14 Proprotein Convertase Subtilisin/Kexin Type 9: Functional Role in Lipid Metabolism and Its Therapeutic Inhibition

Peter P. Toth

Introduction

A proprotein convertase is a proteolytic enzyme that converts an inactive precursor molecule into an active one [1]. An example of this is the conversion of a zymogen (a proenzyme) into a mature bioactive enzyme with catalytic activity in some aspect of intermediary metabolism [2]. There is a family of nine proprotein convertase subtilisin kexins (PCSK). Eight of the nine members are catalytically active and are responsible for the proteolytic conversion of a wide variety of molecules (including receptors, hormones, enzymes, transcription factors) into their bioactive forms [3]. The ninth member of this family (PCSK9) is the most recently discovered and is atypical. PCSK9 like other PVSKs is a serine protease. Once translated in the endoplasmic reticulum (ER), its signal peptide is cleaved by a signal peptidase to form zymogen proPCSK9 [4] (Fig. 14.1). In order to leave the ER and enter the cytosol, it catalyzes the autocleavage of a prosegment from itself. Subsequent to this step PCSK9 is no longer able to engage in a catalytic activity because the cleaved prosegment remains associated and it causes steric hindrance of this enzyme's active site [5]. Enzymatically, PCSK9 has only a single molecular target: its own prosegment.

Low-density lipoprotein particles (LDL-P) are principally cleared from the systemic circulation by the LDL receptor (LDLR) [6]. LDL receptors are expressed along the surface of hepatocytes and are concentrated in clathrin-coated pits within cell membranes. Once an LDLR binds an LDL-P, it is configured within the clathrin-coated pit by LDLR adaptor protein 1 (aka clathrin associated sorting protein), though there is evidence that the protein disabled homolog adaptor protein 2 (dab-2) can also perform this role [7, 8]. An endosome forms and is covered with a clathrin polyhedral lattice [9] (Fig. 14.2). The endosome is released into the cytosol, the clathrin dissociates, and the internal milieu of the endosome is acidified. The drop in pH potentiates the dissociation of the LDLR from LDL-P. Through a mechanism that is yet to be defined, the LDL-P is specifically translocated into the lysosome for destruction by cathepsins and lipases. The LDLR is routed back to the hepatocyte cell surface to initiate another round of LDL-P uptake and catabolism (Fig. 14.3).

PCSK9 regulates the expression of the LDLR (Fig. 14.3). Once in the extracellular milieu, PCSK9 can bind to the epidermal growth factor-like repeat A domain of LDLR [10]. LDLR bound to both an LDL-P and PCSK9 are also concentrated in clathrin-coated endosomes. However, in this instance, the PCSK9 holds the

P. P. Toth (✉)
Cicarrone Center for the Prevention of Cardiovascular Disease, Johns Hopkins University School of Medicine, Baltimore, MD, USA
e-mail: Peter.Toth@cghmc.com

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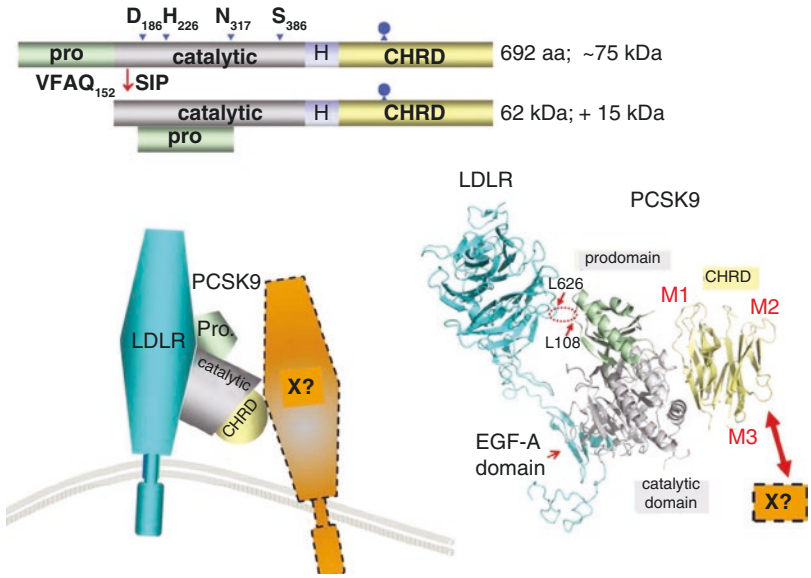

Fig. 14.1 Schematic representation of proprotein convertase subtilisin kexin 9 (PCSK9) zymogen processing and binding to the low-density lipoprotein receptor (LDLR). (**a**) The autocatalytic zymogen processing of proPCSK9 (75 kDa) at Val-Phe-Ala-Gln152↓Ser-Ile-Pro (VFAQ152↓SIP) into the (prosegment (15 kDa) ≡ PCSK9 (62 kDa)) complex is emphasized, together with the positions of the active site Asp186, His226, and Ser386 and the oxyanion hole Asn317. The C-terminal hinge domain (H) and cys-his-rich domain (CHRD) are shown. (**b**) Cartoon representation of the cell surface interaction of the catalytic domain of PCSK9 with the epidermal growth factor-A (domain of the LDLR, as well as the suspected interaction of the prosegment with the β-barrel domain of the LDLR and the CHRD with a putative membrane-bound protein X. (**c**) Crystal structure of the ectodomain of the LDLR with PCSK9 emphasizing the interaction between them and the three subdomains in the CHRD (M1, M2, and M3). The interaction of protein X is presumed to be with one of the latter subdomains, possibly M2. (From Seidah et al. [4])

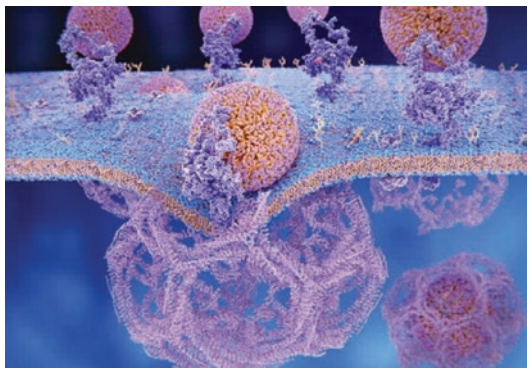

Fig. 14.2 Endosome formation and uptake into the cytosol via a polyhedral clathrin cage. (From Trialsitenews.com)

LDLR and LDL-P tightly together, and they do not dissociate as the intra-endosomal pH decreases [11]. The PCSK9 functions as a chaperone molecule of the LDLR-LDL-P complex into the lysosome. This results in LDLR catabolism and reduced cell surface expression of this receptor vital to LDL-P clearance.

The *PCSK9* gene localizes to chromosome 1 and, like the genes for *LDLR* and apoprotein B (*apo B*), is a locus for familial hypercholesterolemia [12, 13]. The expression of PCSK9 is regulated by the nuclear transcription factor sterol regulating element-binding protein-2 [14]. Gain-of-function mutations in *PCSK9* such as D374Y and R496W lead to reduced LDLR expression, increased serum levels of LDL-P, and heightened risk for ASCVD [15, 16]. Such loss-of-function (LOF) mutations in *PCSK9* as Y142X and C679X in persons of African descent [17] and Q152H in French Canadians [18] lead to increased LDLR expression, lower serum LDL-C, and reduced risk for ASCVD. Major prospective longitudinal

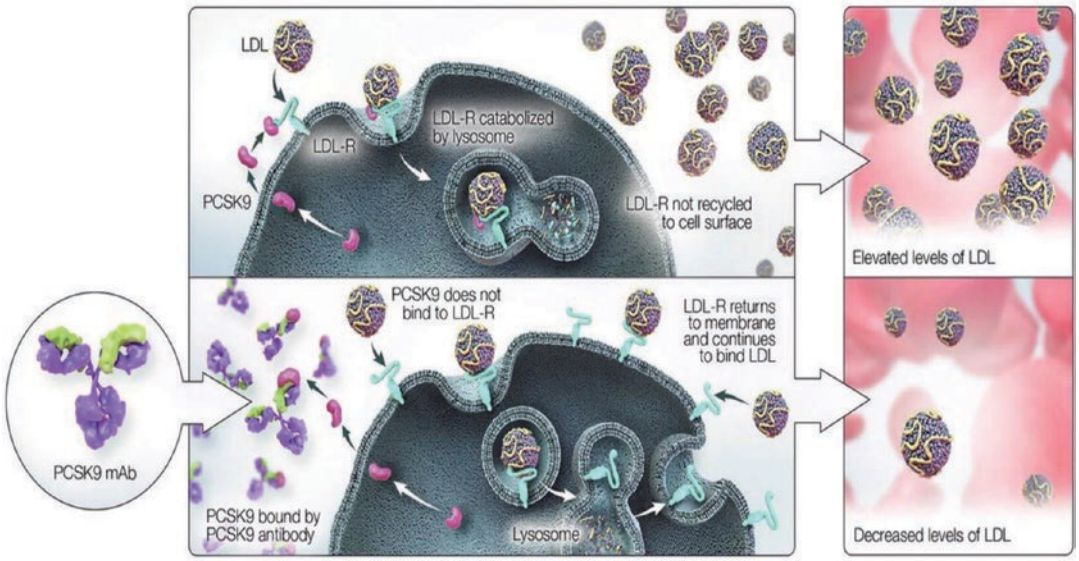

Fig. 14.3 LDL recycling, PCSK9 function, and effect of PCSK9 inhibition. Mechanism of action of PCSK9 inhibition for reduction of serum cholesterol concentration. *Top panel*: PCSK9 secreted by hepatocytes binds to LDL-R on the hepatocyte surface. Upon subsequent binding of the receptor by LDL, the PCSK9/LDL/LDL-R complex is internalized within an endosomal vesicle. The endosome fuses with a lysosome, and the PCSK9 chaperones the LDL/LDL-R complex into the lysosome for destruction. As a result, the number of LDL-Rs is decreased, resulting in less clearance of LDL from the circulation and elevated LDL concentration. *Bottom panel*: Monoclonal antibody binds to PCSK9 and prevents it from engaging the LDL-R. In the absence of PCSK9, the LDL-R is not routed to the lysosome for degradation and is returned instead to the hepatocyte surface. The recycled LDL-R is available for additional LDL binding and clearance, resulting in decreased levels of LDL. LDL, low-density lipoprotein; LDL-R, low-density lipoprotein receptor. (From Toth [96]. With permission from Elsevier)

cohorts confirm that LOF mutations in both men and women result in substantially lower LDL-C levels compared to patients with wild-type alleles for PCSK9 [19] (Fig. 14.4).

Lipoprotein receptor physiology is complex. PCSK9 also regulates cell surface expression of other lipoprotein receptors, possibly impacting serum levels of other lipoproteins and their subfractions (Fig. 14.5). PCSK9 regulates the expression of the very low-density lipoprotein receptor (VLDLR), the apolipoprotein E2 receptor, and the LDL receptor-related protein-1, all of which can participate in the clearance of various apo B-containing lipoproteins [20–23]. In addition, PCSK9 regulates the expression of a cluster of differentiation 36 (CD 36), which is a fatty acid translocase in adipocytes and hepatocytes [24]. The D374Y GOF mutation also suggests that PCSK9 upregulates Nieman Pick C1-like protein without impacting the expression of SR-BI or ABCG5/G8 [25].

Therapeutic Strategies for Inhibiting PCSK9

Monoclonal Antibodies

A monoclonal antibody is a highly specific antibody directed toward a single molecular target [26]. Given the fact that PCSK9 is a secreted protein, it can be targeted by a monoclonal antibody (mAb) in an effort to neutralize its activity. Two fully human mAbs (evolocumab and alirocumab) directed against PCSK9 have been developed for treating primary hyperlipidemia and familial hypercholesterolemia. They are fully human in an effort to reduce the risk of autoimmune responses and the generation of antibodies against them. This also reduces the risk of tachyphylaxis. These agents can be used independently of hepatic and renal function because they have no dependence on hepatic uptake and metabolism,

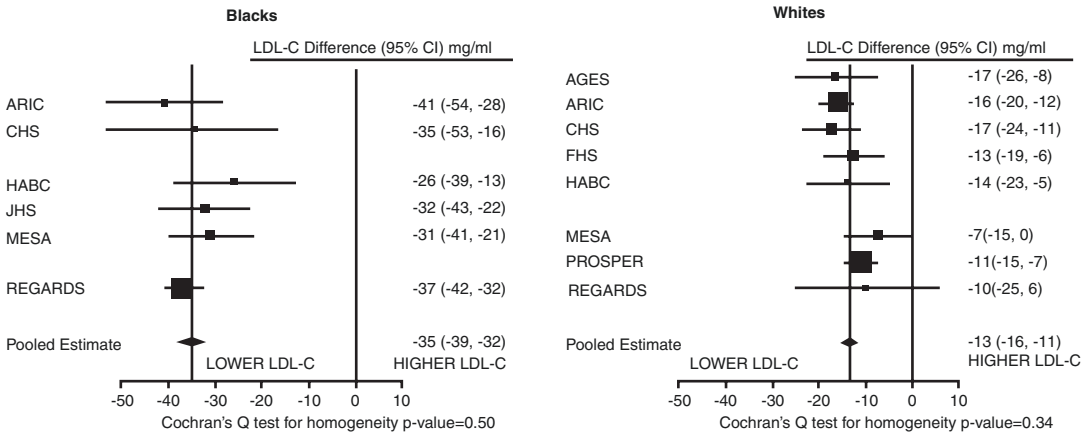

Fig. 14.4 Difference in low-density lipoprotein cholesterol (LDL-C) among participants with vs without *PCSK9* loss-of-function (LOF) variants in individual studies and pooled analyses. AGES indicates Age, Gene, Environment, Susceptibility Study–Reykjavik; ARIC, Atherosclerosis Risk in Communities Study; CHS, Cardiovascular Health Study, CI confidence interval; FHS, Framingham Heart Study; Health ABC, Health, Aging, and Body Composition Study; JHS, Jackson Heart Study; LDL-C, low-density lipoprotein cholesterol; MESA, Multi-Ethnic Study of Atherosclerosis; PROSPER, PROspective Study of Pravastatin in the Elderly at Risk for vascular disease; REGARDS, REasons for Geographic and Racial Differences in Stroke Study. LDL-C differences are comparing participants with *PCSK9* LOF variants to those without *PCSK9* LOF variants (*PCSK9* LOF variant minus no *PCSK9* LOF variant). Models for each participating study include adjustment for age, sex, region/center, and statin use. Pooled analyses are performed using inverse-variance-weighted fixed-effect models. (From Kent et al. [19])

and they do not depend on renal elimination for their clearance [27–29]. The antibody complexes formed between these agents and extracellular PCSK9 are cleared by the reticuloendothelial system (Kupffer cells, spleen, lymph nodes, bone marrow), and they do not promulgate drug interactions since they have no dependence for activity from organic anion transport proteins, the cytochrome P450 isozymes, or glucuronidation as there is no known antibody uptake pathway for the liver.

Evolocumab

LDL-C Reduction in Primary Hyperlipidemia

In patients with primary hyperlipidemia, evolocumab is a highly safe and efficacious therapy for reducing atherogenic lipoprotein burden in serum. It can be dosed at 140 mg subcutaneously (SQ) every 2 weeks or 420 mg SQ every 4 weeks. When used as monotherapy, evolocumab induces a 55%–57% reduction in LDL-C compared to placebo [30] (Table 14.1). When added to statin therapy (atorvastatin 10 mg or 80 mg; rosuvastatin 5 mg or 40 mg; simvastatin 40 mg), evolocumab provides a mean incremental reduction in LDL-C of 63% [31]. Among patients intolerant to two or more statins evolocumab provides a 38% reduction in LDL-C compared to ezetimibe [32]. For patients at various levels of ASCVD risk, the addition of evolocumab to ongoing lipid-lowering therapy (atorvastatin 10 mg or 80 mg daily or the combination of atorvastatin 80 mg daily with ezetimibe) provided a 48–62% incremental reduction of LDL-C compared to placebo [33].

LDL-C Reduction in Familial Hypercholesterolemia

Among patients with familial hypercholesterolemia (FH), as long as the patient is not a homozygote for a null mutation in *PCSK9*, mAbs directed against circulating PCSK9 would be expected to provide some degree of LDL-C reduction. In the

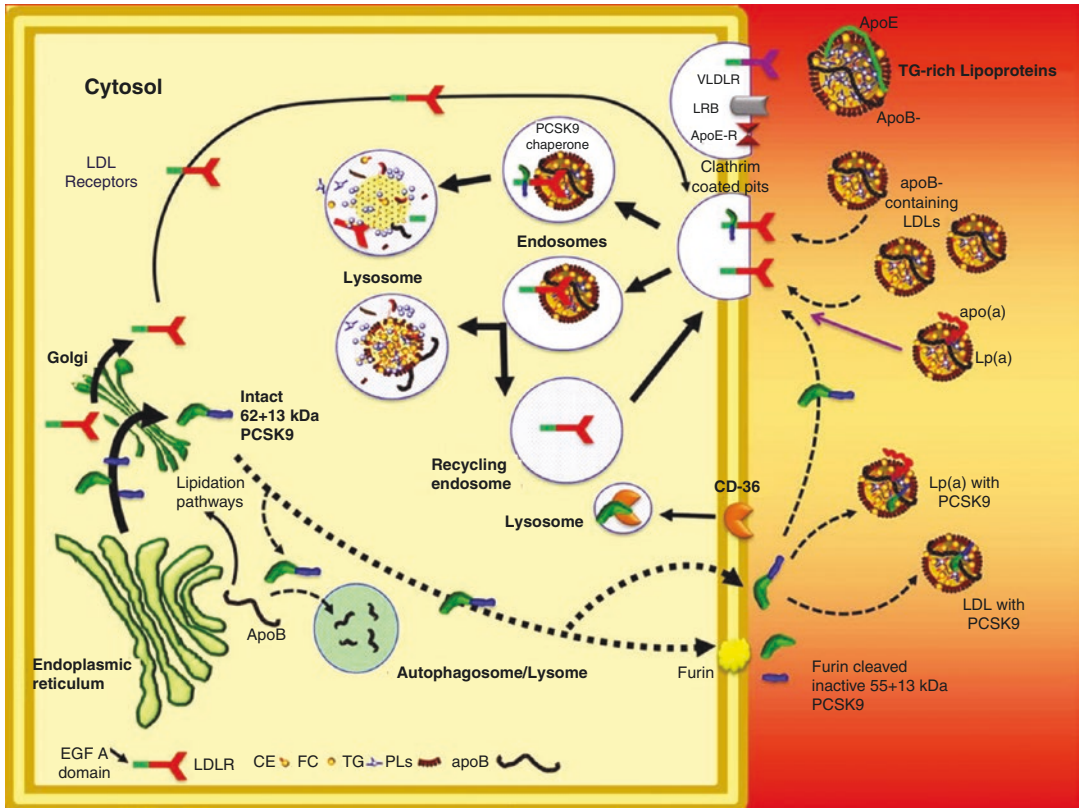

Fig. 14.5 Proprotein convertase subtilisin kexin type 9 (PCSK9) and lipoprotein trafficking. Subsequent to release from the Golgi apparatus, low-density lipoprotein receptors (LDLR) translocate to the cell membrane where they are concentrated in clathrin-coated pits. Hepatocytes secrete PCSK9 into the extracellular space which can bind to the epidermal growth-factor-like repeat A (EGF-A) domain of LDLR. Complexes composed of LDLR and LDL particles are internalized via endosomes. If the LDLR–LDL-P complex is bound with PCSK9, the complex is chaperoned into the lysosome for hydrolytic destruction. The LDLR in this case is not recycled to the cell surface. When LDLR–LDL-P complexes are not bound to PCSK9, the complex dissociates in response to a drop in pH within the endosome. The LDL-P is translocated to the lysosome for destruction, whereas LDLR is spared and recycled to the cell surface to initiate another round of LDL-P binding and uptake. The monoclonal antibodies directed against PCSK9 decrease LDLR translocation into the lysosome, increase LDLR surface expression, and significantly reduce plasma levels of LDL-P. Recent investigations demonstrate that PCSK9 can also regulate the expression of other cell surface receptors, such as a very-low-density lipoprotein receptor (VLDLR), the LDLR-related protein (LRP), an apoprotein E receptor, and cluster of differentiation 36 (CD36). In addition to its impact on cell surface receptor expression, PCSK9 potentiates the production of apo B and increases VLDL secretion by inhibiting the catabolism of apoB via an autophagosome/lysosome-dependent pathway. The serine protease furin (which is found in both membrane-bound and free forms) can cleave active, intact PCSK9 (62 kDa) into an inactive 55-kDa fragment. A small percentage of total circulating LDL and lipoprotein(a) (Lp(a)) can bind PCSK9. Although some Lp(a) is cleared by a pathway that is independent of LDLR, some is demonstrably cleared by LDLR. (From Toth [97])

reduction of LDL-C with PCSK9 Inhibition in Heterozygous Familial Hypercholesterolemia Disorder Study-2 (RUTHERFORD-2), evolocumab when dosed at either 140 mg every 2 weeks or 420 mg dosed every 4 weeks reduced LDL-C by 59% and 60%, respectively [34]. This result suggests that the upregulation of LDLR is so robust that patients with heterozygous FH (HeFH) respond to evolocumab with as much LDL-C reduction as patients with primary hyperlipidemia. As shown in the Trial Evaluating PCSK9 Antibody in Subjects with LDL Receptor

Table 14.1 Lipoprotein and remnant particle concentrations and percent change from baseline to week 52

Variable	Placebo		Evolocumab 420 mg QM	
	n		*n*	
LDL-P total				
Mean ± SD, (nmol/L)	246	1110.3 ± 326.2	300	609.8 ± 336.9
Percent change from baseline, mean [95% CI]	236	6.4 [2.9, 9.9]	294	–44.1* [−47.2, −40.9]
HDL-P total				
Mean ± SD, (μmol/L)	246	35.4 ± 6.1	300	37.5 ± 6.2
Percent change from baseline, mean [95% CI]	236	−0.1 [−1.6, 1.4]	294	9.4* [7.5, 11.4]
Large LDL-P				
Median (Q1, Q3) (nmol/L)	246	362.5 (231.0, 532.0)	300	91.5 (33.0, 180.5)
Percent change from baseline, median (Q1, Q3)	233	5.1 (−22.8, 43.4)	292	−73.7* (−89.8, −50.9)
Small LDL-P				
Median (Q1, Q3) (nmol/L)	246	615.0 (460.0, 775.0)	300	367.0 (274.0, 507.5)
Percent change from baseline, median (Q1, Q3)	236	3.8 (−16.9, 29.0)	294	−35.4* (−56.7, −11.4)
VLDL-P and chylomicron total				
Median (Q1, Q3) (nmol/L)	246	49.4 (32.3, 75.4)	300	35.8 (25.1, 53.7)
Percent change from baseline, median (Q1, Q3)	236	−0.3 (−26.3, 31.7)	294	−15.3** (−39.3, 15.4)
Large VLDL-P and chylomicron				
Median (Q1, Q3) (nmol/L)	246	3.1 (1.4, 6.0)	300	3.1 (1.6, 6.4)
Percent change from baseline, median (Q1, Q3)	236	1.0 (−41.8, 60.4)	294	10.5 (−26.1, 100.0)
Medium VLDL-P				
Median (Q1, Q3) (nmol/L)	246	18.2 (10.3, 31.0)	300	15.1 (8.2, 25.3)
Percent change from baseline, median (Q1, Q3)	235	7.1 (−36.3, 50.8)	292	−15.2 (−47.7, 48.3)
Small VLDL-P				
Median (Q1, Q3) (nmol/L)	246	26.2 (16.2, 37.0)	300	16.8 (10.8, 25.1)
Percent change from baseline, median (Q1, Q3)	236	−7.5 (−33.1, 30.4)	293	−29.0* (−54.1, 18.3)
IDL-P				
Median (Q1, Q3) (nmol/L)	246	74.0 (44.0, 125.0)	300	45.5 (26.0, 79.0)
Percent change from baseline, median (Q1, Q3)	236	0 (−47.4, 87.5)	294	−36.2* (−69.8, 22.0)

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P values reported are for treatment differences (evolocumab versus placebo) tested using two-sample t-test for LDL-P and HDL-P. All other parameters were analyzed using the Wilcoxon rank sum test

HDL-C high-density lipoprotein cholesterol, *IDL-P* intermediate-density lipoprotein particle concentration, *LDL* low-density lipoprotein, *LDL-P* LDL particle concentration, *Q1, Q3* first and third quartiles, *QM* once every month, *SD* standard deviation, *VLDL-P* very-low-density lipoprotein particle concentration

*$P < 0.0001$

**$P < 0.001$

Abnormalities Part B (TESLA), among patients with homozygous FH (HoFH), evolocumab reduces LDL-C 31% compared to placebo [34].

Apo B, Non-HDL-C, and Lipoprotein(a) Reduction

Apo B and non-HDL-C are important measures of atherogenic lipoprotein burden in serum and are highly predictive of risk for ASCVD events [35, 36]. Lipoprotein(a) (Lp(a)) is an LDL-P modified with the covalent addition of apoprotein(a) to apoB [37, 38]. Lp(a) is highly proatherogenic, is an important delivery platform for oxidized phospholipid into the arterial wall, is prothrombotic, and is an established risk factor for ASCVD [39–41]. The mechanism(s) by which Lp(a) is cleared from the circulation are as yet undefined [42]. In a pooled analysis that

includes 4943 participants in 15 phase 2 and phase 3 clinical trials, evolocumab dosed at 140 mg every 2 weeks or 420 mg every 4 weeks reduces median Lp(a) 22%–38% and 20%–33%, respectively [43]. Apo B is reduced by the 140 mg and 420 mg doses by 46%–52% and 40–48%, respectively. Non-HDL-C (defined as total cholesterol minus HDL-C) is reduced by the 140 mg and 420 mg doses of evolocumab by 49–56% and 48–52%, respectively. Compared to either placebo (most patients on intensive statin therapy except in patients with statin intolerance) or ezetimibe therapy, evolocumab dramatically increases the capacity to attain a non-HDL-C < 100 mg/dL (2.6 mmol/L) or an apo B < 80 mg/dL in patients with primary hyperlipidemia/mixed dyslipidemia, statin intolerance, HeFH, type 2 diabetes mellitus, and variable levels of ASCVD risk (in the DESCARTES trial) (Fig. 14.6).

Triglyceride-Enriched Lipoproteins and Lipoprotein Subfractions

Evolocumab promotes a range of changes in lipoprotein particles and subfractions. LDL-P number [44, 45] and size [46, 47], triglyceride-enriched lipoproteins and their remnants [48, 49], and HDL subfractions [50, 51] have been implicated as important risk factors for ASCVD. The apo B/apo A-I ratio is viewed as a strong predictor of CHD risk [52, 53]. Total LDL-P as well as both large and small LDL particles decrease significantly in response to evolocumab therapy [54] (Table 14.1). With the addition of evolocumab to statin therapy, the total LDL-P burden decreases from 1110 to 610 nmol/L. Total HDL particles increase significantly. In addition, the sum of chylomicron and very-low-density lipoprotein particles (VLDL-P) decrease, and both small VLDL-P and intermediate-density lipoprotein particles decrease significantly (Table 14.1). In this same study, HDL-C and apo A-I increased modestly compared to placebo by 5.7% and 2.5%, while VLDL-C and apoB/Apo A-I decreased by 13.0% and 43.2%, respectively [54]. These broad-spectrum changes in lipoproteins and their subfractions are generally viewed as beneficial.

Impact of Evolocumab on Coronary Atherosclerosis

The Global Assessment of Plaque Regression with a PCSK9 Antibody as Measured by Intravascular Ultrasound (GLAGOV) trial tested whether or not treatment of patients with evolocumab (dosed at 420 mg monthly) reduced the progression of atherosclerosis in coronary target lesions using intravascular ultrasonography [55]. When compared to placebo, evolocumab treatment reduced percent atheroma volume by −1.0% ($p < 0.001$), total atheroma volume decreased by −4.9 mm^3 ($p < 0.001$), and more patients experienced plaque regression (17% and 12.5% more experienced reductions in percent atheroma volume and total atheroma volume, respectively (both $p < 0.001$)). Among patients who achieved LDL-C < 70 mg/dL, evolocumab therapy compared to placebo had an even more favorable impact on plaque features: percent atheroma volume decreased −1.62%, and the percentage of patients with regression of percent atheroma volume was 33.2% (both $p < 0.001$). Hence, evolocumab therapy potentiates significant plaque regression, and the lower the LDL-C was reduced, the greater the improvement in atherosclerotic plaque features.

Impact of Evolocumab on Cardiovascular Events

The Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk (FOURIER) trial evaluated the efficacy and safety of evolocumab therapy compared to placebo in 27,564 patients with established ASCVD already being treated with moderate or high intensity statin therapy [56]. Evolocumab reduced LDL-C by a mean of 59% (from 92 to 30 mg/dL). The primary composite endpoint was comprised of CV mortality, MI, stroke, hospitalization for unstable angina, or coronary revascularization. The secondary composite endpoint was comprised of CV death, MI, and stroke. The primary and secondary composite endpoints were reduced by evolocumab compared to placebo by 15% ($p < 0.001$) and 20% ($p < 0.001$), respectively. Myocardial infarction was reduced by 27% ($p < 0.001$), stroke was reduced by 21%

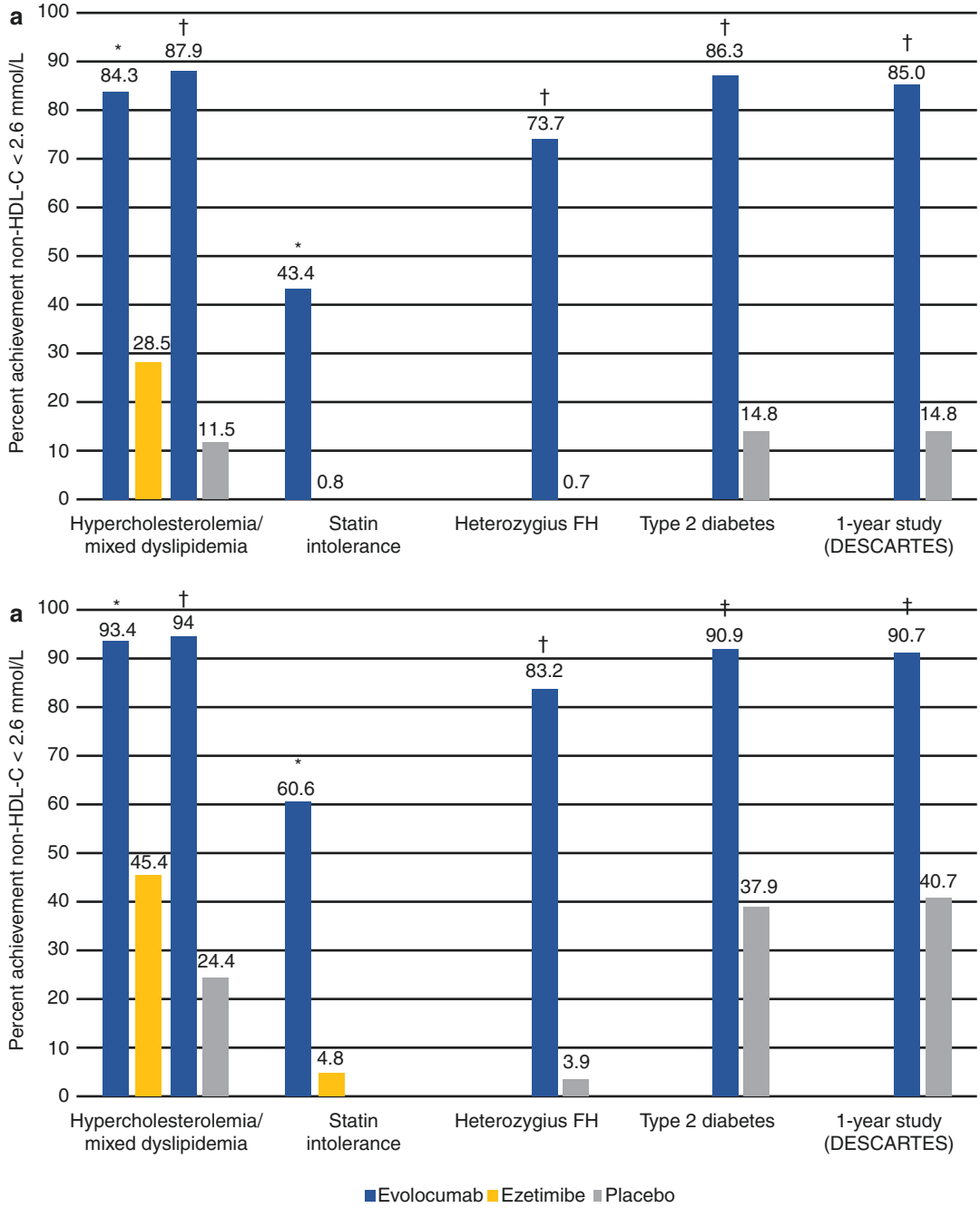


Fig. 14.6 Percent achievement in placebo or ezetimibe-controlled phase 2 and phase 3 evolocumab studies of (**a**) non-HDL-C < 100 mg/dL (2.6 mmol/L) and (**b**) ApoB <80 mg/dL. The percentages of patients who achieved non-HDL-C < 100 mg/dL (**a**) and ApoB <80 mg/dL (**b**) with evolocumab, ezetimibe, or placebo are depicted in this plot for all studies with a placebo or ezetimibe comparator. Results are shown separately for each patient population examined (hypercholesterolemia/mixed dyslipidemia, type 2 diabetes mellitus, heterozygous FH, and statin intolerance), all 12 weeks in duration, as well as for the 1-year study (DESCARTES). ApoB indicates apolipoprotein B; FH, familial hypercholesterolemia; non-HDL-C, non-high-density lipoprotein cholesterol. *Evolocumab-treated patients with ezetimibe comparator arm; †Evolocumab-treated patients with placebo comparator arm. (From Toth et al. [43])

($p < 0.01$), and coronary revascularization was reduced by 22% ($p < 0.001$). The larger the LDL-C reduction, the bigger the benefit. There was continuous benefit all the way down to <10 mg/dL of LDL-C. When comparing an attained LDL-C of <10 mg/dL to >100 mg/dL, there was a 41% relative risk reduction for mortality, MI, and stroke ($p = 0.02$). Neither cardiovascular nor all-cause mortality was reduced by evolocumab. There was no heterogeneity for benefit among prespecified subgroups. Hepatic, skeletal muscle, neurocognitive, and other adverse events in general were not different between groups with the exception of injection site reactions, which occurred more frequently in patients treated with evolocumab.

In a post hoc analysis of the FOURIER trial, the impact of evolocumab therapy on CV event rates in 22,351 patients with a prior MI was evaluated with respect to time from most recent MI, number of prior MIs, and whether or not a patient had residual multivessel CAD (≥40% stenosis in ≥2 large vessels) [57]. For patients who sustained a qualifying MI < 2 years ago or ≥2 years ago, the composite of CV mortality, MI, and stroke were reduced by 24% ($p < 0.001$) and 13% ($p = 0.04$), respectively (Fig. 14.7). For patients who sustained ≥2 previous MIs vs 1 MI, this composite was reduced by 21% ($p = 0.006$) and 16% ($p = 0.008$), respectively. Having or not having multivessel disease was associated with a 30% ($p < 0.001$) and 11% ($p = 0.055$) reduction in this composite endpoint, respectively.

In the setting of peripheral arterial disease (PAD), evolocumab appears to be particularly beneficial. When comparing evolocumab therapy to placebo in patients with and without PAD in the Fourier trial, the composite of CV death, MI, and stroke were reduced by 27% ($p = 0.004$) and 19% ($p < 0.001$), respectively [58]. For the trial as a whole, major acute limb events (defined as a composite of acute limb ischemia, major amputation (above the knee or below the knee, excluding forefoot or toe), and urgent revascularization (thrombolysis or urgent vascular intervention for ischemia) were reduced by evolocumab by 42% ($p = 0.0093$) (Fig. 14.8). Among patients with established PAD, evolocumab reduced major acute limb events by 37%, but this finding was not statistically significant. However, the event curves for the two groups separate in a compelling way (Fig. 14.8). For patients without a prior history of PAD, evolocumab reduced major acute limb events by 63% ($p = 0.0197$). In all three analyses, event curve separation is immediate and increases as a function of time. Benefit also increased as LDL-C decreased, even below 10 mg/dL.

In a subgroup analysis of 11,033 participants with diabetes in the FOURIER trial, there was an 18% ($p = 0.0021$) and 22% reduction in the composite of CV death, MI, and stroke for patients with and without diabetes [59]. Evolocumab did not increase the incidence of diabetes in patients with either no diabetes or prediabetes. In addition, hemoglobin a1c and fasting plasma glucose levels remained unchanged between groups showing no disturbance in glycemic control induced by evolocumab.

As noted above, evolocumab reduces serum levels of Lp(a). Evolocumab reduced the risk of CV death, MI, or urgent revascularization by 23% (hazard ratio, 0.77; 95% CI, 0.67–0.88) in patients with a baseline Lp(a) >median and by 7% (hazard ratio, 0.93; 95% CI, 0.80–1.08; *P* interaction = 0.07) in those ≤median [60]. Hence, it is plausible to conclude that the Lp(a) reduction promoted by evolocumab therapy contributes to overall risk reduction.

Evolocumab and Neurocognitive Impairment

There has been lingering concern that lipid lowering, especially with statins, may be associated with cognitive impairment. Although largely based on speculation, there is particular concern that if LDL-C is lowered below 50 mg/dL, this may precipitate adverse changes in the brain resulting in memory impairment or frank dementia. While it is imperative that vigilance always be maintained for adverse side effects from any pharmacologic intervention, there has historically been no prospective clinical trial evidence that aggressive LDL-C reduction or statin therapy per se cognitive impairment. Cholesterol metabolism behind the blood-brain

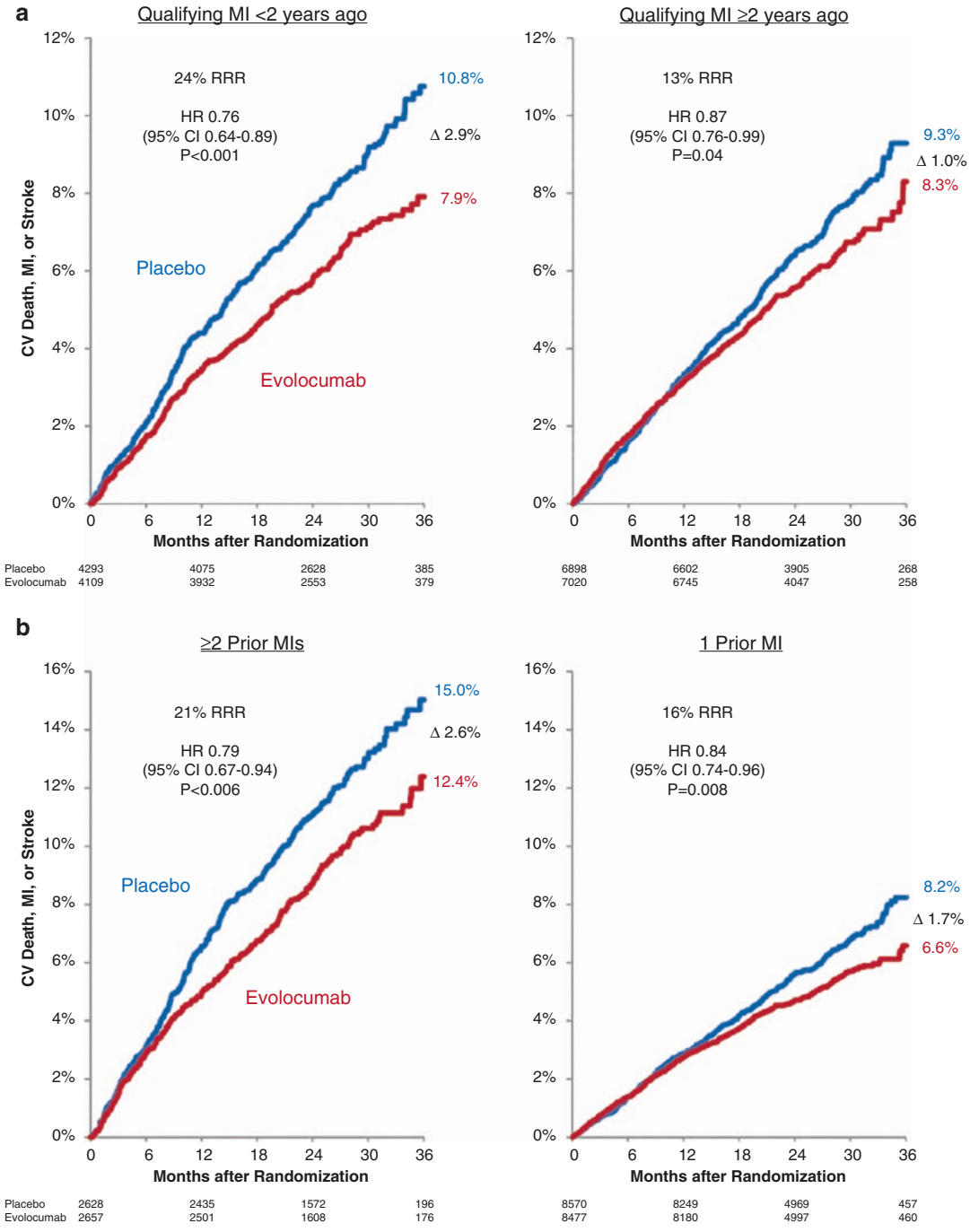


Fig. 14.7 Incidence of the key secondary end point in patients stratified by high-risk features. Cumulative incidence curves for the key secondary end point by treatment arm in patients stratified by time from qualifying myocardial infarction (MI; **a**), number of prior MIs (**b**), and presence of residual multivessel coronary artery disease (**c**). *P* values for interactions between treatment and subgroups were 0.18, 0.57, and 0.03, respectively. CI indicates confidence interval; CV, cardiovascular; HR, hazard ratio; and RRR, relative risk reduction. (From Sabatine et al. [57])

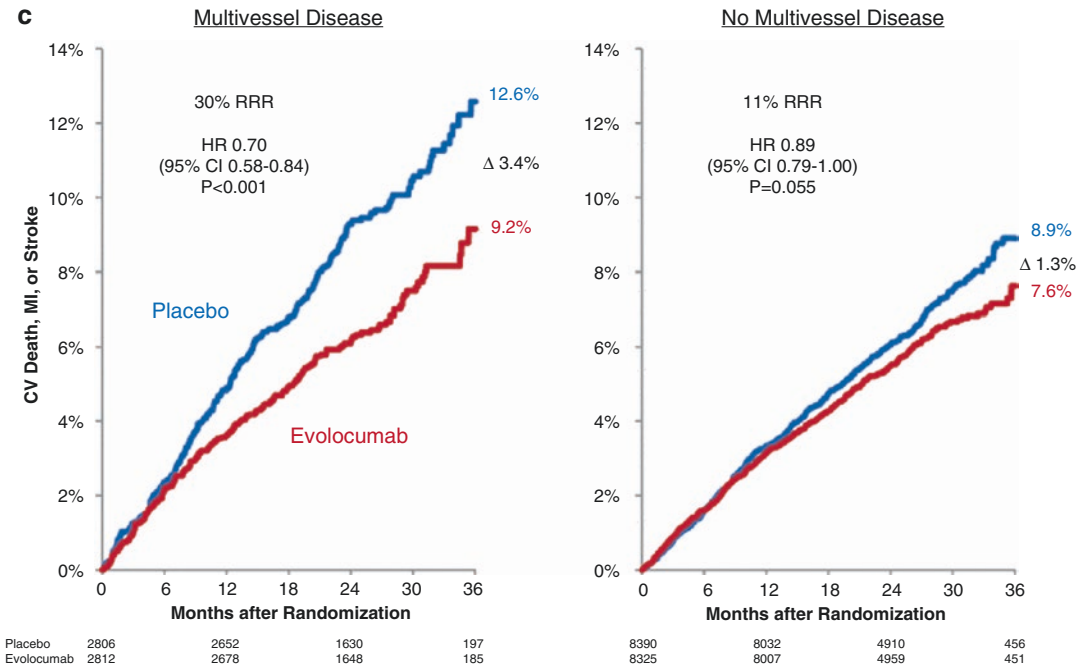


Fig. 14.7 (continued)

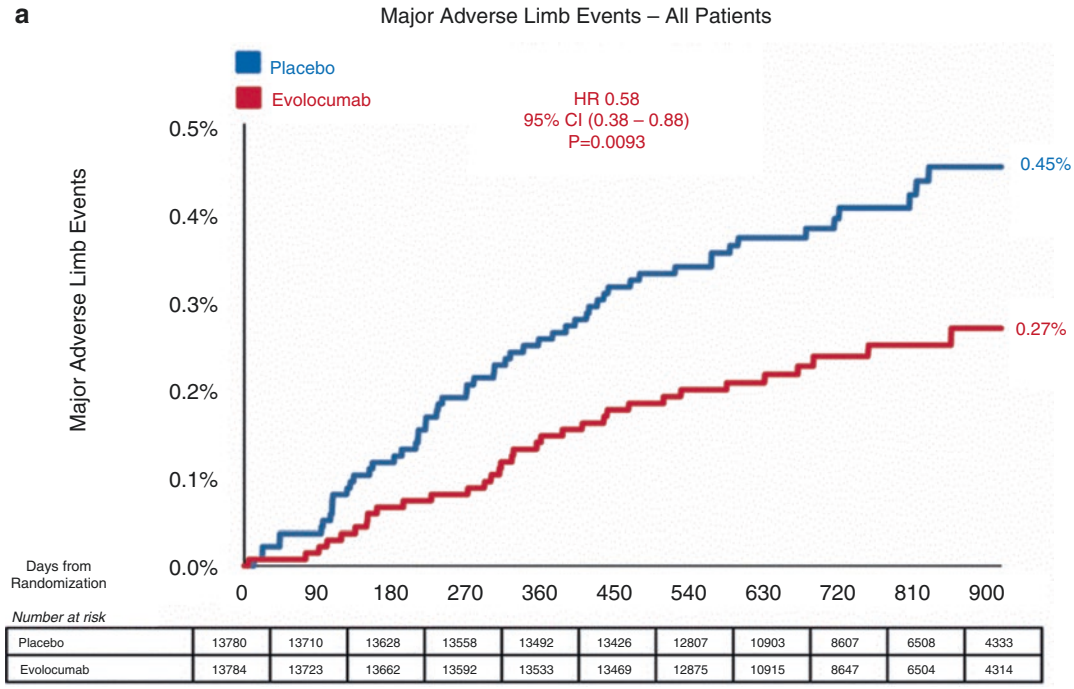
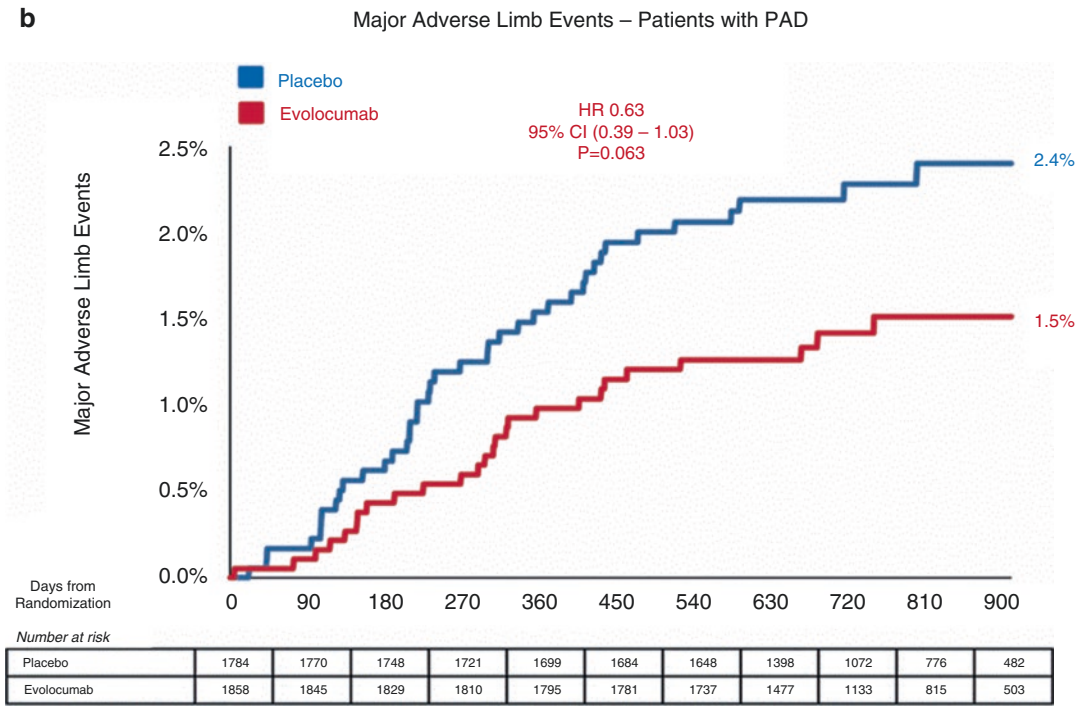


Fig. 14.8 Major adverse limb events. Major adverse limb events (composite of acute limb ischemia, major amputation, or urgent revascularization) by treatment (evolocumab in red, placebo in blue) in all randomly assigned patients (**a**), in patients with symptomatic PAD (**b**), and in patients with no known PAD (**c**). CI indicates confidence interval, HR hazard ratio, and PAD peripheral artery disease. (From Bonaca et al. [58])



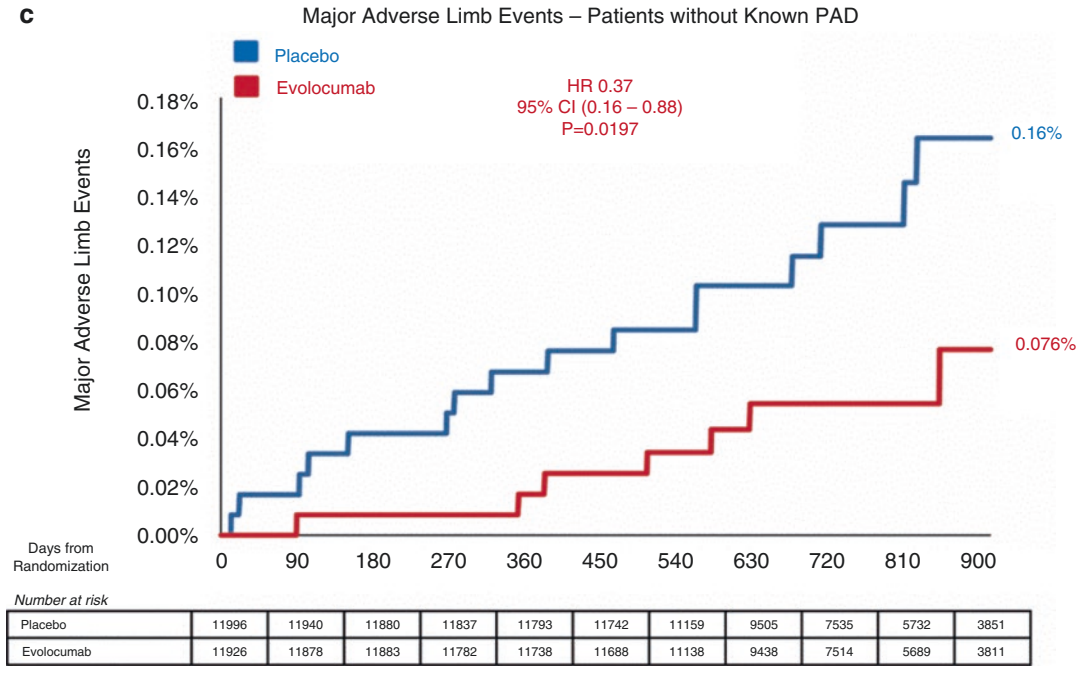


Fig. 14.8 (continued)

barrier is completely segregated from the central circulation. Within the brain, oligodendrocytes and astrocytes provide all of the cholesterol necessary for myelin formation and normal neuronal function [61, 62]. Neither cholesterol nor lipoproteins cross the blood brain barrier.

The National Lipid Association's Statin Safety Task Force concluded that statins as a class are not associated with adverse effects on cognition (strength of recommendation: A) [63]. Neither the Heart Protection Study [64] nor the Prospective Study of Pravastatin in the Elderly at Risk [65] trials were unable to demonstrate any adverse impact of statin therapy on cognitive capacity in patients with dyslipidemia. An early meta-analysis suggested that statins reduce risk of neurocognitive impairment [66]. In a prospective cohort study, after adjusting for education, smoking status, the presence of at least one APOE ε4 allele, and history of stroke or diabetes at baseline, participants treated with statins had a 48% lower risk of developing dementia compared to those who had not been treated with a statin [67]. The Cardiovascular Health Study showed a similar 44% lower risk of Alzheimer's type dementia compared to patients not taking lipid-lowering therapy [68]. Much of the concern surrounding lipid lowering and dementia relies on case reports, anecdote, and unconfirmed submissions to the adverse event reporting system of the Food and Drug Administration.

The Evaluating PCSK9 Binding Antibody Influence on Cognitive Health in High Cardiovascular Risk Subjects (EBBINGHAUS) study prospectively evaluated whether or not lipid-lowering therapy with a statin ± evolocumab very low LDL-C are associated with increased risk for cognitive impairment [69].

The Evaluating PCSK9 Binding Antibody Influence on Cognitive Health in High Cardiovascular Risk Subjects (EBBINGHAUS) study investigated whether or not lipid-lowering therapy with statins and evolocumab or low levels of LDL-C induce neurocognitive impairment. A 1204 patient subgroup of the FOURIER trial prospectively underwent assessment of their cognitive function using the Cambridge Neuropsychological Test Automated Battery (CANTAB). The CANTAB is a computer-based test that is valid independent of language and culture. Four components of cognition were evaluated: (1) spatial working memory strategy index of executive function, (2) spatial working memory between errors, (3) paired associates learning, and (4) reaction time. Patients also self-evaluated everyday cognition at the conclusion of the study. The EBBINGHAUS investigators were unable to detect any change in either individual components of the CANTAB or a global score between the beginning and end of the FOURIER trial. In addition, based on self-assessment, there was no between group differences in self-reported cognitive capacity or changes therein. These results applied even to patients who achieved ultra-low LDL-C of <10 mg/dL.

Overall Safety of Evolocumab

In addition to its efficacy, evolocumab has been shown to be safe. The most commonly occurring adverse events occurring in >5% of patients and more often than in placebo treated patients are nasopharyngitis, influenza-like reaction, upper respiratory infection, and injection site reactions [70]. In an analysis of over 6000 patients treated with evolocumab, the incidence of muscle, liver, and kidney related adverse events was similar to that observed with placebo (Table 14.2). Neurocognitive adverse events were also rare and on par with placebo, consistent with the Ebbinghaus trial (Table 14.3). Evolocumab therapy also does not increase risk for impaired glucose tolerance or diabetes mellitus [56, 70]. The side-effect profile of evolocumab is unchanged when comparing patients who achieve LDL-C levels on therapy of >40 mg/dL, 25–40 mg/dL, or <25 mg/dL [70].

Table 14.2 Laboratory investigations for muscle injury, liver function, and renal function

	Integrated parent Studies		Integrated interim extension studies Year 1 SoC-controlled period	
	Control[a]	Evolocumab	SoC	Evolocumab
	(*N* = 2080)	(*N* = 3946)	(*N* = 1489)	(*N* = 2976)
CK				
Number of patients with any post-baseline CK measurement	2055	3892	1472	2962
CK >5 × ULN, *n* (%)	14 (0.7)	27 (0.7)	17 (1.2)	17 (0.6)
CK >10 × ULN, *n* (%)	5 (0.2)	9 (0.2)	9 (0.6)	7 (0.2)
Liver function tests				
Number of patients with any post-baseline liver function test measurement	2055	3893	1477	2968
ALT or AST >3 × ULN, *n* (%)	20 (1.0)	17 (0.4)	18 (1.2)	31 (1.0)
ALT or AST >5 × ULN, *n* (%)	7 (0.3)	6 (0.2)	3 (0.2)	10 (0.3)
Total bilirubin >2 × ULN, *n* (%)	3 (0.1)	6 (0.2)	2 (0.1)	8 (0.3)
(ALT or AST >3 × ULN) and (total bilirubin >2 × ULN), *n* (%)	0	0	0	1 (<0.1)
Renal function tests				
Serum creatinine				
Baseline mean (SD), mg/dL	0.9 (0.2) (*n* = 302[b])	0.9 (0.2) (*n* = 599[b])	0.9 (0.2)	0.9 (0.2)
Number of patients evaluated at week 52	273	533	402	833
Mean (SD) change from baseline at week 52, mg/dL	−0.01 (0.1)	0.01 (0.1)	−0.01 (0.1)	−0.01 (0.1)
Blood urea nitrogen				
Baseline mean (SD), mg/dL	15.8 (4.5) (*n* = 302[b])	15.7 (4.3) (*n* = 599[b])	16.1 (4.8)	16.2 (4.4)
Number of patients evaluated at week 52	273	533	402	883
Mean (SD) change from baseline at week 52, mg/dL	0.1 (3.3)	0.2 (3.9)	−0.03 (3.8)	0.26 (3.9)

From Toth et al. [70]

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *CK* creatine kinase, *SoC* standard of care, *ULN* upper limit of normal

[a]Control includes placebo and ezetimibe treatment groups

[b]For the parent trials, week 52 renal function data are available from the DESCARTES study, which enrolled 901 patients (evolocumab plus background therapy, *n* = 599; placebo plus background therapy, *n* = 302)

Alirocumab

LDL-C Reduction in Primary Hyperlipidemia

Alirocumab can be dosed at 75 mg or 150 mg SQ every 2 weeks or 300 mg SQ every 4 weeks. In patients with primary hyperlipidemia, the 75 mg and 150 mg doses induce mean LDL-C reductions of 43% and 58%, respectively. When specifically evaluated in high-risk patients on maximally tolerated statin therapy given 75 mg every 2 weeks, mean LDL-C reduction was 48.2%, and patients in the alirocumab/statin treatment arm achieved LDL-C < 70 mg/dL 75% of the time after 6 months of therapy compared to 9% for placebo treatment [71]. Among patients with type 1 and type 2 diabetes mellitus (T1DM, T2DM) treated with maximally tolerated statin therapy, alirocumab treatment (either at 75 mg or 150 mg every 2 weeks if LDL-C not controlled with the lower dose) for 6 months decreased LDL-C by 49% and 47.8% in T2DM and T1DM patients, respectively, compared to placebo [72]. In addition,

Table 14.3 Neurocognitive adverse events

	Integrated parent Studies		Integrated interim extension studies Year 1 SoC-controlled period	
	Control[a]	Evolocumab	SoC	Evolocumab
	(*N* = 2080)	(*N* = 3946)	(*N* = 1489)	(*N* = 2976)
Any neurocognitive-related AE[b], *n* (%)	6 (0.3)	5 (0.1)	5 (0.3)	27 (0.9)
Amnesia	0	2 (0.1)	2 (0.1)	8 (0.3)
Disorientation	2 (0.1)	1 (<0.1)	0	1 (<0.1)
Memory impairment	1 (<0.1)	1 (<0.1)	3 (0.2)	7 (0.2)
Delirium	0	1 (<0.1)	0	0
Cognitive disorder	1 (<0.1)	0	0	1 (<0.1)
Dementia with Lewy bodies	1 (<0.1)	0	0	0
Disturbance in attention	1 (<0.1)	0	0	0
Dementia	0	0	0	3 (0.1)
Confusional state	0	0	0	2 (0.1)
Mental impairment	0	0	0	2 (0.1)
Alzheimer's type dementia	0	0	0	2 (0.1)
Illusion	0	0	0	1 (<0.1)
Transient global amnesia	0	0	0	1 (<0.1)
Neurocognitive-related AEs leading to study drug discontinuation, n (%)	1 (<0.1)	1 (<0.1)	N/A	3 (0.1)

From Toth et al. [70]
Neurocognitive events are listed in decreasing order of frequency in the evolocumab arm of the parent trials
AE adverse event, *N/A* not applicable, *SoC* standard of care
[a]Control includes placebo and ezetimibe treatment groups
[b]Neurocognitive events were identified using deliria (including confusion), cognitive and attention disorders and disturbances, dementia and amnestic conditions, disturbances in thinking and perception, and mental impairment disorders in high-level group terms

LDL < 70 mg/dL was achieved in 76.4% and 70.2% of T2DM and T1DM patients, respectively. In both groups, fasting blood glucose and glycated hemoglobin levels remained unchanged in the two treatment groups.

LDL-C Reduction in Familial Hypercholesterolemia

Alirocumab is indicated for the treatment of HeFH. In the Efficacy and Safety of Alirocumab Versus Placebo on Top of Lipid-Modifying Therapy in Patients with Heterozygous Familial Hypercholesterolemia Not Adequately Controlled with Their Lipid-Modifying Therapy (ODYSSEY FH I and FH II), patients were treated for 78 weeks with the highest tolerated dose of a statin ± other lipid-lowering therapy and then randomized to either alirocumab (75 mg every 2 weeks and then increased to 150 mg every 2 weeks if LDL-C > 70 mg/dL) [73]. The mean LDL-C decreased from 144.7 to 71.3 mg/dL (57.9% reduction compared to placebo) in FH I and decreased from 134.6 to 67.7 mg/dL (51.4% reduction compared to placebo) in FH II [73]. These changes in LDL-C were maintained through 78 weeks of treatment. In ODYSSEY High FH (HeFH with LDL-C > 160 mg/dL despite maximally tolerated statin therapy ± other lipid-lowering therapy), alirocumab dosed at 150 mg every 2 weeks reduced mean LDL-C by 90.8 mg/dL at week 24, and this change was maintained through 78 weeks of treatment [74]. Alirocumab is not yet indicated for the treatment of HoFH.

Apo B, Non-HDL-C, and Lipoprotein(a) Reduction

In a pooled analysis of ten phase 3 ODYSSEY studies which included 4983 participants, alirocumab dose at 75 or 150 mg every week reduced non-HDL-C by approximately 42%–51% and 40% in patients who did and did not receive concomitant statin therapy, respectively [74]. Compared to statin monotherapy control arms, the addition of alirocumab to ongoing statin therapy increased the attainment of non-HDL-C < 100 mg/dL to 70–80% from 7%–10% (Fig. 14.9). Similarly, use of alirocumab at 75/150 mg every 2 weeks added to ongoing statin therapy reduced apoB by 40%–52%; among patients not treated with a statin, alirocumab decreased apoB by 36.5%. Alirocumab increased the goal attainment rate for apo B < 80 mg/dL to 78%–85% from approximately 20% on placebo (Fig. 14.9). For patients not on a statin alirocumab induced apo B <80 mg/dL in approximately 70% of patients.

Alirocumab reduces Lp(a) significantly. In a pooled analysis of ten phase 3 studies including 4915 participants, alirocumab reduced Lp(a) from baseline by 23% to 27% with alirocumab 75/150-mg Q2W and 29% with alirocumab 150-mg Q2W (both $p < 0.0001$ vs placebo) at 6 months [75]. These reductions in Lp(a) were sustained over 78–104 weeks and were independent of race, gender, the presence of familial hypercholesterolemia, baseline Lp(a) and LDL-C concentrations, or use of statins.

Triglyceride-Enriched Lipoproteins and Lipoprotein Subfractions

A comprehensive analysis of changes induced by alirocumab in lipoproteins and their subfractions as well as apo B, apoA1, ApoCII, and ApoCIII are summarized in Table 14.4 [76]. $LDL_{1–2}$ are larger, more buoyant LDL species, while $LDL_{3–4}$ are smaller, denser LDL species. Remnant lipoproteins are defined as $VLDL_3$-C + IDL-C. The ratio of apo B/apoA1 is a well-recognized marker of CV risk.

Alirocumab induces substantive reductions in triglycerides, LDL-C subfractions, VLDL-C and its subfractions, IDL-C, LDL_R-C (LDL_{real} = LDL-C – Lp(a)-C – IDL-C), and remnant lipoprotein cholesterol (RLP-C) (Table 14.4). In addition, alirocumab therapy correlates with significant reductions in apoB/apoA1 ratio, ApoCII, and ApoCIII. All of these changes would be expected to be both advantageous and beneficial with regard to ASCVD risk.

Impact of Alirocumab on Cardiovascular Events

The clinical efficacy of alirocumab was evaluated in the ODYSSEY OUTCOMES trial, which included 18,924 patients who sustained an ACS

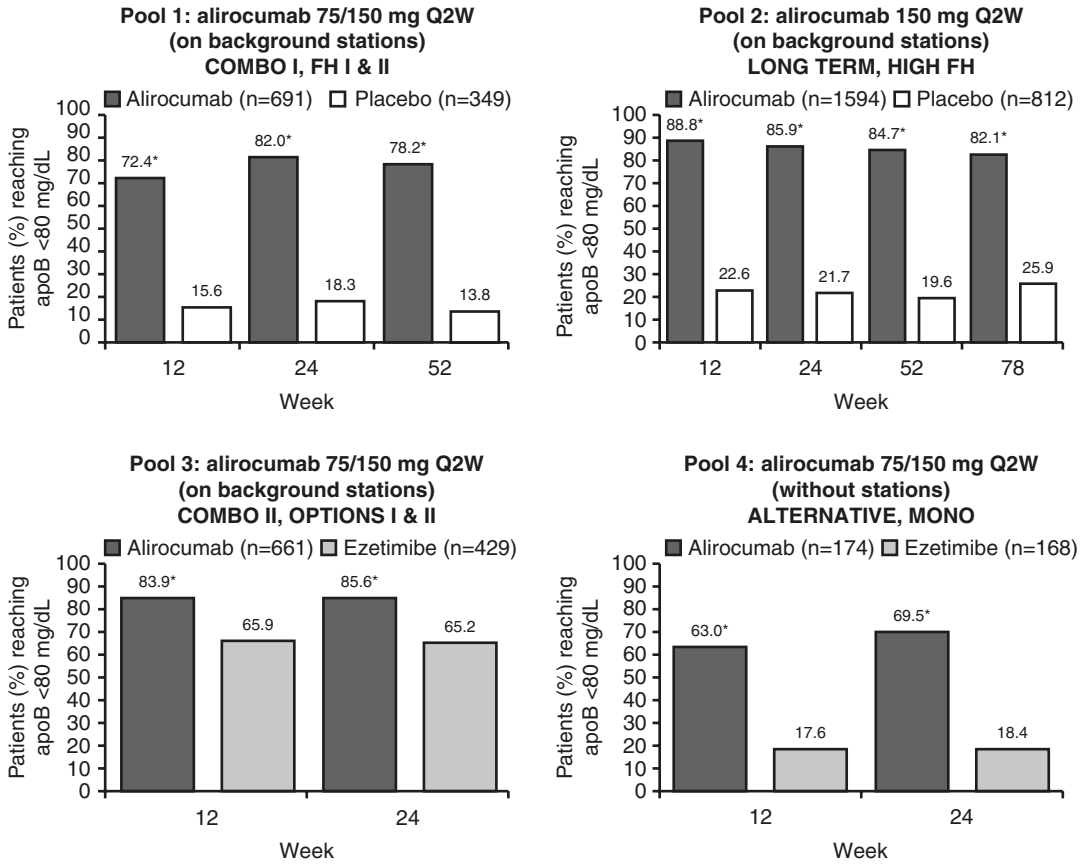


Fig. 14.9 Percent of patients achieving apoB levels<80 mg/dL during the studies (modified intention to treat population). $P < 0.0001$ vs control group at all time points and in all study pools. ApoB indicates apolipoprotein B, Q2W every 2 weeks. (From Bays et al. [98])

within the year previous to enrollment [77]. All patients were treated with either high-intensity statin therapy of the highest dose of a statin they could tolerate. Patients were randomized to either alirocumab 75/150 mg every 2 weeks with the goal of an attained LDL-C on therapy of 25–50 mg/dL. The median duration of follow-up was 2.8 years. The primary composite endpoint included death from coronary heart disease, nonfatal MI, unstable angina requiring hospitalization, and fatal or nonfatal ischemic stroke. Alirocumab reduced the primary composite endpoint compared to placebo by 15% ($p < 0.001$). The composite of all-cause mortality, nonfatal MI, and nonfatal ischemic stroke was reduced by 14% ($p < 0.001$). Other endpoint reductions included nonfatal MI 14%, fatal or nonfatal ischemic stroke 27%, unstable angina requiring hospitalization 39%, and ischemia-driven revascularization 12%. Neither coronary nor cardiovascular mortality was reduced significantly.

A variety of additional analyses of ODYSSEY OUTCOMES demonstrate broad benefit from alirocumab. The number needed to treat to prevent major adverse cardiovascular events (MACE) over a period of 3 years decreases as a function of age: 43 at age 45 years, 26 at age 75 years, and 12 at age 85 years [78]. Relative risk reduction for MACE was consistent for person above or below the age of 65 years. Alirocumab decreases risk of any stroke (28%) without increasing the risk of hemorrhagic stroke, irrespective of attained LDL-C or history of cerebrovascular disease (Fig. 14.10). This is a highly

Table 14.4 Pooled data from three studies of alirocumab for changes from baseline in cholesterol content of lipoprotein subfractions, apoB/apoA1 ratio, and levels of apo CII and CIII using vertical auto profiling (ultracentrifugation)

	Pooled data	
Lipoprotein subfractions, mg/dL	Placebo $n = 72$[a]	Alirocumab 150 mg Q2W $n = 100$[a]
LDL total		
Baseline	121.8 (29.1)	120.9 (28.8)
Posttreatment[b]	110.3 (33.1)	42.8 (22.9)
Mean (SD) change from baseline, %	−7.5 (25.1)	−64.1 (18.4)
P-value		<0.0001
LDL-R		
Baseline	95.7 (25.6)	94.4 (25.1)
Posttreatment[b]	86.1 (27.7)	27.9 (19.7)
Mean (SD) change from baseline, %	−7.2 (28.4)	−70.6 (19.4)
P-value		<0.0001
LDL_1-C		
Baseline	19.8 (7.6)	19.5 (8.5)
Posttreatment[b]	17.1 (8.8)	4.9 (4.3)
Mean (SD) change from baseline, %	−7.4 (47.6)	−64.0 (110.0)
P-value		0.0001
LDL_2-C		
Baseline	26.2 (14.3)	25.3 (14.2)
Posttreatment[b]	20.8 (14.5)	4.9 (7.5)
Mean (SD) change from baseline, %	−8.3 (98.9)	−82.8 (21.4)
P-value		<0.0001
LDL_3-C		
Baseline	39.4 (14.6)	37.9 (14.3)
Posttreatment[b]	35.9 (13.0)	11.3 (9.0)
Mean (SD) change from baseline, %	−0.2 (45.0)	−68.4 (25.7)
P-value		<0.0001
LDL_4-C		
Baseline	10.4 (7.9)	11.7 (10.0)
Posttreatment[b]	12.3 (8.6)	6.8 (3.3)
Mean (SD) change from baseline, %	78.9 (182.9)	13.7 (213.3)
P-value		0.1596
LDL_{1+2}-C		
Baseline	46.0 (19.7)	44.8 (20.2)
Posttreatment[b]	37.9 (21.4)	9.8 (11.0)
Mean (SD) change from baseline, %	−11.1 (46.4)	−62.3 (169.1)
P-value		0.0201
LDL_{3+4}-C		
Baseline	49.7 (18.5)	49.7 (21.6)
Posttreatment[b]	48.2 (17.1)	18.1 (11.1)
Mean (SD) change from baseline, %	6.0 (46.3)	−60.3 (25.5)
P-value		<0.0001
ApoB/A1		
Baseline	0.7 (0.1)	0.7 (0.2)
Posttreatment[b]	0.7 (0.2)	0.4 (0.1)
Mean (SD) change from baseline, %	−4.5 (17.2)	−47.3 (14.5)
P-value		<0.0001

(continued)

Table 14.4 (continued)

Lipoprotein subfractions, mg/dL	Pooled data	
	Placebo $n = 72$[a]	Alirocumab 150 mg Q2W $n = 100$[a]
VLDL-C		
Baseline	24.5 (20.0–32.5)	23.0 (18.0–30.5)
Posttreatment[b]	23.5 (18.0–29.5)	17.0 (14.0–20.0)
Mean (SD) change from baseline, %	−3.9 (−22.5 to 19.4)	−27.1 (−38.9 to −15.8)
P-value		<0.0001
$VLDL_{1+2}$-C		
Baseline	9.9 (7.7–13.2)	9.5 (7–12.7)
Posttreatment[b]	9.4 (7.2–12.1)	7.1 (5.5–8.8)
Mean (SD) change from baseline, %	−1.6 (−28.0 to 24.4)	−27.8 (−42.9 to −12.2)
P-value		<0.0001
$VLDL_3$-C		
Baseline	14.5 (12.0–18.0)	14.0 (11.0–17.0)
Posttreatment[b]	13.5 (11.0–18.0)	10.0 (9.0–12.0)
Mean (SD) change from baseline, %	−5.1 (−18.8 to 10.8)	−25.8 (−35.7 to −15.4)
P-value		<0.0001
IDL-C		
Baseline	16.5 (13.0–21.0)	17.0 (12.0–21.0)
Posttreatment[b]	15.0 (10.5–20.5)	7.0 (5.0–9.0)
Mean (SD) change from baseline, %	−10.6 (−28.6 to 13.9)	−57.1 (−68.6 to −42.3)
P-value		<0.0001
Triglycerides		
Baseline	135.0 (102.5–202.0)	132.0 (97.0–179.0)
Posttreatment[b]	137.0 (99.5–190.0)	101.0 (82.0–151.5)
Mean (SD) change from baseline, %	0.2 (−24.7 to 23.4)	−21.7 (−35.8 to 3.8)
P-value		0.0008
RLP-C		
Baseline	32.5 (25.5–40.0)	30.5 (23.0–36.0)
Posttreatment[b]	28.5 (23.0–37.0)	17.5 (14.0–21.0)
Mean (SD) change from baseline, %	−7.2 (−21.0 to 11.0)	−42.5 (−52.9 to −31.3)
P-value		<0.0001
ApoCII		
Baseline	4.8 (2.0)	4.9 (2.0)
Posttreatment[b]	4.8 (2.3)	3.9 (1.4)
Mean (SD) change from baseline, %	2.8 (32.5)	−17.1 (25.7)
P-value		<0.0001
ApoCIII		
Baseline	11.3 (4.3)	11.2 (4.1)
Posttreatment[b]	11.3 (4.8)	9.1 (2.7)
Mean (SD) change from baseline, %	4.0 (33.6)	−15.0 (19.3)
P-value		<0.0001
ApoCII/VLDL-C		
Baseline	0.2 (0.1)	0.2 (0.1)
Posttreatment[b]	0.2 (0.1)	0.2 (0.1)
Mean (SD) change from baseline, %	6.0 (23.5)	10.7 (25.6)
P-value		0.0866
ApoCIII/VLDL-C		
Baseline	0.4 (0.1)	0.5 (0.2)
Posttreatment[b]	0.5 (0.1)	0.5 (0.1)

Table 14.4 (continued)

Lipoprotein subfractions, mg/dL	Pooled data	
	Placebo $n = 72$[a]	Alirocumab 150 mg Q2W $n = 100$[a]
Mean (SD) change from baseline, %	7.4 (23.8)	15.5 (23.1)
P-value		0.0072

From Toth et al. [76]

Mean (SD) are reported for continuous normally distributed variables, while median (interquartile range) are reported for nonnormally distributed variables. Units are mg/dL

Q2W every 2 weeks, *Apo* apolipoprotein, *IDL-C* intermediate-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *LDLr* "LDL real" (i.e. total LDL fraction minus Lp(a) and intermediate density lipoprotein), *Lp(a)* lipoprotein (a), *RLP-C* remnant-like particle cholesterol, *SD* standard deviation, *VLDL-C* very low-density lipoprotein cholesterol

[a]Pooled data for pool of studies 565, 566, and 1003. Patients included from studies 565 and 1003 all received either placebo or alirocumab 150 mg Q2W. Patients in study 566 were randomized to one of three arms and received (1) placebo with increase in ATV dose from 10 to 80 mg at start of randomized treatment period, (2) alirocumab 150 mg Q2W plus ATV 10 mg, or (3) alirocumab 150 mg Q2W with increase in ATV dose from 10 to 80 mg at start of randomized treatment period

[b]Study 565, week 12; study 566, week 8; study 1003, week 6

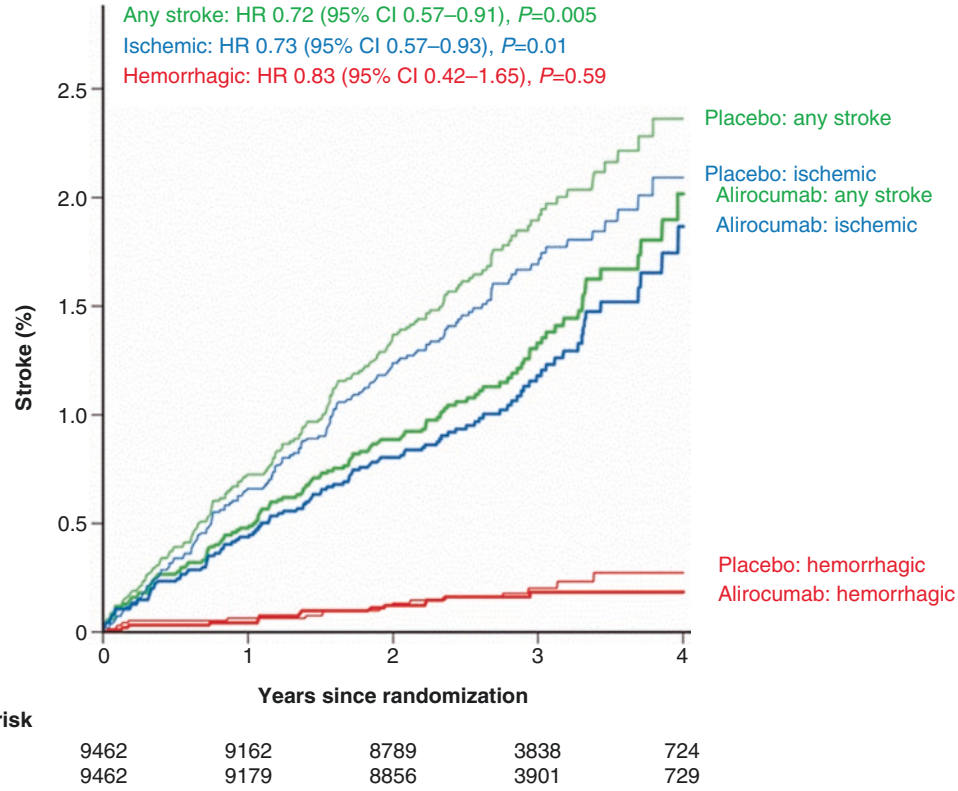


Fig. 14.10 Kaplan-Meier curves for any stroke, ischemic stroke and hemorrhagic stroke. CI indicates confidence interval and HR hazard ratio. (From Jukema et al. [99])

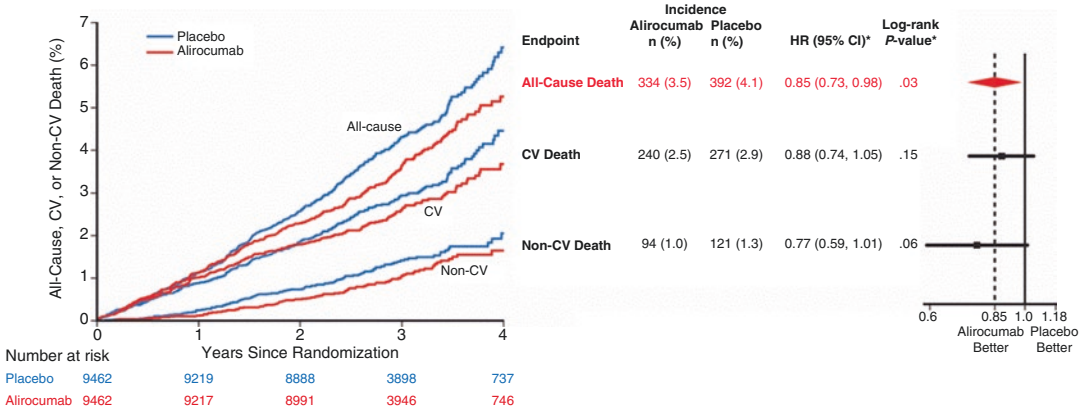


Fig. 14.11 All-cause, cardiovascular, and non-cardiovascular death (intention-to-treat population) shown as Kaplan-Meier curves (left panel) and in a forest plot (right panel). CV indicates cardiovascular and HR hazard ratio. (From Steg et al. [80])

reassuring finding given the longstanding concern that low LDL-C may correlate with increased risk for hemorrhagic stroke [79]. Although alirocumab therapy is not associated with a reduction in CV mortality, it is associated with a reduction in all-cause mortality (15%, $P = 0.03$) [80] (Fig. 14.11). This association must, however, be regarded as *nominally* significant because all-cause mortality followed CV and CHD mortality in the prespecified hierarchy of principal secondary endpoints. Among patients with a history of prior coronary artery bypass grafting prior to their qualifying ACS, alirocumab significantly reduced risk for MACE by 23% [81]. As observed in the FOURIER trial, the reduction of Lp(a) by alirocumab was shown to contribute to overall MACE reduction in the ODYSSEY OUTCOMES trial [82].

Overall Safety of Alirocumab

The side-effect profile of alirocumab is similar to that of evolocumab. The most frequently occurring adverse events are skin reactions at the injection site. Nasopharyngitis, influenza-like reaction, and diarrhea occur slightly more frequently than placebo [83]. Neurocognitive adverse events are similar to placebo. When evaluating adverse events as a function of LDL-C < 15 mg/dL, < 25 mg/dL, or > 25 mg/dL, there are no substantive differences at these different LDL-C thresholds, including for neurocognitive side-effects [84]. In ODYSSEY OUTCOMES, there were no between group differences in hepatic, skeletal muscle, or renal side effects or toxicity [77]. Risk of worsening diabetes or new onset diabetes was not different between groups.

Inclisiran

A rapidly evolving field of novel pharmacologic therapeutic agents are single-stranded and double-stranded ribonucleic acid (ssRNA and dsRNA, respectively) oligonucleotides that antagonize the translation of specific gene products. Mipomersen is an example of an ssRNA oligonucleotide) [85] (Fig. 14.12). Mipomersen enters hepatocytes and binds to a complementary nucleotide sequence according to Watson-Crick base pairing along the messenger RNA (mRNA) for apoB. This interrupts mRNA translation along the ribosome and leads to reduced apo B and, hence, VLDL production, ultimately also resulting in lower serum levels of LDL-C and Lp(a) [86]. Inclisiran is an example of a dsRNA interfering or "silencing" RNA (siRNA). DNA replication and transcription are highly regulated processes within the nucleus of a cell. However, it has become increasingly clear that gene expression is also significantly impacted by microRNAs and siRNAs that inhibit or silence gene/mRNA expression posttranscriptionally [87]. Interfering

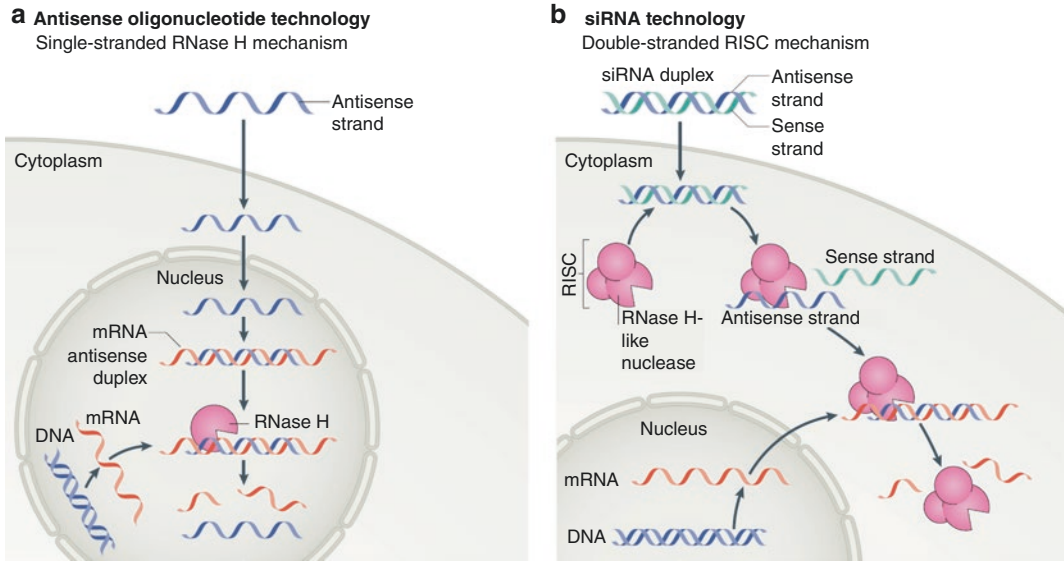


Fig. 14.12 Silencing RNA (siRNA)-based versus antisense oligonucleotide-based approaches for reducing PCSK9 expression. (**a**) Antisense oligonucleotide technology employs a single-stranded RNase H mechanism. (**b**) By contrast, small interfering RNA (siRNA) technology utilizes a double-stranded RNA-induced silencing complex (RISC) mechanism. (From Nordestgaard et al. [100])

RNAs are 20–30 nucleotides long and are composed of both an antisense strand that is complementary to a target sequence in the mRNA for a specific gene and a passenger strand [88]. The antisense strand is used to inhibit mRNA translation [89]. This, however, requires complex molecular machinery. The antisense strand is incorporated into the RNA induced silencing complex (RISC; Fig. 14.12). The RISC is a molecular complex that can be used by a cell to silence the expression of virtually any gene by three possible mechanisms: (1) interrupting mRNA translation, (2) promoting the degradation of mRNA, and (3) promoting the formation of heterochromatin or even inducing DNA elimination [87]. The antisense strand binds to an Argonaute protein which is critical for aligning the antisense strand with a target mRNA so that it can form a complementary Watson-Crick helix. Glycine-tryptophan protein of 182 kDa (GW182) promotes both translational suppression and the recruitment of CCR4–NOT deadenylase complex4 which hydrolyzes the RNA complex [89].

Inclisiran is an example of gene silencing technology that suppresses the expression of PCSK9 leading to the reduction of PCSK9 in both the intra- and extracellular compartments of the hepatocyte. Inclisiran is very specifically targeted to hepatocytes by being covalently bound to triantennary*N*-acetylgalactosamine [90]. This allows inclisiran to very specifically bind to asialoglycoprotein receptors on the hepatocyte surface with high affinity [91]. In the Trial to Evaluate the Effect of Inclisiran Treatment on Low Density Lipoprotein Cholesterol (LDL-C) (ORION-1) trial, inclisiran induced a dose-dependent reduction in serum LDL-C; inclisiran dosed at 300 mg SQ on days 1 and 90 induced the following reductions by day 180 compared to baseline and placebo: LDL-C 52.6% ($p < 0.001$), non-HDL-C 46% ($p < 0.001$), triglycerides 14.2% ($p < 0.05$), VLDL 16% ($p < 0.01$), apo B 40.9% ($p < 0.001$), Lp(a) 25.6%, and PCSK9 69% ($p < 0.001$) [92]. Injection site reactions occurred in 5% of patients receiving inclisiran. Inclisiran had a comparable rate of liver and skeletal muscle related side effects relative to placebo. On this regimen, 48% of participants achieved an LDL-C < 50 mg/dL, and 66% achieved an LDL-C < 70 mg/dL. Because of its pharmacokinetic profile and mechanism of action, inclisiran can be dosed every 6 months and provide durable, stable reductions in LDL-C [93]. Inclisiran provides identical levels of LDL-C reducing capacity to diabetics and nondiabetics

[94]. The clinical efficacy for reducing cardiovascular events by inclisiran is being evaluated in the ORION-4 trial which includes approximately 15,000 patients 55 years of age or older and with established ASCVD. It is anticipated the trial will require 5 years to complete [95].

Conclusions

1. PCSK9 is an important regulator of LDL particle uptake and catabolism.
2. PCSK9 impacts serum levels of multiple lipoprotein species and their subfractions by impacting the expression of multiple members of the LDLR family.
3. PCSK9 monoclonal antibodies (evolocumab and alirocumab) reduce LDL-C markedly and dramatically increase goal attainment rates for LDL-C, apo B, and non-HDL-C.
4. The PCSK9 mAbs have an excellent safety profile and are well tolerated.
5. The PCSK9 mAbs impact risk for CV events significantly when used in combination with statins. The risk for MI, stroke, and need for revascularization are all significantly reduced. There is no increase in risk for hemorrhagic stroke with these agents.
6. The reduction in Lp(a) by the PCSK9 mAbs contributes to ASCVD risk reduction.
7. Inclisiran suppresses the translation of PCSK9 mRNA and provides substantial capacity for reducing LDL-C as well as VLDL, apo B, and non-HDL-C. Its unique mechanism of action allows for dosing this medication twice per year.

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