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RARE DISEASES AND LIPID METABOLISM (JAG LÓPEZ, SECTION EDITOR)

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

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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

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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

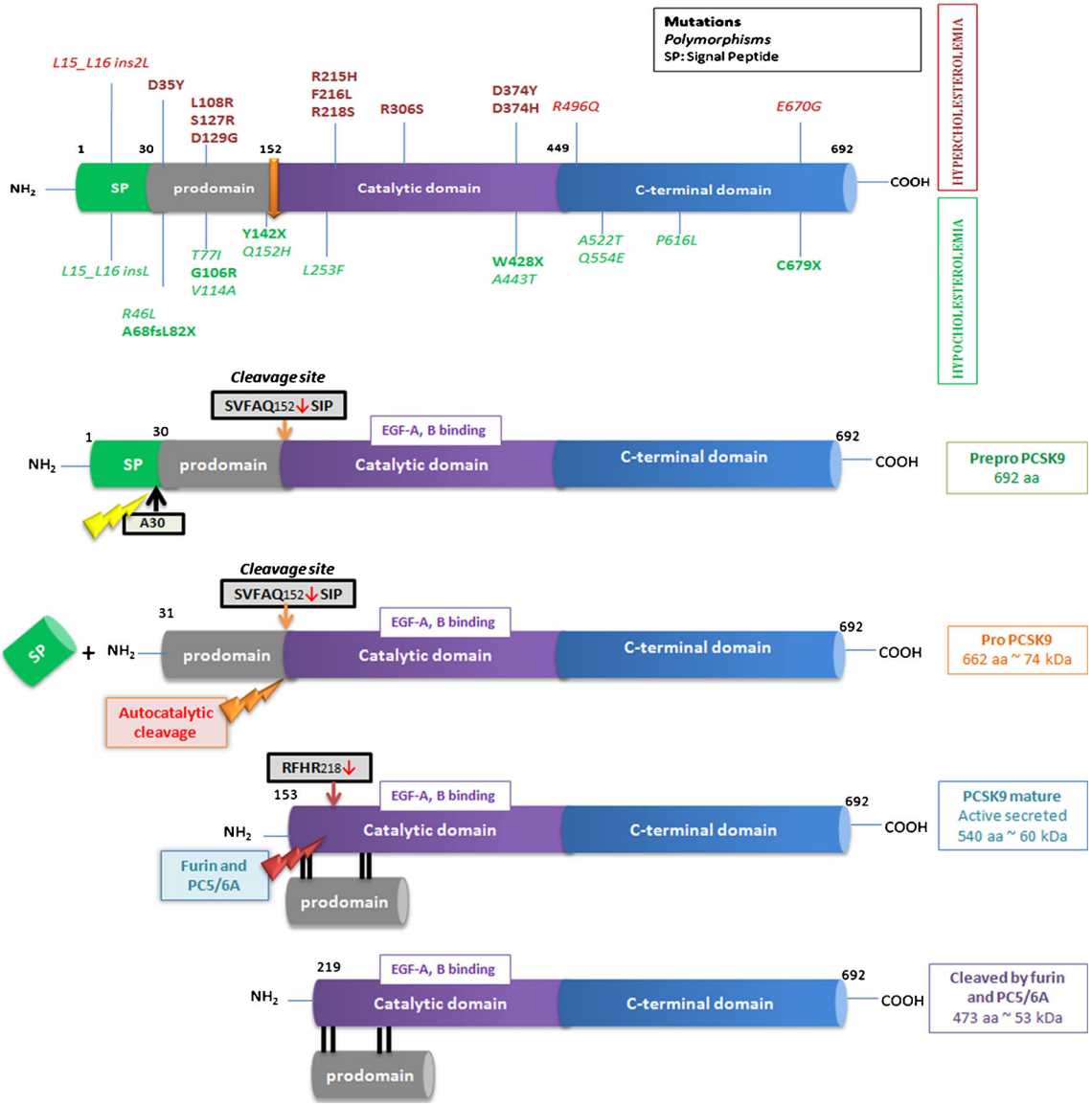

Fig. 1 Structure, processing of proprotein convertase subtilisin/kexin type 9 (*PCSK9*), and impact of PCSK9 main variants and mutations. The PCSK9 structure is characterized by a signal sequence (amino acids 1–30), a prodomain (amino acids 31–152), and a catalytic domain, followed by a 243 amino acid 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 hypocholesterolemia 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 Hypocholesterolemia

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 hypocholesterolemic [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 hypocholesterolemic 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/m^2) 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]. Hypocholesterolemia due to a deficiency of PCSK9 appears to be benign, in contrast to other Mendelian forms of severe hypocholesterolemia 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*)

Study	Subjects/method	Objectives	Results	Conclusion
LCAS (Chen et al. [46])	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	Determine the effects of *PCSK9* variants on plasma LDL-C levels, severity of coronary atherosclerosis, and response to statin therapy	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 > EG > 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. LDL-C lowering effect of fluvastatin is not associated with *PCSK9* variants. Haplotype 3, representative of the E670G mutation, is modestly associated with minimum lumen diameter tertiles ($p = 0.045$)	E670G a common SNP, an important determinant of plasma LDL-C concentration, is associated with the severity of coronary atherosclerosis in the LCAS population
ARIC (Cohen et al. [35])	3,363 blacks, 9,524 whites, age 45–64 years, 4 American communities	Compare the incidence of CHD over a 15-year interval according to the presence or absence of *PCSK9* sequence variations (Y142X, C679X, and R46L)	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. The mean IMT was slightly but significantly lower among carriers than among noncarriers in both black and white subject groups	Moderate lifelong reduction in LDL-C levels is associated with a substantial reduction in the incidence of coronary events
European study, University Hospital Hamburg, Germany (Evans and Beil [48])	506 patients attending the lipid clinic	Study the incidence of the E670G SNP in *PCSK9*	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. In a European population, E670G is associated with increased LDL-C levels in men but not in women	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
Bogalusa Heart Study (Hallman et al. [49])	478 African Americans, 1,086 white Americans, age 4–38 years	Analyze the relation between R46L, Y142X, and C679X *PCSK9* variants and serum LDL-C levels (in childhood and adulthood)	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)	These *PCSK9* variants are associated with significantly lower LDL-C levels starting in childhood
Belgium Stroke Study (Abboud et al. [50])	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.	Find a potential link between *PCSK9* and the risk of ischemic stroke or intracranial atherosclerosis	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.	*PCSK9* is associated with the risk of the large-vessel atherosclerosis stroke subtype, and this risk is mediated by the severity of intracranial atherosclerosis

Table 1 (continued)

Study	Subjects/method	Objectives	Results	Conclusion
	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)		In multivariate analysis, the minor allele (G) appeared as a significant predictor of large-vessel atherosclerosis In autopsy subjects, the G-allele carriers had severer atherosclerosis in the large intracranial cerebral arteries (EE=4.71vs G+=5.97)	
Second Northwick Park Heart Study (Scartezini et al. [51])	2,444 healthy UK men (age 50–61 years), 275 UK men with CHD (15 years of follow-up), 597 UK FH patients from the Simon Broome Register	Determine the relative frequency of *PCSK9* variants (R46L, I474V, and E670G) and their association with plasma lipid levels and CHD	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	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
PROSPER (Poliseck et al. [45])	5,804 subjects (2,804 men and 3,000 women), mean age 75.3 years, selected for having a history of vascular disease or CHD risk factors (smoking, hypertension, or diabetes), randomized to receive either pravastatin at 40 mg/day (n=2,891) or placebo (n=2913) and were followed for a mean of 3.2 years	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 with follow-up for 3.2 years	3.5 % were carriers of R46L, and these subjects had significantly (p<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.	R46L significantly lowers LDL-C levels, but does not greatly reduce CHD risk in an elderly population with a high prevalence of cardiovascular disease
Myocardial Infarction Genetics Consortium (Kathiresan [52])	1,454 cases of early-onset myocardial infarction (in men aged 50 years or younger or women aged 60 years or younger) ; 1617 age- and sex-matched controls free of myocardial infarction (from Boston, Seattle, Sweden, Finland, and Spain)	Test the hypothesis that R46L is associated with the risk of early-onset myocardial infarction	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	The *PCSK9* loss-of-function allele provides protection against MI in humans and is a valid target for pharmacologic therapy
ARIC (Folsom et al. [53])	3,472 blacks, 10,162 whites, age 45–64 years	Determine whether specific *PCSK9* (blacks Y142X, C679X; whites R46L) variants are associated with reduced prevalence and incidence of PAD	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.	This study provides mixed evidence that variation in *PCSK9* may contribute to genetic risk of PAD

Table 1 (continued)

Study	Subjects/method	Objectives	Results	Conclusion
Coronary Artery Risk Development in Young Adults (Huang et al. [54])	1,750 African Americans and 1,828 whites, age 18–30 years	Association of 6 genetics variants of *PCSK9* with LDL-C and IMT over 20 years, from young adulthood to middle age	White carriers of the R46L variant, blacks with 3 genetic variants (L253F, 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
PLIC study (Norata et al. [47])	1,541 middle-aged Caucasian subjects; 1,351 subjects were enrolled to confirm the results for the PLIC population	Study the effects of E670G and I474V on the IMT of the common carotid artery and the possible relation to apoE polymorphisms	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, $p<0.05$). 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
3 independent studies and meta-analyses: CCHS, CGPS, and CIHDS (Benn et al. [55])	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 ($n=66,698$)	Examine the association of R46L with LDL-C levels, and risk of IHD, MI, and death	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 te risk of IHD	The reduction in risk of IHD was larger than predicted by the observed reduction in LDL-C levels alone
Italian population (Guella et al. [56])	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)	Association of the *PCSK9* R46L allele with premature MI and plasma lipid levels in the Italian population	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	Highlights the importance of exposure to higher cholesterol concentrations in the older population to reveal the protective effect of the *PCSK9* variant against MI

Table 1 (continued)

Study	Subjects/method	Objectives	Results	Conclusion
			1.04 %), and a clear trend toward a protective effect was observed, although the association with lower MI risk was not significant, whereas in the older control population it was significantly associated with a reduced risk of MI (OR 0.67, 95 % CI 0.46–0.97; p=0.036)	
POLCA, OLIVIA, CORONA, and 60YO (Chernogubova et al. [57])	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	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	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	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
PROSPER 2013 (Postmus et al. [58])	5,804 subjects with preexisting vascular disease or at increased risk were randomly assigned to receive pravastatin or placebo	Assess the association between the *PCSK9* SNP rs11591147 (R46L), cognitive performance, activities of daily living, and noncardiovascular clinical events	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>0.1)	It is unlikely that medication lowering LDL-C levels via inhibiting PCSK9 will affect cognitive performance, functional status, or risk of noncardiovascular clinical events
ARIC (Folsom et al. [59])	7,082 men and 8,710 women aged 45–64 years were recruited; whites and African Americans	Examine whether *PCSK9* variants (Y142X, C679X, and R46L) linked to lifetime low LDL-C concentrations are associated with cancer risk	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)	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

apoB apolipoprotein B, *apoE* apolipoprotein E, *ARIC* Atherosclerosis Risk in Communities, *CCHS* Copenhagen City Heart Study, *CGPS* Copenhagen General Population Study, *CI* confidence interval, *CIHDS* Copenhagen Ischemic Heart Disease Study, *FH* familial hypercholesterolemia, *HR* hazard ratio, *IHD* ischemic heart disease, *IMT* intima–media thickness, *LCAS* Lipoprotein Coronary Atherosclerosis Study, *LDL-C* LDL cholesterol, *Lp(a)* lipoprotein (a), *MI* myocardial infarction, *OR* odds ratio, *PAD* peripheral artery disease, *PCSK9* proprotein convertase subtilisin/kexin type 9, *PROSPER* Prospective Study of Pravastatin in the Elderly at Risk, *SNP* single-nucleotide polymorphism

LDLR [62]. We showed that *PCSK9* might constitute a modifier gene in FH: in Lebanese FH patients sharing the *LDLR* p.C681X mutation, p.Leu21dup, in exon 1 of *PCSK9*, known to be associated with lower LDL-C levels in general populations, is also associated with a reduction of LDL-C levels in FH [62]. Furthermore, additive effects of mutations of *LDLR* and gain-of-function mutations of *PCSK9* on the phenotype of FH have been reported in several studies [26, 63] and might be associated with a severe phenotype. It is noteworthy that the p.R496Q variant in *PCSK9* was identified [33] 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 [64]. 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 [66].

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 depletion [70], inflammation, administration of insulin, and statin therapy [71]. *PCSK9* is downregulated 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 ezitimibe 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, rosuvastatin, 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 A_2 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

Characteristics/subjects/administration	Results	Adverse effects
Adnectins: BMS-962476, Bristol-Myers Squibb, phase I		
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 a 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	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 %	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
RNA interference: ALN-PCS02, Alnylam Pharmaceuticals, phase I		
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	The 0.400 mg/kg group showed a mean 70 % reduction in circulating PCSK9 plasma protein levels (p <0·0001) and a mean 40 % reduction in LDL-C levels from the baseline relative to placebo (p <0·0001)	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
Specific antibodies or competing peptides		
RG7652 (MPSK3169A), Genentech (Roche), phase I		
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]	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	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
Bococizumab (RN316/PF-04950615), Pfizer, phase II		
A 24-week, multicenter, randomized, double-blind, placebo-controlled trial.	Bococizumab significantly reduced LDL-C levels across all doses.	Adverse events were similar across placebo and bococizumab groups.

Table 2 (continued)

Characteristics/subjects/administration	Results	Adverse effects
Statin-treated subjects aged 18 years or older with a diagnosis of hypercholesterolemia and LDL-C level of 80 mg/dl or higher 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/day or lower. Ballantyne et al. [99], ClinicalTrials.gov identifier NCT01592240	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 35 % 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	Few subjects discontinued treatment owing to treatment-related adverse events. The every 14 days regimen is being evaluated in larger trials
AMG 145 (evolocumab), Amgen, phase I		
Interventional, randomized, double-blind, placebo-controlled, ascending multiple dose trial. 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 NCT01133522	Phase Ia: up to 64 % reduction of LDL-C levels after 1 dose of AMG 145 of 21 mg or greater compared with placebo ($p<0.0001$); up to 55 % reduction of apoB levels ($p<0.0001$); reduction of free PCSK9 levels. Phase Ib: up to 81 % reduction of LDL-C levels compared with placebo ($p<0.001$) at nadir; up to 59 % reduction of apoB levels ($p<0.001$); reduction of mean serum levels of Lp(a) by 27 % (35 mg weekly for 6 weeks, $p<0.033$) to 50 % (heterozygous FH cohort 140 mg every 2 weeks for3 weeks, $p<0.001$) versus placebo; reduction of free PCSK9 levels	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. 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
AMG 145 (evolocumab), Amgen, phase II		
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 NCT01375751	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	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
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	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,	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

Table 2 (continued)

Characteristics/subjects/administration	Results	Adverse effects
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 of4.5 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 NCT01375777	−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	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
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	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/apoAI ratios. Modest increase of HDL-C levels from 6 % to 12 % compared with 1 % with placebo/ezetimibe. Increase of apoAI 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	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
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	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 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	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,

Table 2 (continued)

Characteristics/subjects/administration	Results	Adverse effects
		erythema, hematoma, or pain), of which 3 (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
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	At 52 weeks, there was a 52.3 % reduction of LDL-C levels, a 42 % reduction in apoB levels, a1/3 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
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); subcutaneus 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	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 with 420 mg AMG 145 were 22.7 % and 87.6 %, respectively	6 of the 8 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
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/1) 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 th 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 (unadjusted P<0.001) It may be that patients who have already been treated with high-dose statins or combination	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

Table 2 (continued)

Characteristics/subjects/administration	Results	Adverse effects
Blom et al. [109], ClinicalTrials.gov identifier NCT01516879	lipid-lowering therapy may have slightly less capacity to further upregulate LDL receptor activity with PCSK9 inhibition or may require higher doses of antibody	
MENDEL-2 is a double-blind, randomized, placebo- and ezetimibe-controlled, multicenter study, 614 patients aged 18–80 years with fasting LDL-C levels grater than or equal to 100 mg/dl and below 190 mg/dl and Framingham risk scores of 10 % or lower were randomized (1:1:1:1:2:2) 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. Koren et al. [110], ClinicalTrials.gov identifier NCT01763827	AMG 145 treatment reduced LDL-C levels from baseline, on average, by 55–57 % more than placebo and 38 %–40 % more than ezetimibe (P <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	Treatment-emergent adverse events, muscle-related adverse events, and laboratory abnormalities were comparable across treatment groups. In 2 cases, local investigators considered events related to the study drug: acute pancreatitis in a patient with a history of cholestectomy, long-term alcohol intake, and concomitant use of valproate semisodium receiving AMG 145 monthly; and levels of aminotransferases and creatine kinase 8 times above the upper limit of normal in a patient receiving AMG 145 biweekly that returned to normal after study drug discontinuation
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	AMG 145 reduced LDL-C levels from the baseline by 53–56 %, corresponding to treatment differences versus ezetimibe of 37 % to 39 % (p<0.001)	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
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) 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)		
REGN727 (SAR236553) (alirocumab), Regeneron Pharmaceuticals and Sanofi-Aventis, phase I		
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 ascending 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.	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	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

Table 2 (continued)

Characteristics/subjects/administration	Results	Adverse effects
Stein et al. [112], Crunkhorn [113], ClinicalTrials.gov identifier NCT01026597		REGN727 subcutaneously, who had a small-bowel obstruction that was diagnosed on study day 75.
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.	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	In the single-dose intravenous study, a 54-year-old man who received REGN727 at 12 mg/kg had a slightly elevated total bilirubin level at screening and throughout most of the trial. A 26-year-old man who received REGN727 at 0.3 mg/kg had a transient elevation of serum creatine kinase level to more than 10 times the upper limit of the normal range.
Stein et al. [112], ClinicalTrials.gov identifier: NCT01074372		Multiple-dose trial:
Randomized, double-blind, placebo-controlled, multiple-dose study. 21 FH and 30 non-FH patients (aged 18–65 years) receiving atorvastatin with LDL-C levels above 100 mg/dl. 10 non-FH patients receiving a modified diet only with LDL-C levels above 130 mg/dl. Subcutaneous administration of different doses of REGN727 (50, 100, or 150 mg) or placebo on days 1, 29, and 43. Study duration, 148 days.	For REGN727 plus atorvastatin versus placebo, LDL-C levels were significantly reduced ($p<0.001$ 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.	5 subjects who were receiving REGN727 plus atorvastatin had brief elevations in creatine kinase level to more than 3 times the upper limit of the normal range. No subject had a serious adverse event, and all subjects completed all visits. Headache was the commonest adverse event. No clear evidence of drug-related adverse events Given the small number of subjects and the short duration of exposure, the ability to evaluate the safety profile of REGN727 in these trials was limited
Stein et al. [112], ClinicalTrials.gov identifier NCT01161082	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	
REGN727 (SAR236553) (alirocumab) Regeneron Pharmaceuticals and Sanofi-Aventis, phase II		
Randomized, double-blind, parallel-group, 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.	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 ($P<0.001$) 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	Of patients receiving 80 mg of atorvastatin plus REGN727, 1 patient discontinued treatment owing to a hypersensitivity reaction and rash, both occurring 12 days after the 2nd injection of REGN727. This responded to treatment with an antihistamine. 1 serious adverse event of dehydration; the event was not thought to be treatment-related. The patient recovered fully. Transient increase in the aspartate aminotransferase level (between more than 3times and less than 5 times the upper limit of the normal range in 1 patient with mildly elevated aspartate aminotransferase level before randomization 1 patient receiving REGN727 and 2 patients receiving 80 mg of atorvastatin plus placebo had an injection-site reaction. Antibodies against REGN727 were detected at low titer in 7 of the 56 patients in the REGN727 groups at week 8
Roth et al. [114], ClinicalTrials.gov identifier NCT01288469		
Multicenter, randomized, double-blind, 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.	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 ($p=0.0035$); 42.53 % for 300 mg every 4 weeks ($p<0.0001$); 67.9 % for 150 mg every 2 weeks ($p<0.0001$) compared with 10.65 % with placebo	No increases of more than 3 times the upper limit of the normal range for hepatic aminotransferases or creatinine kinase concentrations Common adverse event: injection-site reaction 1 patient in the 300 mg every 4 weeks group terminated the study after 1 dose because of an adverse event—injection-site reaction—

Table 2 (continued)

Characteristics/subjects/administration	Results	Adverse effects
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 1:1:1:1:1). Stein et al. [112], ClinicalTrials.gov identifier NCT01266876	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	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
Double-blind, randomized, parallel-group, placebo-controlled, multicenter study. 183 patients (aged 18–75 years) with primary hypercholesterolemia with LDL-C levels of 100 mg/dl (2.59 mmol/l) or higher receiving stable-dose atorvastatin (10, 20, or 40 mg) for 6 weeks or more. 5 subcutaneous doses and 2 dose regimens of REGN727: placebo every 2 weeks; REGN727 at 50, 100, or 150 mg every 2 weeks; or REGN727 at 200 or 300 mg every 4 weeks alternating with placebo to mimic the every 2 weeks dosing, for 12 weeks. Study duration, 12 weeks. McKenney et al. [115], ClinicalTrials.gov identifier NCT01288443	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	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 by 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
REGN727 (SAR236553) (alirocumab), Regeneron Pharmaceuticals and Sanofi-Aventis, phase III, ODYSSEY studies		
An important program of studies, the "ODYSSEY studies," has been initiated. It concerns a large number of patients in short-term or long-term trials and targets several populations :		
Patients with high cardiovascular risk, with hypercholesterolemia not adequately controlled with their lipid-modifying therapy: ODYSSEY COMBO I (306 subjects, 52 weeks, versus placebo, 2014, ClinicalTrials.gov identifier NCT01644175), ODYSSEY LONG TERM (2,100 subjects, background of statin therapy or lipid-modifying therapy, 78 weeks, October 2014, ClinicalTrials.gov identifier NCT01507831), ODYSSEY COMBO II		

Table 2 (continued)

Characteristics/subjects/administration	Results	Adverse effects

(660 subjects, 24 weeks, subcutaneous administration of REGN727 with oral administration of ezetimibe or placebo, ClinicalTrials.gov identifier NCT01644188).

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).

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

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 we 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 involoved in anti-PCSK9 studies with Regeneron and Sanofi.

Sandy Elbitar, Petra El Khoury, Youmna Ghaleb, Mélody 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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- • Of importance
- •• Of major importance

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