subcutaneous administration of 1 ml of REGN727 every 2 weeks plus 10 mg of atorvastatin or placebo daily REGN727 was supplied at a concentration of 150 mg/ml. Study duration, 8 weeks. Roth et al. [114], ClinicalTrials.gov identifier NCT01288469</td>
<td>The least-squares mean (±standard error) percent reduction from the baseline in LDL-C concentration was (73.2±3.5 %) with 80 mg of atorvastatin plus REGN727 versus (17.3±3.5 %) with 80 mg of atorvastatin plus placebo (P&lt;0.001) and (66.2±3.5 %) with 10 mg of atorvastatin plus REGN727. A reduction of 31.0 % in Lp(a) level was seen in patients receiving 80 mg of atorvastatin plus REGN727 versus 2.7 % in patients receiving 80 mg of atorvastatin plus placebo. A small increase in HDL-C level in patients receiving 80 mg of atorvastatin plus REGN727. All the patients who received REGN727, as compared with 52 % of those who received 80 mg of atorvastatin plus placebo, attained LDL-C levels of less than 100 mg/dl, and at least 90 % of the patients who received REGN727, as compared with 17 % who received 80 mg of atorvastatin plus placebo, attained LDL-C levels of less than 70 mg/dl The LDL-C concentration reductions from the baseline to week 12 were as follows: 28.9 % for 150 mg every 4 weeks (p=0.0113); 31.54 % for 200 mg every 4 weeks (p=0.0058); 42.53 % for 300 mg every 4 weeks (p&lt;0.0001); 67.9 % for 150 mg every 2 weeks (p&lt;0.0001) compared with 10.65 % with placebo.</td>
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<td>REGN727 (SAR236553) (alirocumab) Regeneron Pharmaceuticals and Sanofi-Aventis, phase II</td>
<td>Randomized, double-blind, parallel-group, placebo-controlled study 77 patients with heterozygous FH (aged 18–75 years) with LDL-C levels of 2.6 mmol/l or higher receiving a stable diet and a statin dose with or without ezetimibe for 12 weeks. Multicenter, randomized, double-blind, placebo-controlled study</td>
<td>The LDL-C concentration reductions from the baseline to week 12 were as follows: 28.9 % for 150 mg every 4 weeks (p=0.0113); 31.54 % for 200 mg every 4 weeks (p=0.0058); 42.53 % for 300 mg every 4 weeks (p&lt;0.0001); 67.9 % for 150 mg every 2 weeks (p&lt;0.0001) compared with 10.65 % with placebo.</td>
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<td>Different subcutaneous doses of REGN727: 150–200 or 300 mg every 4 weeks or 150 mg every 2 weeks or placebo every 2 weeks (ratio 1:1:1:1). Stein et al. [112], ClinicalTrials.gov identifier NCT01266876</td>
<td>Reductions in apoB levels were consistent with those recorded for LDL-C levels: a least-squares mean reduction at week 12 ranging from 20.91 % to 50.19 % compared with a reduction of 6.39 % with placebo. HDL-C showed consistent increases in least-squares means from the baseline to week 12. Dose–response relationship with respect to percent LDL-C concentration lowering for administration every 2 weeks and administration every 4 weeks: 40 % with 50 mg every 2 weeks, 64 % with 100 mg every 2 weeks, and 72 % with 150 mg every 2 weeks, and 43 % with 200 mg every 4 weeks and 47 % with 300 mg every 4 weeks. ApoB level reduction: 27–56 %, proportional with the changes in LDL-C level. Non-HDL-C level reduction: 34–63 %. Lp(a) level reduction: 13–29 %. Increases in both HDL-C and apoA1 levels were variable but greater with all REGN727 regimens than with placebo. The effects of REGN727 on the levels of triglycerides were minimal (but the 150 mg every 2 weeks regimen reduced the levels of triglycerides by 19 %). 89–100 % of REGN727 recipients versus 16 % of placebo recipients achieved a target LDL-C level of less than 100 mg/dl and generalized pruritus, which was identified as being related to the study drug. 1 serious adverse event was reported with placebo and none were reported with REGN727</td>
<td>Generally well tolerated. 5 serious adverse events occurred in 4 patients during the study: a 64-year-old placebo-treated man required back surgery; a 68-year-old woman assigned to REGN727 at 200 mg every 4 weeks underwent elective right knee total arthroplasty; a 69-year-old woman with a history of chronic obstructive pulmonary disease, assigned to REGN727 at 100 mg every 2 weeks, was hospitalized during the follow-up period for worsening disease; and a 57-year-old man who, after the initial dosage of REGN727 at 300 mg every 4 weeks, developed diarrhea followed by a rash on his arms, legs, and abdomen, and was diagnosed with biopsy with leukocytoclastic vasculitis. Prednisone treatment led to full resolution. The investigator considered this a significant medical event. 6 patients prematurely discontinued REGN727 treatment owing to adverse events: 1 each in the 100 mg every 2 weeks arm (neutropenia) and 150 mg every 2 weeks arm (fatigue), 3 in the 200 mg every 4 weeks arm (injection-site rash, chest pain, and combined headache and nausea), and 1 in the 300 mg every 4 weeks arm (leukocytoclastic vasculitis considered as a serious adverse event) but who responded rapidly to steroid therapy. No antidrug antibodies were found following the vasculitis, but the week 20 follow-up assessment found a minimally detectable level (titer of 30) of antidrug antibodies. Mild injection-site reactions (this group term included erythema, pruritus, swelling, discoloration, hematoma, and rash) were the commonest adverse events. These occurred in REGN727 recipients only, and were commoner with every 2 weeks than with every 4 weeks dosing. Elevated creatine kinase level more than 10 times the upper limit of normal occurred in 1 patient (placebo-treated); no patients had levels of hepatic aminotransferases more than 3 times the upper limit of normal or significant changes in other laboratory values. Muscle complaints were infrequent and similar across treatment groups.</td>
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<td>(660 subjects, 24 weeks, subcutaneous administration of REGN727 with oral administration of ezetimibe or placebo, ClinicalTrials.gov identifier NCT01644188)</td>
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<td>Patients with heterozygous FH not adequately controlled with their lipid-modifying therapy: ODYSSEY FH I (471 subjects, 78 weeks, end 2014, ClinicalTrials.gov identifier NCT01623115), ODYSSEY FH II (249 subjects, 24 weeks, REGN727 plus statin, January 2015, ClinicalTrials.gov identifier NCT01709500), ODYSSEY ALTERNATIVE (314 patients with primary hypercholesterolemia who are intolerant to statins, 24 weeks, ClinicalTrials.gov identifier NCT01709513), ODYSSEY CHOICE I (700 patients with primary hypercholesterolemia, 24 weeks, ClinicalTrials.gov identifier NCT01926782), ODYSSEY CHOICE II (200 patients with primary hypercholesterolemia not treated with a statin, 24 weeks, December 2016, ClinicalTrials.gov identifier NCT02023879), ODYSSEY HIGH FH (105 patients, 78 weeks, REGN727 or placebo with background statin therapy or other lipid-lowering therapy, January 2015, ClinicalTrials.gov identifier NCT01617655), ODYSSEY OLE (1,200 patients with heterozygous FH, long-term efficacy, 120 weeks, July 2016, ClinicalTrials.gov identifier NCT01954394).</td>
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<td>Finally, ODYSSEY OUTCOMES (ClinicalTrials.gov identifier NCT01663402) is a very long term study (64 months; estimated study completion date, January 2018) estimating the effect of REGN727 on the occurrence of cardiovascular events. It will include 18,000 patients (40 years and older) who have recently experienced an acute coronary syndrome</td>
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**apoA** apolipoprotein A, **HDL-C** HDL cholesterol

Many phase I and phase II studies have been published recently in several interesting articles for two antibodies targeting and inhibiting PCSK9 interaction with LDL receptor: AMG 145 (evolocumab) developed by Amgen (Thousand Oaks, CA, USA), and SAR236553/REGN727 (alirocumab) developed by Regeneron Pharmaceuticals (Tarrytown, NY, USA) and Sanofi-Aventis (Paris, France). These antibodies and related patents were given in our previously published review on PCSK9 patents [95], but the details of the clinical trials, the doses given every 2 or 4 weeks subcutaneously, the results, and the adverse events are given in Table 2 [100–108, 109**, 110, 111, 112**, 113–115].

Phase III studies have been initiated by Amgen and Sanofi and Regeneron. The results of two of these phase III studies with evolocumab have been published and are detailed in Table 2, and several other phase III studies have been launched but have not been published yet. For alirocumab, an important program (ODYSSEY) concerning a large number of patients in short-term or long-term trials and targeting several populations has also been initiated. The design of these studies is summarized in Table 2 as well. Long-term studies that will involve 20,000 patients for both evolocumab and alirocumab will provide results regarding the long-term efficacy, safety, and tolerability of these anti-PCSK9 antibodies that are eagerly awaited.

**Other PCSK9 Interactions and Studies in Other Diseases**

PCSK9 interactions and the possibility of the involvement of PCSK9 in several diseases such as liver diseases, obesity, Alzheimer disease, cognitive performance [58] and cancer [59] have been studied (Table 1). Jonas et al. [117] showed that overexpression of PCSK9 in cells decreased cellular levels of BACE1, a membrane protease responsible for the production of toxic β-amyloid peptides that accumulate in neuritic plaques of Alzheimer disease brains. However, Liu et al. [118] found that PCSK9 does not have a role in regulating LDL receptor family members or BACE1 protein levels in the adult mouse brain and that the development of PCSK9 therapies for CHD is probably not to be hampered by potential CNS adverse effects. Devay et al. [119] discovered recently that PCSK9 interacts via its C-terminal domain directly and in a pH-dependent manner with amyloid precursor protein as well as amyloid-precursor-protein-like protein 2. It is notable that no genetic association was found between PCSK9 polymorphisms and Alzheimer disease and plasma cholesterol level in Japanese patients studied by Shibata et al. [120]. PCSK9 reduces the protein levels of LDL receptor in mouse brain during development and after ischemic stroke [121]. In vivo, endogenous PCSK9 regulates VLDL receptor protein and triglyceride accumulation in visceral adipose tissue. In a clinical perspective, because Pcsk9−/− mice do not develop liver steatosis and are not prone to obesity, the administration of a PCSK9 inhibitor developed for hypercholesterolemia treatment should not result in adverse effects [122]. A potential role of PCSK9 in the pancreas is also controversial. PCSK9 deficiency reduces liver metastasis by its ability to lower cholesterol levels and by possibly enhancing TNFα-mediated apoptosis [123]. Furthermore studies in Xenopus oocytes and in epithelia showed that PCSK9 noncatalytically reduced the abundance of the epithelial Na+ channel, a major contributor to blood pressure control [124]. PCSK9 interacts with annexin A2 [125]. Possible other unknown functions of PCSK9 and unidentified binding partners could exist; thus, it is important for the safety of new cholesterol-lowering therapy to target specifically PCSK9 action on the LDL receptor. An antiviral effect of circulating liver PCSK9 on hepatitis C virus in cells has recently been shown, and PCSK9 downregulates in vitro the level of expression of mouse liver CD81, a major
hepatitis C virus receptor [126]. Conditional knockout mice lacking PCSK9 in hepatocytes have impaired liver regeneration after a partial hepatectomy, suggesting that on hepatic damage, patients lacking PCSK9 could be at risk [127]. Thus, liver problems, hepatitis, or muscle problems are taken into consideration before inclusion or exclusion and are closely monitored during clinical trials. In clinical trials, anti-PCSK9 antibodies seem well tolerated, with no clinically significant safety findings in phase I and phase II/III studies, the most commonly reported adverse events being nasopharyngitis, injection-site pain, headache, skin burning sensation, upper respiratory tract infection, influenza, and back pain [100–108, 109••, 110, 111, 112••, 113–115]. Longer-term studies will provide the highly awaited long-term efficacy, safety, and tolerability of these anti-PCSK9 antibodies.

Conclusions

Reduction of PCSK9 levels or inhibition of PCSK9 is especially interesting in patients with hypercholesterolemia or an atherogenic lipid profile who fail to reach their individual cholesterol goal from classic lipid-lowering treatment, patients at high risk of developing side effects from statins, poor responders to statin therapy alone, and patients with severe hypercholesterolemia, particularly some carriers of a mutation of the LDLR, APOB, or PCSK9 gene. The tremendous commitment from all the centers of the French Research Network for Hypercholesterolemia that helped us in recruiting French patients and the enormous amount of genetic and molecular work performed were very important in our pioneering step linking PCSK9 to LDL-C metabolism and paving the way for the work of several other teams. Finally, the PCSK9 story is a wonderful example of how collaboration between teams (Boileau’s and Seidah’s teams) conducting research in completely different fields can be initiated and prove to be highly successful. It is also a fine example of the power of genetic research strategies in revealing new therapeutic targets.

The results of the phase III studies using the anti-PCSK9 antibodies with or without statins or other hypocholesterolemic drugs are highly awaited, with the hope that this new class of blockbuster candidates will keep its promises in helping lowering cholesterol levels and fighting against cardiovascular disease.

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Compliance with Ethics Guidelines

Conflict of Interest Marianne Abifadel is member of the advisory board of Amgen and is involved in anti-PCSK9 studies and trials with Amgen and with Regeneron and Sanofi. Jean-Pierre Rabès and Catherine Boileau are involved in anti-PCSK9 studies with Regeneron and Sanofi.

Sandy Elbitar, Petra El Khoury, Youmna Ghaleb, Melody Chémaly, Marie-Line Moussalli, and Mathilde Varret declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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Papers of particular interest, published recently, have been highlighted as:

• Of importance
•• Of major importance


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