Cholesterol-Lowering Agents

PCSK9 Inhibitors Today and Tomorrow

Robert S. Rosenson, Robert A. Hegele, Wolfgang Koenig

Abstract: **Loss-of-function variants in PCSK9 (proprotein convertase subtilisin-kexin type 9) are associated with lower lifetime risk of atherosclerotic cardiovascular disease) events. Confirmation of these genetic observations in large, prospective clinical trials in participants with atherosclerotic cardiovascular disease has provided guidance on risk stratification and enhanced our knowledge on hitherto unresolved and contentious issues concerning the efficacy and safety of markedly lowering LDL-C (low-density lipoprotein cholesterol). PCSK9 has a broad repertoire of molecular effects. Furthermore, clinical trials with PCSK9 inhibitors demonstrate that reductions in atherosclerotic cardiovascular disease events are more effective in patients with recent myocardial infarction, multiple myocardial infarctions, multivessel coronary artery disease, and lower extremity arterial disease. The potent LDL-C lowering efficacy of PCSK9 inhibitors provides the opportunity for more aggressive LDL-lowering strategies in high-risk patients with atherosclerotic cardiovascular disease and supports the notion that there is no lower limit for LDL-C. Aggressive LDL-C lowering with fully human PCSK9 monoclonal antibodies has been associated by a safety profile superior to that of other classes of LDL-lowering agents. These clinical trials provide evidence that LDL lowering with PCSK9 inhibitors is an effective therapy for lowering cardiovascular events in high-risk patients with LDL-C levels ≥70 mg/dL on maximally tolerated oral therapies, including statins and ezetimibe. (*Circ Res*. 2019;124:364-385. DOI: 10.1161/CIRCRESAHA.118.313238.)**

Key Words: cardiovascular disease ■ cholesterol ■ myocardial infarction ■ patients ■ subtilisin

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Numerous prospective cohort studies, natural randomization (also called Mendelian randomization) studies, and randomized clinical trials (RCTs) confirm a causal relationship between absolute exposure of vessels to LDL-C (low-density lipoprotein cholesterol) and the risk of atherosclerotic cardiovascular disease (ASCVD) events.[1] LDL-C is lowered by reduced intake of total calories in obese individuals, by reduced intake of saturated fat, and by several classes of cholesterol-lowering therapies.[2,3] Of the multiple cholesterol-lowering therapies, statins (HMG-CoA [3-hydroxy 3-methyl glutaryl coenzyme A] reductase inhibitors) constitute the most extensively studied therapeutic class for lowering LDL-C and ASCVD events.[4] However, maximally tolerated statin therapy may be inadequate to achieve the LDL-C lowering needed to reduce ASCVD risk in certain patients.[5] Furthermore, incremental lowering of LDL-C by 24% with ezetimibe in statin-treated patients after an acute coronary syndrome (ACS) reduced the risk of recurrent cardiovascular events to 32.7% in the simvastatin-ezetimibe group as compared to 34.7% in the simvastatin-monotherapy group, corresponding to an absolute risk reduction (ARR) of 2% (hazard ratio [HR], 0.936) after 7 years of treatment.[6] The introduction of PCSK9 (proprotein convertase subtilisin kexin type 9) inhibitors has opened new options for patients at high risk to achieve unprecedented low LDL-C levels for further residual risk reduction.[7] These data have emerged from large-scale clinical outcome trials with monoclonal antibodies directed against PCSK9.[8–13]

In this state-of-the-art review, we review (1) the proatherogenic mechanisms of PCSK9 in experimental models and human studies; (2) the importance of human genetics in linking PCSK9 mutations to altered ASCVD risk; and (3) clinical trial data with PCSK9 inhibitors in patients with cardiovascular disease and other high-risk individuals.

PCSK9 and Lipoprotein Metabolism

PCSK9 is a zymogen that after autocatalysis acts both intracellularly and extracellularly to ultimately inhibit the recycling of the LDLR (LDL receptor), primarily in the liver.[14–18] The LDLR normally clusters within clathrin-coated pits at the hepatocyte surface and binds LDL particles via the receptor-binding region of circulating apoB, initiating receptor-mediated endocytosis, which also engages LDLAP1 (LDLR adaptor protein 1 or ARH) as a chaperone.[15] Within late endosomes and lysosomes, as the pH increases, the LDL particle is degraded, whereas the receptor

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Nonstandard Abbreviations and Acronyms

ACS	acute coronary syndrome
ANITSCHKOW	Effects of Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) Inhibition on Arterial Wall Inflammation Study in Patients With Elevated Lipoprotein(a)
ApoER2	ApoE receptor 2
ASCVD	atherosclerotic cardiovascular disease
CRP	C-reactive protein
EPIC-HIV	Effect of PCSK9 Inhibition on Cardiovascular Risk in Treated HIV Infection
FH	familial hypercholesterolemia
FOURIER	Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk
GLAGOV	Global Assessment of Plaque Regression With a PCSK9 Antibody as Measured by Intravascular Ultrasound
GRP	glucose-regulated protein
HMG-CoA	3-hydroxy 3-methyl glutaryl coenzyme A
hs-CRP	high-sensitivity CRP
IL	interleukin
LDL	low-density lipoprotein
LDL-C	low-density lipoprotein cholesterol
LDLAP1	LDLR adaptor protein 1
LDLR	LDL receptor
LOX-1	lectin-like oxidized LDLR-1
Lp(a)	lipoprotein (a)
LRPs	LDLR-related protein
MCP	monocyte chemoattractant protein
NARC1	neural apoptosis-regulated convertase-1
NF-κB	nuclear factor κB
NLRP3	NLR family pyrin domain-containing 3
ODYSSEY Outcomes	Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab
ox-LDL	oxidized LDL
PCSK9	proprotein convertase subtilisin/kexin type 9
RCT	randomized clinical trials
REPRIEVE	Evaluating the Use of Pitavastatin to Reduce the Risk of Cardiovascular Disease in HIV-Infected Adults
SHARP	Study of Heart and Renal Protection
SIP	site 1 protease
SKI-1	subtilisin/kexin-isoenzyme 1
SPIRE	Studies of PCSK9 Inhibition and Reduction of Vascular Events
SREBP-2	sterol regulatory element-binding protein-2
TGF	transforming growth factor
TLR	toll-like receptor
TNF	tumor necrosis factor

separates and recycles back to the cell surface, where receptor-mediated endocytosis begins anew and is repeated hundreds of times over the receptor's lifetime.[15] When low intracellular levels of cholesterol are sensed, SREBP-2 (sterol regulatory element-binding protein-2) is upregulated, initiating transcription of both the receptor and the precursor Pro-PCSK9.[16,17] After autocatalytic cleavage within the endoplasmic reticulum, the prodomain is released but then reamalgamates with PCSK9 and in so doing activates it.[17] An additional check at the Golgi stage is provided by furin, which to a variable degree cleaves and inactivates PCSK9 before it reaches the cell surface.[17] Intracellular PCSK9 can interact with some LDLRs before they are even secreted, directing them towards an abbreviated route towards lysosomal degradation.[17] Once it is secreted into plasma, PCSK9 serves as one of many potential ligands for the LDLR.[7] Furthermore, about half of circulating PCSK9 is bound to either LDL or the related Lp(a) (lipoprotein [a]) particle. After endocytosis, if PCSK9 is entwined with the LDL-LDLR complex, the receptor fails to exit the lysosome, becomes degraded with its other contents, and fails to recycle.[16] The huge molar excess of receptors to PCSK9 means that only rarely can an individual PSCK9 molecule insinuate itself into the LDLR-LDL complex; most often PCSK9 is absent, allowing unimpeded repeated recycling of the receptor.[7] There is also a suggestion that intracellular PCSK9 may itself sometimes be recycled so that a single PCSK9 molecule might contribute multiple times to receptor degradation.[7]

PCSK9 and Atherosclerosis

The effects of PCSK9 on atherosclerosis include global effects on LDL metabolism, local effects on LDL metabolism within an atherosclerotic lesion, and effects on other atherogenic mechanisms such as inflammation (Figure 1). Atherosclerosis is a chronic inflammatory disease of the arterial wall that is characterized by endothelial cell activation, accumulation of mononuclear cells, and vascular smooth muscle cells (VSMCs). Atherosclerotic lesions are site-specific and develop preferentially in regions of low wall shear stress.[19] Low wall shear stress augments human and mouse endothelial cell and smooth muscle cell PCSK9 expression, which is mediated by NADPH oxidase–induced reactive oxygen species production.[20] The associations between PCSK9 expression and mononuclear cell chemotaxis and adhesion in low wall shear stress regions suggest an association between PCSK9 and inflammation.[20,21]

A central mechanism whereby PCSK9 contributes to atherogenesis is through its fundamental role in governing plasma LDL-C concentration, which is now accepted as a direct causative factor in atherosclerosis, via several mechanisms which have been well-reviewed.[1] In addition, plasma PCSK9 can freely distribute within an atherosclerotic plaque, and both plaque macrophages and VSMCs can secrete and respond to PCSK9; LDLRs within the plaque are subject to local regulation by PCSK9.[7] If PCSK9 is locally inhibited, enhanced cholesterol internalization from increased LDLR activity can be deleterious for the plaque.

Preclinical studies establish that PCSK9 also participates in multiple steps and processes that contribute to arterial inflammation (Figure 2). In hypercholesterolemic *ApoE*$^{-/-}$ mice fed a high-fat diet, associated monocytosis increases the circulating Ly-6C^{hi} monocyte subset (phenotypically Gr-$^{1+}$CCR2^{+}CX3CR1^{hi}) and lesional macrophages.[22] Ly-6C^{hi} monocytes are more effective than Ly-6C^{lo} monocytes (phenotypically Gr-$^{1+}$CCR2^{+}CX3CR1^{lo}) in adhering to activated endothelium, infiltrating atherosclerotic lesions, transition of monocytes to classically activated macrophages, and generation of inflammatory responses, including increased production of the proinflammatory cytokines IL

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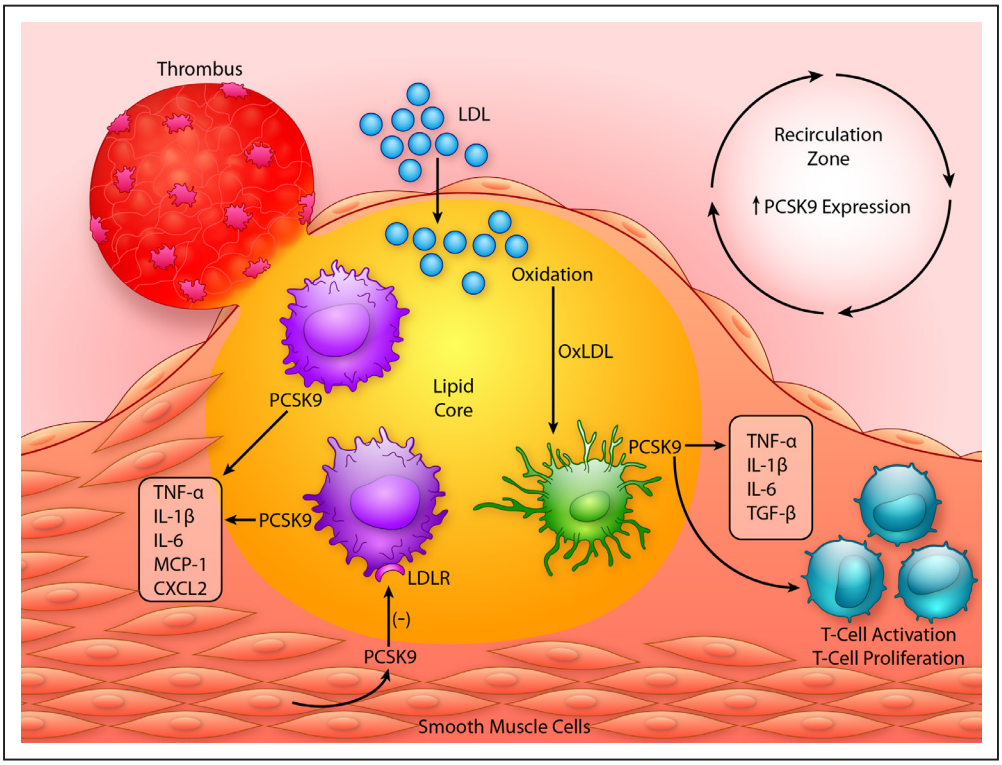

Figure 1. PCSK9 (proprotein convertase subtilisin-kexin type 9) induces release of intraplaque cytokines from macrophages, dendritic cells, and smooth muscle cells. The elaboration of PCSK9 from smooth muscle cells degrades macrophage LDLR (low-density lipoprotein receptor), which increases activation of the p65 subunit of NF-κB (nuclear factor κB) via the signaling pathways JAK (Janus kinase) and SREBP (sterol regulatory element-binding protein). Activation of NF-κB signaling results in increased production of TNF (tumor necrosis factor)-α, IL (interleukin)-1β, IL-6, MCP (monocyte chemoattractant protein)-1 and CXCL2 (chemokine ligand 2). The macrophage ApoER2 (ApoE receptor 2) inhibits NF-κB signaling and suppresses activation of TNF-α, IL-1β, IL-6. PCSK9 increases degradation of ApoER2. Dendritic cells are immune competent cells that participated in plaque complications and rupture. ox-LDL (oxidized LDL) activates dendritic cells and T cells in plaque resulting in increased expression of PCSK9, the costimulatory molecules CD80, CD83, CD86, and HLA-DR (human leukocyte antigen-DR isotype), and the SOCS1 (suppressor of cytokine signaling 1) signaling pathway that induces the proinflammatory cytokines TNF-α, IL-1β, IL-6, and TGF (transforming growth factor)-β. ox-LDL induces dendritic cells to induce the scavenger cell receptors CD36 and LOX-1 (lectin-like oxidized LDLR-1). Illustration Credit: Ben Smith.

(interleukin)-1β and TNF (tumor necrosis factor)-α.[20] Transgenic mice expressing human PCSK9 on the background of apoE$^{-/-}$ and LDLR$^{-/-}$ have increased aortic lesion size. Lesion Ly-6C^{hi} monocytes are increased in apoE$^{-/-}$ mice, but not *Ldlr*$^{-/-}$ mice.[23] Thus, these effects of human PCSK9 are dependent on the presence of the LDLR.

In the early stages of atherosclerosis, lipid-rich macrophages secrete inflammatory mediators that stimulate VSMC migration and proliferation. VSMC PCSK9 secretion degrades macrophage LDLR.[24,25] Transplantation of bone marrow from transgenic mice expressing human PCSK9 into *Ldlr*$^{-/-}$ and *ApoE*$^{-/-}$ mice fed a hypercholesterolemic diet, increased atherosclerotic lesion inflammation in an LDLR-dependent mechanism.[21] It has been postulated that the anti-inflammatory actions of PCSK9 inhibition may be counteracted by upregulation of macrophage LDLRs in plaque cells leading to increased cholesterol accumulation in the atheroma.[25,26] As mentioned, theoretically, therapeutic blockade of PCSK9 via monoclonal antibodies may facilitate cholesterol entry into macrophage via increased LDLR expression.

PCSK9 regulates the concentration of LDL-C by binding to hepatic LDLR, targeting them to the endolysosomal pathway, and interfering with their return to the cell surface.[27,28] Additionally, PCSK9 targets other members of the LDLR superfamily[29] and LRPs (LDLR-related proteins;Figure 2).[21,30–32] LRP1 regulates inflammatory responses in the atheroma through apoE-dependent and apoE-independent pathways.[32,33] ApoE is present in cells involved in atherogenesis, including platelets, endothelial cells, and monocytes and macrophages.[34] Genetic inactivation of *Pcsk9* reduces atherosclerosis in *ApoE*$^{-/-}$ mice.[35] ApoER2 (ApoE receptor 2) or LR7/8B (LDL receptor gene family member [LR] 7/8B) expression reduces cholesterol accumulation in macrophages, decreases atherosclerotic plaque development,[36] and inhibits NF-κB (nuclear factor κB) activation and proinflammatory cytokine production by macrophages.[37] PCSK9 promotes inflammation by degrading ApoER2[38] (Figure 2). Expression of the LOX-1 (lectin-like oxidized LDLR-1) in aortic endothelial cells and VSMCs is increased by NADPH oxidase–dependent reactive oxygen species. Inhibition of reactive oxygen species production by siRNA (short interferring RNA) transfection of p47^{phox} and gp91^{phox} reduced PCSK9 expression. Correspondingly, inhibition of PCSK9, LOX-1, and reactive oxygen species generation reduced NF-κB expression.[20] The elaboration of PCSK9 from smooth muscle cells degrades macrophage LDLR, which increases activation of the p65 subunit of NF-κB via the signaling pathways JAK (Janus kinase) and SREBP.[21] Activation of NF-κB signaling results in increased production of TNF-α, IL-1β, IL-6, MCP-1 (monocyte chemoattractant protein 1), and CXCL2 (chemokine ligand 2).[21] The

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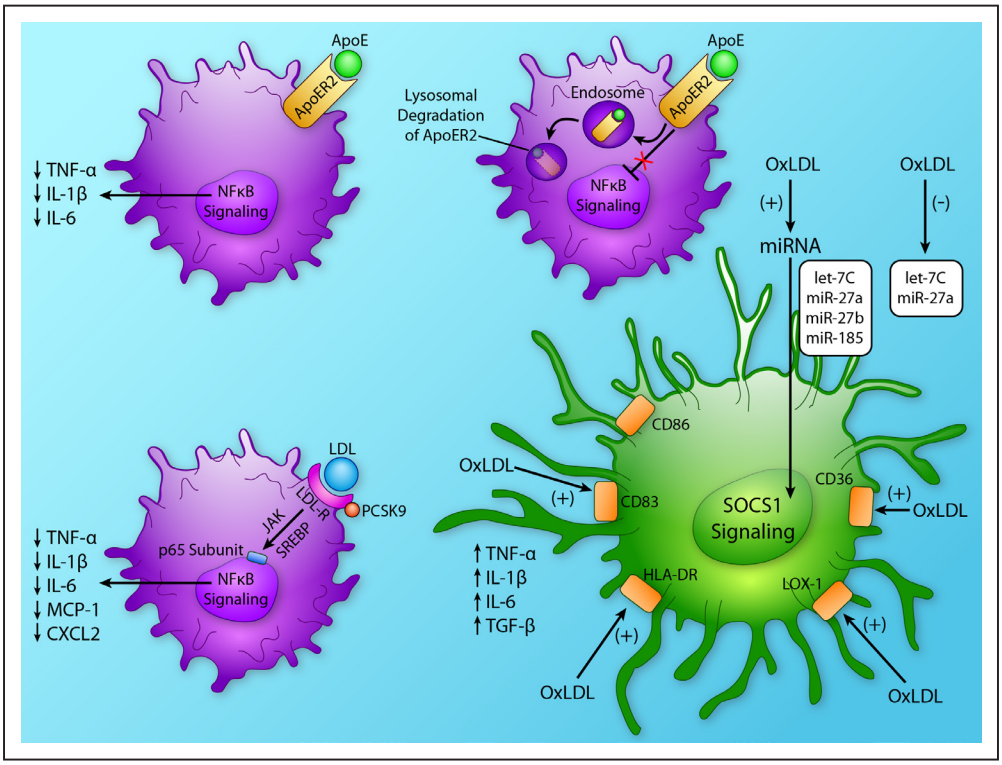

Figure 2. PCSK9 (proprotein convertase subtilisin-kexin type 9) targets several members of the LDLR (low-density lipoprotein receptor) superfamily and LRPs (LDLR-related proteins) to promote inflammation in experimental models. PCSK9 promotes inflammation by degrading the ApoER2 (ApoE receptor 2) or LR7/8B (LDL receptor gene family member [LR] 7/8B) receptor. ApoER2 expression reduces cholesterol accumulation in macrophages and inhibits NF-κB (nuclear factor κB) signaling and suppresses activation of TNF (tumor necrosis factor)-α, IL (interleukin)-1β, IL-6. ox-LDL (oxidized LDL) activates SOCS1 (suppressor of cytokine signaling 1) signaling in dendritic cells, and this activation is influenced by let-7c (lethal-7 gene family member-7c), miR (microRNA)-27a, miR 27b, and miR-18S. Expression of the LOX-1 (lectin-like oxidized LDLR-1) in aortic endothelial cells and vascular smooth muscle cells is increased by NADPH oxidase–dependent reactive oxygen species (ROS). Inhibition of ROS production by siRNA (short interfering RNA) transfection of $p47^{phox}$ and $gp91^{phox}$ reduced PCSK9 expression. Inhibition of PCSK9, LOX-1, and ROS generation reduced NF-κB expression. Illustration Credit: Ben Smith. JAK indicates Janus kinase; MCP, monocyte chemoattractant protein; and SREBP, sterol regulatory element-binding protein.

macrophage ApoER2 receptor inhibits NF-κB signaling and suppresses activation of TNF-α, IL-1β, and IL-6. PCSK9 increases degradation of ApoER2.

Ox-LDL (oxidized LDL) induces PCSK9 expression in dendritic cells, the costimulatory molecules CD80, CD83, CD86, and HLA-DR (human leukocyte antigen-DR isotype), and the SOCS1 (suppressor of cytokine signaling 1) signaling pathway that induces the proinflammatory cytokines TNF-α, IL-1β, IL-6, and TGF (transforming growth factor)-β (Figure 2).[39,40] Ox-LDL induces dendritic cells to induce the scavenger cell receptors CD36 and LOX-1. Silencing of PCSK9 (for instance by siRNA) in proinflammatory T cells and dendritic cells abrogates Ox-LDL–mediated proinflammatory effects of PCSK9, including the costimulatory molecules (CD83, CD86, and HLA-DR), scavenger receptors (LOX-1 and CD36), and polarization of T cells to Th1 andTh17 subsets (Figure 2).[39]

Silencing of PCSK9 in macrophages inhibits redox-sensitive transcription factor–induced NF-κB inflammatory responses[41] and human endothelial cell ox-LDL–induced apoptosis via blocked caspase-3 and -9 activation.[42] Treatment with an anti-PCSK9 monoclonal antibody reduces CCR2 (chemokine receptor 2) expression and migration of circulating monocytes in patients with familial hypercholesterolemia (FH) patients' attenuates proinflammatory cytokine production.[43] The Metchnikoff trial (NCT pending) is a mechanistic trial designed to describe how PCSK9 inhibition alters the circulating monocyte populations and to systematically profile the transcriptional and proteomic responses to TLRs (toll-like receptors) and how these responses are modulated by PCSK9 inhibition in peripheral blood mononuclear cells) of patients with atherosclerotic coronary arterial disease and controlled type 2 diabetes mellitus. In a coronary intravascular ultrasound (IVUS) virtual histology study, PCSK9 levels were linearly associated with the volume of necrotic core tissue, independent of LDL-C level.[44] The reduction in proinflammatory mediators from cells in the arterial wall may contribute to local effects that may mediate a reduction in atherosclerosis progression and cardiovascular events. The neutral effect of PCSK9 inhibitors on hepatic synthesis of inflammatory mediators, such as CRP (C-reactive protein), suggests a reduction in inflammatory pathways independent of the NLRP3 (NLR family pyrin domain-containing 3) inflammasome/IL-1β pathway.

The above complex distal and local effects of PCSK9 on multiple atherogenic mechanisms could have clinical implications: for instance, circulating monoclonal antibodies may have different net effects on PCSK9 within atherogenic plaques compared with antisense oligonucleotides.[7] This complexity emphasizes the need for randomized controlled trials of each particular agent to evaluate its integrated effects on clinical ASCVD end points.

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Genetics of PCSK9

Molecular genetics has played a central role both in discovering PCSK9 and then linking it to cholesterol metabolism.[14,17] While searching for members of the proprotein convertase family of serine proteases, Seidah et al[18] screened nucleotide databases and identified a cDNA sequence showing homology to SIP (site 1 protease), also known as SKI-1 (subtilisin/kexin-isoenzyme 1); this new gene was originally designated NARC1 (neural apoptosis-regulated convertase-1) because of the fact that it was upregulated when apoptosis was induced in primary cultures of cerebellar neurons. Soon renamed PCSK9, it became defined as a member of the proteinase K family of subtilases, with genomic structure comprised of 12 exons transcribed into an mRNA of 3710 bp (NM_174936.3) and translated into a protein of 692 amino acids (NP_777596.2) with liver being the primary site of expression.[18] The gene was mapped to chromosome 1p32, which coincided with a region linked to dominant inheritance of hypercholesterolemia in French and American families who had no causative mutations in either *LDLR* or *APOB* genes.[45,46] Suspecting that genetic variation in *PCSK9* might be related to hypercholesterolemia, Seidah persuaded Boileau's group to undertake DNA sequencing in their affected French families. This led to identification of rare heterozygous missense mutations in *PCSK9* (p.S127R and p.F216L), which in their respective families cosegregated impeccably with hypercholesterolemia.[47] The Utah kindred had a different *PCSK9* rare variant—p.D374Y—that likewise showed cosegregation with elevated LDL-C.[48]

PCSK9 is now recognized as the third gene causing FH, with rare variants presently accounting for <1% of FH cases compared with 80% to 90% and 5% to 10% of cases resulting from *LDLR* loss-of-function mutations and *APOB* receptor–binding defective mutations, respectively.[49]

A key observation tying a PCSK9 gain-of-function mechanism to FH came from Maxwell and Breslow,[50] who showed that simple transgenic overexpression of PCSK9 in mice caused an FH-like phenotype because of increased intracellular degradation of the LDLR.[27] This implied that FH in humans was caused by gain-of-function mutations in PCSK9, which resulted in more avid degradation of the LDLR, with reduced capacity for internalization of LDL and consequent accumulation of LDL in plasma. That a gain-of-function rather than loss-of-function in PCSK9 leads to hypercholesterolemia has been confirmed by reports over the past 15 years of dozens of disease-causing mutations, mostly rare heterozygous nonsynonymous variants that occur in virtually every domain of the protein[16] and result in either increased transcription, impaired autocatalysis, or increased affinity of PCSK9 for the receptor.[17,51] Recently, a unique gain-of-function mechanism was reported in 2 unrelated Canadian families in which the entire *PCSK9* gene was duplicated.[52] One affected patient had the highest plasma levels of PCSK9 ever reported in a human being—a >20-fold increase over normal levels. Carrying 3 copies of the *PCSK9* gene cosegregated with severe hypercholesterolemia, aggressive ASCVD, and resistance to available pharmacotherapies, including PCSK9 inhibitors.

Proof that loss-of-function mutations in *PCSK9* lowered LDL-C in humans was reported in 2005, after Cohen et al[53] and Kotowski et al[54] sequenced the *PCSK9* gene in individuals from the Dallas Heart Study who had either extremely high or low LDL-C levels. Among individuals from different geographic ancestries with extremely low LDL-C levels, they identified certain uncommon *PCSK9* coding variants that were not seen in individuals with high levels. Some of these variants, for example, p.Y142X or p.C679X, encoded a severely truncated version of the protein that was clearly predicted to impart a loss-of-function to PCSK9. They next showed, in a landmark example of a Mendelian randomization experiment, that individuals with *PCSK9* loss-of-function variants who had lifelong depressed LDL-C levels also had reduced ASCVD risk.[55] Specifically, of 3363 blacks, 2.6% of individuals carried 1 of 2 heterozygous *PCSK9* nonsense variants, which were associated with 28% and 88% reductions in LDL-C and ASCVD risk, respectively. Of the 9524 white subjects examined, 3.2% had the *PCSK9* p.R46L missense variant that was associated with 15% and 47% reductions in LDL-C and ASCVD risk, respectively. These results were essentially confirmed in subsequent studies performed in much larger cohorts.[56,57] A meta-analysis of 3 Scandinavian cohorts totaling >66 000 individuals showed that heterozygotes for the PCSK9 p.R46L allele had 12% and 28% reductions in LDL-C and ASCVD risk, respectively.[57] This particular missense variant has been shown to compromise secretion, phosphorylation, and binding affinity for the LDLR.[58–60] Similarly, the nonsynonymous *PCSK9* p.R93C variant, which is specific to Asian subpopulations and is predicted to be deleterious, had an allele frequency of 1.4% in 14 473 Chinese individuals and was associated with reduced LDL-C and 24% reduced risk of ASCVD.[61]

Currently, at least 20 probable loss-of-function *PCSK9* variants have been reported, of which most are either missense or nonsense changes.[52] These are situated within all functional domains of PCSK9 and to varying degrees lead to reduced PCSK9 function through accelerated degradation, compromised intracellular transport, aberrant autocatalysis, or reduced affinity for the LDLR.[52] The loss-of-function variants are instructive in several ways. First, they provided evidence for the potential biochemical and clinical efficacy of targeted reduction in PCSK9 using a pharmacological modality to mimic the effects of naturally occurring loss-of-function. Furthermore, the Mendelian (or natural genetic) randomization results implicating PCSK9 has become a paradigm for drug discovery more generally.[62] Second, and just as importantly, close study of complete human knockouts for PSCK9 (eg, individuals with homozygous or biallelic loss-of-function variants) show that they have only depressed LDL-C; these individuals appear healthy in all other respects.[63,64] This supports the potential safety of pharmacologically targeting PCSK9.

Specific human variants both in *PCSK9* and related proteins can shed further light on biology and can possibly have clinical relevance.[51] For instance, both the *PCSK9* p.D374Y gain-of-function variant and the doppelganger *LDLR* p.H306Y loss-of-function variant result in a more tightly associated LDLR-PCSK9 complex.[17] Targeting this interaction could be another approach to prolong the lifespan

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of receptors. Furthermore, the *PCSK9* p.F216L and p.R218S gain-of-function mutations attenuate furin cleavage and inactivation of PCSK9, indicating that approaches to enhance this cleavage step might provide an independent mechanism to reduce PCSK9 activity via a mechanism that is independent of currently available monoclonal antibodies.[17] In addition, the *LDLR* p.R410S variant associates more avidly with PCSK9 at a more acidic pH is associated with reduced delivery of LDL particles to lysosomes, and likely recycles loaded with its LDL contents, suggesting a new class of FH-causing mutation.[65] Also, cellular studies of the PCSK9 p.Q152H loss-of-function variant, which fails to normally exit the endoplasmic reticulum and undergo autocatalytic cleavage, showed altered intracellular interactions with the 94-kDa GRP94 (glucose-regulated protein 94) and 78-kDa GRP78, which are both sensors for the unfolded protein response.[66] Furthermore, knockdown of GRP94 increased the stability of GRP78-PCSK9 complex, resulting in activation of the unfolded protein response. Together, these findings suggest that therapeutic strategies aimed at blocking the autocatalytic cleavage of PCSK9 within the endoplasmic reticulum might represent a new and alternative approach to reduce circulating PCSK9.[66]

Natural Mendelian randomization studies of genetic variants in populations might also provide clues about possible clinically pleiotropic effects of PCSK9 inhibitions, both favorable and unfavorable. For instance, carriers of PCSK9 loss-of-function variants seemed to have increased risk of developing type 2 diabetes mellitus,[67–69] which at first suggested that pharmacological inhibition might similarly increase type 2 diabetes mellitus risk, as has been reported for statin drugs.[70] However, to date, clinical trials with human monoclonal anti-PCSK9 antibodies have not shown any relationship with increased diabetes mellitus risk.[71] Other natural randomization studies found no causal relationship between reduced PCSK9 function and either ischemic stroke,[56,72] neurodegenerative diseases,[73] cognitive impairment,[74] or abdominal aortic aneurysms.[75]

A recent phenome-wide association study performed in >337 000 individuals from the United Kingdom Biobank sample found a protective effect of *PCSK9* p.R46L on hyperlipidemia, coronary heart disease (CHD), ischemic stroke, and cerebral infarction, but increased risk of type 2 diabetes mellitus risk (adjusted for lipid-lowering medication use) and no association with cataracts, heart failure, atrial fibrillation, or cognitive dysfunction.[76] A smaller cohort study showed that *PCSK9* p.R46L carriers had reduced measures of subclinical atherosclerosis and reduced erectile dysfunction.[77] Another report using the phenome-wide association approach confirmed that carriers of *PCSK9* p.R46L had lower cholesterol levels, but also a significant 5.9-fold increased risk of spina bifida.[78] An earlier study in patients with sepsis, the *PCSK9* p.R46L variant was actually associated with better survival and reduction in cytokine levels, whereas the gain-of-function p.E670G variant had the opposite association.[79] However, a prospective study of blacks from the REGARDS study (Reasons for Geographic and Racial Differences in Stroke) did not show a protective effect for PCSK9 loss-of-function variants on the development of septic shock or hospitalizations for severe infections.[80] Additional associations with various clinical phenotypes will likely emerge as p.R46L, and other variants are examined systematically in databases containing information on a wider range of phenotypes.

Few formal pharmacogenetic studies on either *PCSK9* genetic variation or interindividual differences in response to PCSK9 inhibitors have been reported. Small cohort studies are inconsistent with regard to an association between common single nucleotide polymorphism variation in *PCSK9* and response to statin therapy.[81,82] However, response to PCSK9 inhibitors seems relatively uniform across patients with heterozygous rare variants in different genes causing FH.[83,84] In contrast, patients with homozygous FH due to biallelic *LDLR* mutations are generally less responsive to PCSK9 inhibitors,[85] whereas a homozygote for a *PCSK9* variant anecdotally showed a relatively milder baseline phenotype and better response to lipid-lowering therapies.[86] At this time, we cannot recommend making treatment decisions on PCSK9 inhibitors based on a priori genetic information; even in patients with homozygous FH and apparently biallelic *LDLR* null mutations, we recommend an empirical therapeutic trial of PSCK9 inhibition to gauge individual response before deciding against the use of such treatment.

Clinical Trials of PCSK9 Inhibitors

PCSK9 inhibitors reduce cardiovascular events in patients with ASCVD. A summary of the baseline characteristics and primary outcome measures from 3 large clinical end point trials are provided in Tables 1 and 2.[8–13]

Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk

FOURIER (Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk) was a randomized, double-blind placebo-controlled trial conducted in 27 564 clinically stable patients aged 40 to 85 years who were considered high-risk based on established ASCVD, either with prior myocardial infarction (MI), prior stroke or symptomatic lower extremity peripheral arterial disease (LEAD), who, despite maximally tolerated statin treatment with or without ezetimibe failed to attain LDL-C <70 mg/dL or non-HDL (high-density lipoprotein) cholesterol <100 mg/dL[8,9] All screened potentially eligible patients underwent a lipid stabilization phase with optimization of LDL-C lowering before randomization to either evolocumab, a fully human antibody against PCSK9, given subcutaneously at a dose of 140 mg every 2 weeks or 420 mg every 4 weeks or placebo.[8] A total of 2906 participants experienced a primary end point that was comprised of cardiovascular death, MI, stroke, hospitalization for unstable angina, or coronary revascularization.[9] A total of 1829 participants experienced a key secondary end point that included the composite of cardiovascular death, MI, or stroke. The primary end point was completely ascertained in 99.5% of potential patient-years of follow-up, and few participants discontinued drug treatment prematurely and permanently (5.6% per year in the evolocumab group and 5.8% per year in the placebo group). Withdrawal of consent was 0.3% per year, and few participants were lost to follow-up (5 patients in the evolocumab group and 13 patients in the placebo group), confirming an excellent quality study. The baseline characteristics of patients are described in Table 1. Although FOURIER included patients with an index event in any of the 3 major

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Table 1. Baseline Characteristics and Primary Outcome Measures of the 3 PCSK9 Inhibitor Trials

	FOURIER		SPIRE 1/2		ODYSSEY Outcomes	
Monoclonal antibody	Evolocumab (human)		Bococizumab (humanized)		Alirocumab (human)	
Entry LDL-C, mg/dL	≥70		≥70/ ≥100		≥70	
Statin requirement	High-intensity statin preferred, minimum dose atorvastatin 20 mg or equivalent		Atorvastatin 40 or 80 mg, rosuvastatin 20 or 40 mg (or 80 mg if >1 y) or documented intolerance to high-intensity statin (Spire 1 and Spire 2) or documented complete statin intolerance (SPIRE 2)		Atorvastatin 40 or 80 mg, rosuvastatin 20 or 40 mg or maximum-tolerated dose of one of these agents	
High-Risk Secondary Prevention	Yes		Yes		Yes	
High-Risk Primary Prevention	No		Yes		No	
	Evolocumab (N=13 784)	Placebo (N=13 780)	Bococizumab (N=13 720)	Placebo (N=13 718)	Alirocumab (N=9462)	Placebo (N=9462)
Age, y	62.5	62.5	62.9	63.0	58.5	58.6
Male sex, %	75.2	75.5	70.7	70.2	74.7	74.9
Type of atherosclerosis						
Myocardial infarction, %	80.9	81.3			83.2	82.8
Median time from most recent previous myocardial infarction -months	40.8	39.6			2.8	2.8
Nonhemorrhagic stroke	19.5	19.2				
Median time from most recent previous stroke - months	38.4	39.6				
Peripheral artery disease, %	13.5	12.9				
Cardiovascular risk factors						
Hypertension, %	80.1	80.1	81.3	80.4	65.6	63.9
Diabetes mellitus, %	36.7	36.5	48.1	46.9	28.5	29.1
Current cigarette use, %	28.0	28.5	24.7	24.4	24.1	24.1
Statin use, %						
High intensity	69.5	69.1	84.6	84.5	88.6	89.1
Moderate intensity	30.2	30.7			8.8	8.2
Ezetimibe, %	5.3	5.2	9.7	10.3	2.8	3.0
Other cardiovascular medications, %						
Aspirin, $P2Y_{12}$ inhibitor, or both	92.7	92.0				
Aspirin					95.6	95.5
$P2Y_{12}$ inhibitor					87.7	87.1
Beta-blocker	75.8	75.4			84.5	84.5
ACE inhibitor or ARB, aldosterone antagonist, or both	78.4	77.9			77.7*	77.8*
Median lipid measures						
LDL-C, mg/dL	92	92	109.3†	109.1†	87	87
Total cholesterol, mg/dL	168	168	179.4†	179.3†		
HDL cholesterol, mg/dL	44	44	47.2†	47.4†	43	42
Triglycerides, mg/dL	134	133	136.7	135.0	129	129
Lipoprotein(a), mg/dL	37‡	37‡	19.2	19.2	21	22
Outcome measures						
Primary end point (RRR, %)	15		12		15	
Key secondary end point (RRR, %)	20		12		14	

ACE indicates angiotensin-converting enzyme; ARB, angiotensin receptor blocker; FOURIER, Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk; HDL, high-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; ODYSSEY Outcomes, Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab; PCSK9, proprotein convertase subtilisin-kexin type 9; RRR, relative risk reduction; and SPIRE, Studies of PCSK9 Inhibition and Reduction of Vascular Events.

*No data on aldosterone antagonists.

†Mean.

‡nmol/L.

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Table 2. Benefit of Evolocumab Regarding the Key Secondary End Point in Various Subgroups

	AR (%)	ARR (%)	NNT	Hazard Ratio (95% CI)	RRR (%)
<2 y					
Placebo	10.8	2.9	35	0.76 (0.64–0.89)	24
Evolocumab	7.9			*P*<0.001	
≥2 y					
Placebo	9.3	1.0	101	0.87 (0.76–0.99)	13
Evolocumab	8.3			*P*=0.04	
≥2 prior MIs					
Placebo	15.0	2.6	38	0.79 (0.67–0.94)	21
Evolocumab	12.4			*P*=0.006	
1 prior MI					
Placebo	8.2	1.7	60	0.84 (0.74–0.96)	16
Evolocumab	6.6			*P*=0.008	
MVD					
Placebo	12.6	3.4	29	0.70 (0.58–0.84)	30
Evolocumab	9.2			*P*<0.001	
No MVD					
Placebo	8.9	1.3	78	0.89 (0.79–1.00)	11
Evolocumab	7.6			*P*=0.055	
LEAD					
Placebo	13	3.5	29	0.73 (0.59–0.91)	27
Evolocumab	9.5			*P*=0.004	
No LEAD					
Placebo	7.6	1.4	72	0.81 (0.73–0.90)	19
Evolocumab	6.2			*P*<0.001	
<2 y, ≥2 y, time after qualifying MI					

AR indicates absolute risk, ARR, absolute risk reduction; LEAD, lower extremity arterial disease; MI, myocardial infarction; MVD, multivessel disease; NNT, numbers needed to treat; and RRR, relative risk reduction.

vascular beds namely coronary, cerebrovascular, or peripheral arterial, the vast majority namely 81% entered the trial because of a prior MI, 19% had a prior stroke, and 13% had symptomatic LEAD as their index event. Most importantly, this was a truly stable population. Only 5% of participants were treated with ezetimibe in combination with statin treatment, which was mainly attributed to the paucity of clinical outcome data for ezetimibe at the time when FOURIER was initiated.

Evolocumab treatment lowered baseline LDL-C by 59% from a baseline level of 92 mg/dL after 48-week treatment.[9] Of note, 42% of patients in FOURIER achieved LDL-C levels <25 mg/dL. After a median follow-up of 26 months (interquartile range, 22–30), the primary end point was achieved in 9.8% in the evolocumab group versus 11.3% in the placebo group, resulting in a 15% relative risk reduction (RRR; HR, 0.85; 95% CI, 0.79–0.92) and an ARR of 1.5%. Treatment with evolocumab reduced the relative risk of acute MI by 27%, stroke by 21%, and coronary revascularizations by 22%. The reduction in coronary revascularizations was more pronounced for urgent versus elective revascularizations (27% versus 17%, respectively). The key secondary end point consisting of cardiovascular death, MI, and stroke was reduced by 20% (HR, 0.80; 95% CI, 0.73–0.88) with an ARR of 1.5%. Forest plot analyses showed that the efficacy of evolocumab was independent of either age, sex, race, primary affected vascular bed, or baseline LDL-C. Importantly, FOURIER demonstrated efficacy in older participants, including those patients who were aged 85 years at the time of randomization. Efficacy of evolocumab was also independent of whether or not patients received high or moderate intensity statin therapy and also of the dosing regimen. Even study participants with baseline LDL-C levels in the lowest quartile (ie, <80 mg/dL) had a 20% RRR.

Atherosclerosis is a time-dependent process, and thus, planned landmark analyses showed a modest RRR of 16% during the first year for the key secondary end point, but a clearly more pronounced RRR of 25% after 2 years of treatment.[9] The same pattern was seen for the combination of fatal or nonfatal MI or stroke. Importantly, these 2-year data are consistent with published Cholesterol Treatment Trialists' Collaboration meta-analyses of statins.[4] With the exception of

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major vascular events, the effect in FOURIER was somewhat lower than Cholesterol Treatment Trialists' Collaboration, which may simply be because of the fact that patients in FOURIER had been previously treated for a much longer time period with more effective LDL-C–lowering drugs than patients in earlier statin trials. Additionally, FOURIER demonstrated benefit at a median follow-up of 2.2 years versus statin trials that most often required 5 to 6 years to demonstrate improvements in cardiovascular outcomes. Furthermore, there were no significant safety concerns with evolocumab either at the injection site, muscle-related adverse events, new-onset diabetes mellitus, or cognitive dysfunction.[9,87,88] Importantly, only 0.3% of participants exhibited binding antibodies, and there were no neutralizing antibodies seen in this trial.

Although reduction in ASCVD events in FOURIER was consistent with the anticipated reduction based on the Cholesterol Treatment Trialists' Collaboration meta-analysis, it was modest in nature. Thus, identification of patients deriving the greatest benefit from a PCSK9 inhibitor would be of paramount importance. Subgroup analyses in FOURIER have provided important information about risk stratification in high-risk ASCVD patients.[89] The main outcome of interest for these subgroup analyses was the key secondary end point, a composite of cardiovascular death, MI, or stroke. In the placebo group, the event rate in those with a more recent MI was 10.6% versus 9.3% in those with an MI ≥2 years prior, resulting in an HR of 1.19 (95% CI, 1.04–1.37; $P<0.01$). The difference in risk between those participants with at least 1 recurrent MI versus those with a single MI was even more pronounced with an event rate of 15% in those with recurrent MI versus 8.2% in those with a single MI resulting in a HR of 2.04 (95% CI, 1.78–2.35; $P<0.001$). Finally, the presence of residual multivessel disease at baseline was associated with a 12.6% event rate versus 8.9% in those without multivessel disease in patients receiving placebo resulting in an HR of 1.47 (95% CI, 1.27–1.70; $P<0.001$). Thus, these simple clinical criteria clearly identified high-risk subgroups that showed greater benefit in absolute terms from evolocumab.

Efficacy of Evolocumab in Relation to Time From Qualifying MI (<2 Years Versus ≥2 Years)

With respect to the efficacy of evolocumab, those with an index MI <2 years compared with ≥2 years previously had a 24% RRR versus a 13% RRR and an absolute risk of 10.8% versus 7.9% resulting in a number needed to treat (NNT) of 35 in those with a more recent MI compared with an absolute risk of 9.3% versus 8.3% and higher NNT of 101 in those with a MI ≥2 years previously.[89]

Efficacy of Evolocumab in Relation to Number of Prior MIs at Baseline

A subsequent subgroup analysis evaluated patients with recurrent MIs versus those with only one prior MI and found greater efficacy of evolocumab in those with ≥2 prior MIs.[89] The RRR in these patients was 21% (HR, 0.79; 95% CI, 0.67–0.94; $P<0.006$) versus 16% (HR, 0.84; 95% CI, 0.74–0.96; $P<0.008$) in those with just 1 prior MI. In the recent MI group, absolute risk while on evolocumab versus placebo was 12.4% versus 15.0%, respectively, corresponding to an NNT of 38 over 3 years. In contrast, in the remote MI group, absolute risk on evolocumab versus placebo was 6.6% versus 8.2%, respectively, corresponding to an NNT of 60 over 3 years.

Efficacy of Evolocumab in Relation to Presence of Residual Multivessel Disease at Baseline

A third subgroup analysis defined using simple clinical assessment of multivessel disease versus no multivessel disease also showed a dramatic difference in RRR of 30% (HR, 0.70; 95% CI, 0.58–0.84; $P<0.001$) versus 11% (HR, 0.89; 95% CI, 0.79–1.00; $P<0.055$) in the multivessel versus no multivessel disease group, respectively.[89] In the multivessel disease group, AR on evolocumab versus placebo was 9.2% versus 12.6%, respectively, corresponding to an NNT of 29 over 3 years. In contrast, in the single vessel disease group, AR on evolocumab versus placebo was 7.6% versus 8.9%, respectively, corresponding to an NNT of 78 over 3 years. Thus, by these simple criteria, high-risk groups could be identified with acceptable NNTs over 3 years compared with other cardiovascular interventions.

Efficacy of Evolocumab in Patients With Stroke as the Index Event

Patients with a recent stroke represent a further high-risk group for recurrent events who were adequately represented in FOURIER with a total of 5337 subjects.[90] Despite some baseline differences between those with and without stroke (eg, male sex, advanced age, presence of hypertension, diabetes mellitus and atrial fibrillation), the overall reduction in LDL-C was similar to the overall cohort. The primary end point was reached in 14.6% in the placebo group and in 13.9% in the evolocumab group resulting in an identical 15% RRR (HR, 0.85; 95% CI, 0.72–1.00; $P<0.047$) as seen in the total population. Thus, evolocumab was equally effective in the subgroup with stroke; furthermore, there was a lower degree of disability poststroke (modified Rankin score) among patients with stroke end point in those treated with evolocumab.

Efficacy of Evolocumab in Patients With Symptomatic LEAD as the Index Event

For patients with a diagnosed LEAD, data are inconsistent regarding the effectiveness of lipid lowering. FOURIER actually represents the largest trial evaluating aggressive LDL-C lowering in patients with symptomatic LEAD (n=3642).[91] Patients with LEAD are well known to have a larger atherosclerotic burden compared with those without; thus, conceivably, these patients were somewhat sicker, possibly reflecting higher rates of such comorbidities as chronic kidney disease (CKD), diabetes mellitus, and coronary artery bypass grafting. The subgroup analysis showed that patients with LEAD had greatest absolute and relative reductions in risk of the primary and secondary endpoints with evolocumab therapy. Specifically, in LEAD patients, the AR of the primary end point in the evolocumab versus placebo group was 13.3% versus 16.8%, respectively, with the 3.5% ARR and a 21% RRR (HR, 0.79; 95% CI, 0.66–0.94; $P<0.0098$), corresponding to a NNT of 29. In patients without LEAD, the ARR was only 1.6%, the RRR was 14% (HR, 0.86; 95% CI, 0.80–0.93; $P<0.001$), and the NNT was 63. Similar findings were seen for the key secondary end point with an identical 3.5% ARR

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in the LEAD group (RRR, 27; HR, 0.73; 95% CI, 0.59–0.91; $P<0.0040$) and an NNT of 29. Those without LEAD showed a 1.4% ARR (RRR, 19%; HR, 0.81; 95% CI, 0.73–090; $P<0.001$) and an NNT of 72.

In addition to the reductions in cardiovascular events, major adverse limb events (acute ischemia, revascularization, and amputation) in patients with LEAD but without MI or stroke were significantly reduced with a RRR of 57% (HR, 0.43; 95% CI, 0.19–0.99; $P<0.042$) and an absolute risk of 2.6% versus 1.3% in placebo versus evolocumab. When considering the total coronary and peripheral arterial clinical endpoints, the ARR in the LEAD group between placebo and evolocumab was even larger with 4.1% with an NNT of 25 (AR, 15% versus 10.9% in placebo versus evolocumab). Similar to what has been shown for the overall FOURIER study population, the reduction in major adverse limb event in LEAD patients showed a linear relationship with achieved LDL-C, down to levels as low as 20 mg/dL. This is the first time that in a lipid-lowering trial in patients with LEAD such strong effects on not only cardiovascular but also peripheral endpoints namely acute ischemia, revascularization, and amputation have been shown.

Efficacy of Evolocumab in Patients With Very Low LDL-C (<10 mg/dL)

In an exploratory analyses of LDL-C levels obtained 4 weeks after initiation of therapy, there was a larger RRR in participants with an attained LDL-C <10 versus ≥10 mg/dL in the primary (31% RRR; HR, 0.69; 95% CI, 0.49–0.97; $P<0.03$) and secondary (41% RRR; HR, 0.59; 95% CI, 0.37–0.92; $P<0.02$) endpoints, with no significant differences in adverse event signals.[92] Such subgroup analyses strongly indicate that extremely low levels of LDL-C are safe and provide evidence that patients so treated can achieve an additional benefit.[93]

Studies of PCSK9 Inhibition and Reduction of Vascular Events Outcomes

The second large clinical trial, SPIRE (Studies of PCSK9 Inhibition and Reduction of Vascular Events) Outcomes,[10,11] evaluated the safety and efficacy of a humanized antibody, namely bococizumab. This trial recruited 27 439 patients at high risk in primary and secondary prevention and consisted of 2 similarly structured substudies, namely SPIRE 1 (n=16 817) and SPIRE 2 (n=10 621). The entry criteria for the 2 studies were somewhat different. SPIRE 1 included individuals on highly effective statin therapy with LDL-C >70 mg/dL but <100 mg/dL, whereas SPIRE 2 recruited only individuals with LDL-C ≥100 mg/dL. After a screening period of 4 weeks, potentially eligible patients were randomized to receive either bococizumab 150 mg subcutaneously or placebo injections every 2 weeks in addition to maximally tolerated statin therapy. At weeks 12 and 25, changes in lipid levels were assessed. The primary end point was almost identical to FOURIER,[8,9] with the exception that only patients with unstable angina requiring urgent nonelective revascularization were included. Ninety-three percent of patients were receiving statin treatment at baseline, and the median follow-up was 10 months owing to early termination of the trial because of high incidence rates of antidrug antibodies. Differences between SPIRE 2 and SPIRE 1 included the proportion of patients with FH (≈7% in SPIRE 2 and 1.7% in SPIRE 1), less frequent statin use in SPIRE 2 (mainly because of statin intolerance), and consequently differences in median LDL-C: 94 mg/dL (2.43 mmol/L) in SPIRE 1 and 134 mg/dL in SPIRE 2.[10,11] Furthermore, hs-CRP (high-sensitivity CRP) levels were slightly higher in SPIRE 2 versus SPIRE 1: 2.3 versus 1.8 mg/L, respectively. The AR for major adverse cardiovascular events was 3.02 and 4.19 per 100 person-years in SPIRE 1 and SPIRE 2, respectively. Combining the data from both studies, at 14 weeks, a pronounced decrease from baseline in LDL-C of 56% was observed in the bococizumab group versus an increase of 2.9% in the placebo group, resulting in a net median reduction of 64% from baseline related to active treatment. Waterfall plots of percent LDL-C changes indicated a significant interindividual variability at both 14- and 25-week time points. The primary end point was not significantly reduced in the lower risk SPIRE 1 group who had been followed for a median of only 7 months, whereas a highly significant 21% RRR was seen in SPIRE 2 after a median follow-up of 12 months. Because of development of antidrug antibodies and specifically neutralizing antibodies, LDL-C reduction was considerably attenuated from a 56% change at 14 weeks to a 42% change at 52 weeks in combined analyses. In addition, and not surprisingly, patients on bococizumab exhibited significant higher serious adverse effects leading to injection site reactions and drug discontinuation. The attenuation in LDL-C reduction was seen in ≈10% to 15% of patients that contrasts virtually no attenuation with the fully human monoclonal antibodies evolocumab and alirocumab. This was linked with another concern about the wide variability in the LDL-C response. Yet, despite the antidrug antibody production, variation in individual response, and early trial termination, analyses showed that bococizumab significantly reduced cardiovascular event rates in the higher risk SPIRE 2 trial in those with an LDL-C level >100 mg/dL. Although the SPIRE program was discontinued, these data mirror the positive findings from the FOURIER trial and lend further strong support for the use of PCSK9 inhibitors in selected patients as an adjunct to aggressive statin therapy. Unfortunately, because of the premature termination of the SPIRE trial, there are no data on PCSK9 inhibitors in high-risk primary prevention patients.

Evaluation of Cardiovascular Outcomes After an ACS During Treatment With Alirocumab

The third large clinical outcomes trial of a PCSK9 inhibitor is ODYSSEY Outcomes (Evaluation of Cardiovascular Outcomes After an ACS During Treatment With Alirocumab),[12,13] which enrolled 18 924 patients who had an ACS during the previous 12 months and had not attained the LDL-C target <70 mg/dL, despite taking high-intensity statin therapy. The primary efficacy outcome was time to first occurrence of CHD death or nonfatal MI, or fatal or nonfatal ischemic stroke, or unstable angina requiring hospitalization. Major secondary efficacy endpoints were tested in a hierarchical sequence, included all the aforementioned endpoints and in addition all-cause death. Eligible patients on high-intensity

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or maximum-tolerated doses of atorvastatin or rosuvastatin were randomized to receive either alirocumab or placebo. The LDL-C target was clamped between 25 and 50 mg/dL but > 15 mg/dL, at which level a blinded back-titration algorithm came into effect. Median follow-up was 2.8 years, which was slightly longer than in FOURIER, whereas rates of premature treatment discontinuation and patients lost to follow-up were similarly low. Importantly, in contrast to FOURIER,[8,9] the time from the index event, in this case, ACS, to randomization was a median of 2.6 months,[13] with the distribution of defining events of almost 50%, 35%, and ≈17% of patients having experienced non–ST-segment–elevation MIs, ST-segment–elevation Mis, and unstable angina, respectively. Altogether, 72% of patients had a revascularization procedure for the index ACS event. Of note, alirocumab was discontinued in ≈8% of patients because of 2 consecutive LDL-C measurements below the prespecified threshold of 15 mg/dL (0.39 mmol/L). After 4 weeks, there was a 62.7% decrease in LDL-C in the alirocumab group, with an absolute reduction of 55.7 mg/dL compared with placebo. After 4 years, this effect decreased to 44.7%, with a difference of 48.1 mg/dL in the overall study sample. By the end of follow-up, the mean LDL-C was ≈13 mg/dL lower in the on-treatment group compared with the intention-to-treat group (which included subjects who had dropped out of the study or discontinued treatment). Although speculative, it is possible that the attenuation in LDL-C lowering may be because of downtitration of alirocumab in a significant number of patients or discontinuation once the LDL-C was <15 mg/dL on 2 occasions.

The RRR in primary efficacy end point in ODYSSEY OUTCOMES[13] was identical to FOURIER,[9] namely 15% (HR, 0.85; 95% CI, 0.78–0.93; P<0.0003) with an ARR of 1.6%. Similar to FOURIER, this decrease in the primary end point was mainly driven by the reductions in nonfatal MI, ischemic stroke, and unstable angina, yet CHD and cardiovascular disease death were also not significantly reduced, as seen in FOURIER.[9] However, all-cause death was significantly reduced: a 15% RRR (HR, 0.85; 95% CI, 0.73–0.98). Despite this interesting finding, because death from CHD and cardiovascular disease death were not significantly reduced, technically, the planned hierarchical statistical analysis should not have proceeded any further, and reduced all-cause death should thus not have been detected or reported. Even considering this statistically doctrinaire algorithm, the question remains as to the cause for the reduced all-cause mortality observed with a seemingly pure LDL-C lowering agent. Interestingly, despite no differences in efficacy of LDL-C lowering across a wide range of prespecified subgroups, such as age above and below 65 years, sex, geographic region, and time from index event to randomization, a trend to a stronger effect could be observed in the subgroup of individuals with LDL-C ≥ 100 mg/dL (≥2.6 mmol/L) at baseline, with a RRR of 24% (HR, 0.76; 95% CI, 0.65–0.87) an AR of 14.9% in the placebo group and 11.5% in the alirocumab group, resulting in an ARR of 3.4%. There were no safety signals. Thus, taken together, 3 large RCTs of outcomes with PCSK9 inhibitors with different antibodies (FOURIER, SPIRE, and ODYSSEY) consistently showed similar reductions in major cardiovascular endpoints, comprising a broad spectrum of atherosclerotic manifestations, in patients with both stable and acute ASCVD.[8–13]

Interplay Between Hypercholesterolemia and Inflammation in Atherosclerosis

Although LDL-C represents a crucial risk factor for atherosclerosis,[1] an abundance of accumulated data from vascular biology research have clearly implicated inflammatory processes as playing a significant role in atherosclerosis and its complications.[94] In addition to convincing and consistent bench experiments, numerous long-term observational studies, in population-based but also clinical cohorts, have suggested that increased levels of various markers of inflammation are associated with worse outcomes.[95] Ultimately, the inflammation hypothesis has been confirmed by the positive results of the CANTOS trial (Canakinumab Antiinflammatory Thrombosis Outcome Study).[96] In >10 000 patients in whom IL-1β was targeted with the selective antibody canakinumab, an overall 15% RRR (HR, 0.85; 95% CI, 0.76–0.96; P<0.007) of major cardiovascular events was observed. The reduction was independent of an effect on the lipid profile and suggests that pharmacological blockade of inflammation provides incremental reduction in ASCVD events over and above modulation of traditional risk factors. However, these results need to be replicated in future studies.

The question of whether inflammatory processes may still be relevant once unprecedented low average LDL-C levels of 30 mg/dL (0.78 mmol/L) are attained (as in FOURIER) was explored in 2 post hoc analyses.[97,98] First, in the FOURIER study, Bohula et al[97] clearly showed that persistent inflammation as indicated by increased hs-CRP levels was associated with higher event rates, even when LDL-C was low. To show this, patients were subdivided by hs-CRP levels <1, 1 to 3, and >3 mg/L. Increased risk of all cardiovascular outcomes was observed with increasing hs-CRP levels but was particularly striking for the combined primary end point and the key secondary end point as well as for all-cause mortality. When response to evolocumab was examined, according to baseline hs-CRP strata, a persistent increase in the difference between placebo and evolocumab was noted. Thus, in those with hs-CRP >3 mg/L, the AR was 15.5% versus 18.1% in evolocumab versus placebo groups, respectively: the RRR of 20% (HR, 0.8; 95% CI, 0.71–0.90), and the ARR was 2.6%, corresponding to a NNT of 38, compared with the group with hs-CRP <1 mg/L, for which the AR was 10.4% versus 12.0% in evolocumab versus placebo groups, respectively: the RRR was 18% (HR, 0.82; 95% CI, 0.70–0.95; P interaction 0.17). and the ARR was 1.6%. Similar differences, albeit to a lower extent, were seen for secondary endpoints. Of note, adjusted 3-year event rates across various categories of achieved LDL-C, demonstrate that hs-CRP further modified risk in those with an LDL-C <20 mg/dL. Event rates for the primary end point at 3 years varied between 9.0% and 13.1% across hs-CRP categories and between 5.3% and 8.9% for the key secondary end point, with consistent increases in risk with higher hs-CRP irrespective of achieved LDL-C levels.

Second, the SPIRE Outcomes program also showed an increased risk for the primary end point across categories of

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hs-CRP on treatment at 14 weeks, even after adjustment for smoking, diabetes mellitus, hypertension, body mass index, use of lipid-lowering agents, and on-treatment LDL-C.[11] In multivariable analyses looking at the association of hs-CRP on treatment and LDL-C on treatment with incident cardiovascular events, hs-CRP still clearly predicted increased events, whereas LDL-C after additional adjustment for hs-CRP on treatment did not. Despite these post hoc subgroup analyses, the data from both trials are consistent and suggest that inflammation further modulates cardiovascular risk even at very low levels of LDL-C achieved with highly potent PCSK9 inhibitors.[98] Yet, even those participants with the lowest levels of LDL-C and hs-CRP had absolute event rates that would qualify as high-risk. These data support the need for additional interventions to prevent atherosclerotic events at an earlier stage or a time before atherosclerosis becomes less modifiable.[99]

Global Assessment of Plaque Regression With a PCSK9 Antibody as Measured by Intravascular Ultrasound

Mechanistic studies using various imaging methods, in particular, IVUS, are able to provide further insight into the effects of drastic LDL-C lowering specifically on atheroma volume. The GLAGOV trial (Global Assessment of Plaque Regression With a PCSK9 Antibody as Measured by Intravascular Ultrasound).[100,101] was conducted in 968 symptomatic, but clinically stable, CHD patients who presented for elective coronary angiography. Eligibility included a culprit lesion, a 20% and 50% stenosis in a nonculprit vessel, and 1 major or 3 minor cardiovascular risk factors. Further criteria included LDL-C levels >80 mg/dL despite maximally tolerated statin therapy or an LDL-C between 60 and 80 mg/dL with additional major risk factors, such as recent MI, noncoronary atherosclerotic vascular lesions, or type 2 diabetes mellitus or minor risk factors, such as low HDL cholesterol, elevated hs-CRP, smoking, or a positive family history of atherosclerosis as minor criteria. During clinically indicated coronary angiography, these patients underwent IVUS in a suitable nonculprit epicardial coronary artery in at least 1 segment of 40 mm length. After the index imaging, patients were randomized to receive statin plus 420 mg evolocumab subcutaneously every 4 weeks versus statin monotherapy. A total of 846 patients underwent a second IVUS at 78 weeks. Baseline characteristics were similar to those seen in PCSK9 inhibitor end point studies, except that diabetes mellitus was less prevalent. The baseline LDL-C of 92 mg/dL was similar to that in FOURIER, and LDL-C reduction was ≈60%, resulting in a mean LDL-C of 36.6 mg/dL throughout the study. Percent atheroma volume and total atheroma volume were assessed by IVUS at 2 time points. After 78 weeks, the primary end point percent atheroma volume was reduced by 0.95% in the evolocumab group ($P<0.001$) compared with baseline with a between-group difference of −1.0% (95% CI, −1.8% to −0.64%; $P<0.001$). Similarly, the secondary end point, total atheroma volume, was also significantly reduced by 5.8 mm^3 in the evolocumab group ($P<0.001$) compared with baseline and a between-group difference of −4.9 mm^3 (95% CI, −7.3% to −2.5; $P<0.001$). A larger proportion of patients showed regression of plaque measures during evolocumab treatment (64.3% in the statin plus evolocumab treatment group versus 47.3% in the statin plus placebo group), whereas the percentage of those whose plaque volume progressed was lower albeit still at 35.7% in the statin plus evolocumab group versus 52.7% in the statin plus placebo group. The relationship between mean on-treatment LDL-C versus change in percent atheroma volume was linear with no attenuation of the effect up to on-treatment LDL-C of 20 mg/dL, thus suggesting that reducing LDL-C to these very low levels continues to be effective. In various selected prespecified subgroups, there was no effect on the primary end point of age, sex, baseline non-HDL cholesterol, presence of diabetes mellitus, or statin intensity. Still, the relatively high rate of coronary atheroma progression despite an achieved mean LDL-C of 36 mg/dL is consistent with the idea that other pathophysiological mechanisms, in particular, inflammatory processes, may contribute to the further advancement of this chronic disease.

Other High-Risk Populations

Certain patient populations are at particularly high risk for cardiovascular events.[2] The risk may be because of complex biology of the underlying disease or treatments for the primary condition that limits use of high-intensity statins. Several of these high-risk populations have been the focus subgroup analyses of large outcome trials and short-term efficacy studies that investigate efficacy and safety of therapy with PCSK9 inhibitors.

Familial Hypercholesterolemia

FH is a relatively common genetic disorder that causes early ASCVD.[102] The heterozygous and homozygous forms of FH have a global prevalence of ≈1 in 250 and ≈1 in 250 000, respectively,[49,103,104] with higher prevalence in some founder populations.[105,106] FH is more complex at the molecular genetic level than was previously believed.[107] Most cases result from rare, large-effect high-penetrance variants in the *LDLR* gene or less commonly in the *APOB* or *PCSK9* genes. About 10% of FH patients have a large-scale copy number variant in *LDLR*[108] or another gene,[52] whereas 30% to 40% of patients referred with a diagnosis of possible FH have a polygenic basis for their phenotype.[107] Rare mutations in other genes, such as *LDLRAP1*, *APOE*, *STAP1*, *LIPA*, *ABCG5/8* are found in a handful of phenotypic FH patients.[107] In adults, heterozygous FH is suspected when LDL-C exceeds 190 mg/dL, the certainty of diagnosis increases if xanthelasmas, tendon xanthomas, a family history of early ASCVD or dyslipidemia, or positive DNA test result are present.[109] Untreated heterozygous FH patients have greatly increased ASCVD risk by age 40 years,[103] whereas some homozygous FH patients express ASCVD before age 20 years.[104,105]

For almost 30 years, statins were the therapeutic foundation for heterozygous FH patients, together with diet and in combination with drugs like ezetimibe and cholestyramine.[49,102–104] Although RCTs of ASCVD outcomes in FH patients are lacking, compelling observational data establish that statins have improved these patients' survival.[110] However, even with high-intensity statins and multiple drug regimens, FH patients often fail to reach stringent target

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LDL-C levels[111]; this treatment gap presents an opportunity for PCSK9 inhibitors, which have been extensively studied in FH patients. As long as an FH patient has 1 functional copy of the *LDLR* gene that can be upregulated, there can be a biochemical response to PCSK9 inhibition, often with large clinically relevant LDL-C reductions over and above those achieved with statins alone.

Because FH patients were a targeted population in PCSK9 inhibitor development programs, evolocumab and alirocumab are now widely approved by regulators for this clinical indication. For instance, evolocumab 140 mg every 2 weeks or 420 mg every 4 weeks incrementally reduced LDL-C by 54% to 61% compared with placebo over background therapy in heterozygous FH patients.[112–114] In addition, when given on top of background therapy to 735 patients with heterozygous FH, alirocumab either 75 or 150 mg every 2 weeks reduced LDL-C by 51 to 58% compared with placebo.[115] Furthermore, in 1257 heterozygous FH patients from 4 trials, alirocumab 150 mg every 2 weeks reduced LDL-C by 55% over placebo.[116] Among 1191 molecularly defined heterozygous FH patients, response to alirocumab was similar across all mutation types and classes.[84]

Up to 80% of FH patients on statins treated with either evolocumab or alirocumab achieved LDL-C targets, compared with ≈3% of those randomized to placebo.[84] Because PCSK9 inhibitors allow most heterozygous FH patients to achieve LDL-C targets, subgroup analyses of randomized trials or observational studies in patient registries[117,118] should help solidify their role in reducing ASCVD risk in FH. Indeed, a recent subgroup analysis of RCTs of the terminated drug bococizumab showed that over 11.2 months, reductions in LDL-C and ASCVD event rates were not different among 1578 FH patients and 15 959 patients without FH: ≈55% and ≈17%, respectively.[119] Also, registry-based observational studies report that both PCSK9 inhibitors showed comparable LDL-lowering efficacy and tolerability to those seen in clinical trials of FH patients.[120–122] Larger studies with longer-term follow-up and clinical outcomes are expected in the next few years.

Favorable LDL-C reductions have also been observed in patients with less common molecular causes of FH. For instance, heterozygotes for *PCSK9* gain-of-function mutations had 54% reduced LDL-C with alirocumab compared with placebo.[83] A patient with 2 copies of the binding defective *APOB* p.R3527Q variant had a 56% incremental LDL-C reduction with evolocumab.[123] Interestingly, evolocumab reduced LDL-C in homozygous FH patients by 21% to 31%.[85,124] A study of 20 patients with phenotypic homozygous FH because of various types of biallelic defects, including double heterozygous mutations in 2 different FH-causing genes, had incremental reductions in LDL-C between 22% and 64% on alirocumab versus placebo.[125] A patient with autosomal recessive hypercholesterolemia because of a homozygous nonsense variant in *LDLRAP1* had a 33% incremental LDL-C reduction with evolocumab.[126] The unifying explanation for responsiveness across this spectrum of molecular genetic forms of FH seems to be the presence of at least 1 nonnull allele encoding a partially functioning LDLR that can be upregulated.[85,124–128] Until this is further clarified mechanistically, all patients with apparent homozygous FH or atypical forms of FH should probably receive a therapeutic trial of PCSK9 inhibition, irrespective of their genotype.

Based on studies in FH patients to date, high-intensity statins remain first-line therapy which should be initiated as early as possible to prevent or delay ASCVD, adhering to local treatment guidelines for target levels of LDL-C. The degree of LDL-C elevation and presence of additional risk factors would be primary determinants of the aggressiveness of treatment.[107] Ezetimibe would be an appropriate second-line agent if target LDL-C is not achieved. A subset of FH patients, including those in the categories of secondary prevention and high-risk primary prevention because of presence of additional ASCVD risk factors, may require particularly aggressive intervention.[111] This would include consideration of PCSK9 inhibition at a relatively early stage of treatment.

Lp(a) Excess

Lp(a) is comprised of an LDL-like component, whose apo B_{100} moiety binds to the distinctive glycoprotein apo(a), a plasminogen-like molecule that includes repeated pretzel-shaped domains called kringles.[129] Plasma Lp(a) levels have a profoundly right-skewed distribution and vary >1000-fold between individuals.[129–131] Lp(a) levels are directly related to increased risk of ASCVD, stroke, and aortic stenosis, based on epidemiological and Mendelian randomization data,[130–134] even when LDL-C levels are normal. Lp(a) levels are strongly genetically determined not only by both common single nucleotide variants and copy number variants at the *LPA* locus on chromosome 6q25-26[135,136] but also by other loci, including *SORT1* on chromosome 1p13.1 encoding sortilin.[132] The genomic copy number variants at *LPA* underlie the size polymorphism of Lp(a) isoforms in the population that is because of variable numbers of repeats of kringle IV type 2[133,134]; larger Lp(a) isoforms are associated with lower plasma levels because of their greater instability and propensity for degradation.[129] Lp(a) levels are little influenced by diet, age, sex, obesity, or other extrinsic factors but are elevated in patients with renal failure.[137]

Despite decades of study, Lp(a) production and clearance pathways are incompletely understood.[138] Traditional lipid-lowering therapies have little impact on plasma Lp(a), except for niacin, which lowers it by ≈20%, presumably by increasing catabolism.[139] Newer agents, such as the anti-APOB antisense oligonucleotide mipomersen and the microsomal triglyceride transfer protein inhibitor lomitapide, each of which reduces assembly and secretion of apoB-containing lipoproteins, both reduce Lp(a) levels by ≈20%.[139,140] However, counterintuitively, PCSK9 inhibitors that have a minimal effect on the production of apoB-containing lipoproteins reduce Lp(a) levels by 20% to 30%,[140–142] contradicting the idea that the LDLR does not determine Lp(a) levels.

Although an LDLR-independent mechanism was implied in a study of 2 LDLR-null FH homozygotes in whom evolocumab lowered Lp(a), with no effect on LDL-C,[143] subsequent in vitro experiments showed that overexpression of LDLR increased internalization of Lp(a) and that this was reduced with PCSK9.[144] In vivo human lipoprotein kinetic studies showed that alirocumab had no effect on production, but rather increased catabolism of Lp(a), which again

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implied involvement of the LDLR.[145] But comparable kinetic studies with evolocumab gave somewhat different results.[146] Evolocumab monotherapy lowered the plasma Lp(a) pool size by decreasing Lp(a) production by 36%, with no effect on catabolism. However, when atorvastatin was given concurrently, evolocumab lowered plasma Lp(a) pool size by increasing particle catabolism by 59%. These findings suggested a dual mechanism of PCSK9 inhibition on Lp(a) lowering, comprised of inhibition on Lp(a)-apo(a) production and upregulation of LDLR activity on Lp(a) particle clearance depending on whether a statin was coadministered.

The potential clinical relevance of these complicated mechanistic observations linking PCSK9 inhibition to Lp(a) reduction remains uncertain.[147] In FOURIER, evolocumab reduced Lp(a) by 26.9%, but apparently, this provided no incremental efficacy benefit over and above the reduction in LDL-C.[148] Similarly, a subgroup analysis in ODYSSEY Outcomes has not definitively shown any incremental benefit of Lp(a) reduction on hard outcomes.[149] Extrapolations of findings from a recent genetic analysis may provide some clarity.[150] For instance, evaluation of genetic markers, *LPA* levels, and vascular disease risk showed that a large absolute reduction in Lp(a) cholesterol levels—on the order of 100 mg/dL—would be required for clinical benefit.[150] Thus, the 30% relative reduction in Lp(a) with PCSK9 inhibition would translate to an absolute reduction of 100 mg/dL only in rare individuals with extremely elevated plasma Lp(a), that is, ≥300 mg/dL. Individuals enrolled in FOURIER had a mean baseline Lp(a) of ≈12 mg/dL (ie, 37 nmol/L), a level at which an absolute reduction of 100 mg/dL would be impossible.[147] In addition, observational data suggest that the risk of elevated Lp(a) becomes attenuated when LDL-C is relatively low, for example, <100 mg/dL (2.60 mmol/L).[151]

These recent insights suggest that future trials, whether using PCSK9 inhibitors or other therapies designed to more specifically knockdown Lp(a), should focus on individuals with high plasma Lp(a) to maximize the absolute reduction required.[150] Furthermore, studies should exclude individuals with low LDL-C to attain manageable numbers needed to treat.[150] Such selection criteria have already been applied in the ANITSCHKOW trial (Effects of Proprotein Convertase Subtilisin/Kexin Type 9 [PCSK9] Inhibition on Arterial Wall Inflammation Study in Patients With Elevated Lipoprotein(a); http://www.clinicaltrials.gov. Unique identifier: NCT02729025), which has enrolled patients with Lp(a) >50 mg/dL and LDL-C >100 mg/dL to determine the effect of evolocumab on arterial wall inflammation. Additional challenging features of targeting Lp(a) include the lack of measurement standardization in clinical laboratories[152] and variations in plasma levels and genetic determinants across different geographic ancestries.[129,130,136,153] Identifying patients with high baseline Lp(a) may be another way to reduce the NNT, similar to other high-risk subgroups.

Diabetes Mellitus

FOURIER included 11 031 participants (40%) with (mainly type 2) diabetes mellitus who received treatment with moderate to high-intensity statin therapy.[8,9] In a preplanned subgroup analysis, the HR of a primary end point was reduced similarly in participants with diabetes mellitus (0.83; 95% CI, 0.75–0.93; *P*=0.0008 and without diabetes mellitus (0.87; 95% CI, 0.79–0.96; *P*=0.0052, although absolute event rates were much higher in the patients with diabetes mellitus. The HRs for the secondary end point was 0.82 (95% CI, 0.72–0.93; *P*=0.0021) for participants with diabetes mellitus and was 0.78 (95% CI, 0.69–0.89; *P*=0.002) for those without diabetes mellitus but again event rates were higher in patients with diabetes mellitus. The NNT to prevent 1 primary cardiovascular end point over 3 years was 37 in participants with diabetes mellitus versus 62 in participants without diabetes mellitus. Evolocumab was not associated with a higher risk of new-onset diabetes mellitus (HR, 1.05; 95% CI, 0.94–1.17), including among those participants with impaired fasting glucose (HR, 1.00; 95% CI, 0.89–1.13).

The safety and lipid-lowering efficacy of PCSK9 inhibitors has also been evaluated in several smaller multicenter trials of patients with diabetes mellitus.[154,155] The BANTING trial (Evolocumab Efficacy and Safety in Type 2 Diabetes Mellitus on Background Statin Therapy; http://www.clinicaltrials.gov. Unique identifier: NCT02739984) investigated the efficacy of evolocumab on fasting and postprandial lipemia in high-risk type 2 diabetes mellitus patients with hypercholesterolemia or mixed hyperlipidemia on the background of moderate to high-intensity statin therapy.[155] After 10 to 12 weeks treatment with evolocumab, the mean LDL-C was reduced by 65.0 % from 108 to 38.2 mg/dL. Non–HDL-cholesterol and apoB levels were reduced by 56.1% and 50.2%, respectively. After a mixed meal, participants treated with evolocumab had reductions in chylomicron triglyceride and cholesterol and apo B_{48} levels, which support enhanced chylomicron remnant clearance by the LDLR and suggest the potential for additional incremental benefits in diabetic patients receiving PCSK9 inhibition.

Chronic Kidney Disease

CKD is a risk factor for the development of coronary artery disease, and it is associated with a risk for adverse outcomes in those with existing ASCVD.[156] Treatment of CKD patients not requiring dialysis with a statin leads to a significant reduction in ASCVD events as supported by post hoc subgroup analyses of randomized trials that were not intended to include patients with decreased kidney function. The SHARP trial (Study of Heart and Renal Protection) specifically evaluated cholesterol lowering with a statin and ezetimibe to prevent major vascular events in patients with CKD.[157] The FOURIER trial included study participants with the most severe renal impairment (estimated glomerular filtration rate >15 mL/[min·1.73m2]) of any PCSK9 inhibitor trial.[8,9] As compared to the 8077 patients in FOURIER with estimated glomerular filtration rate ≥90 mL/(min·1.73 m2), the 4443 patients with stage 3 CKD <60 mL/(min·1.73 m^2) had larger RRR for the primary (−16.1% versus 12.2%; *P*<0.01) and secondary endpoints (−12.8% versus 7.1%; *P*<0.001). The ARR was largest for patients with stage 3 CKD (2.5% versus 1.7%).[158]

HIV-Infected People

The widespread availability of combination antiretroviral therapy has increased life expectancy in people living with HIV. Correspondingly, there has been an increase in non-HIV

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morbidity and mortality.[159] Adults living with HIV versus those without HIV have higher rates of MI, stroke, and LEAD[160] in the contemporary era despite more widespread use of ART (antiretroviral therapy) and increased use of statin therapy. Several purported mechanisms for increased ASVD risk include residual ART-associated dyslipidemia and inflammation. Statins inhibit the proinflammatory effects of ox-LDL activation of T cells in human atherosclerotic plaque and blood via repression of microRNA let-7c (lethal-7 gene family member-7c).[40] Statin use in ART-treated HIV patients is often complicated by excessive statin dosing in patients treated with protease inhibitors and the pharmacological enhancer cobicistat.[161] Often, HIV-infected treated with nonnucleoside reverse transcriptase inhibitors have less than expected LDL-C reductions with statins resulting from lower blood statin concentrations with this class of agents.[162] The REPRIEVE trial (Randomized Trial to Prevent Vascular Events; http://www.clinicaltrials.gov. Unique identifier: NCT02344290) is an ongoing trial of primary prevention trial that is investigating the efficacy of pitavastatin.[163]

Plasma PCSK9 levels are increased in ART-naive patients with HIV infection[164] and in ART-treated patients with no detectable viral load and CD4 counts >250 cells/mm^3.[165] Levels of PCSK9 are positively associated with HIV progression (high HIV viral load, low CD4+ cell count,[164] and markers of systemic monocyte activation [soluble CD14, soluble CD163]).[166] After treatment with protease inhibitor regimens, PCSK9 levels remain elevated.[164] In a US cohort of HIV-infected black women, differential binding of has-miR (microRNA)-548t-5p and has-miR-4796-3p to the 3′ UTR (untranslated region) variant of PCSK9 (*rs17111557*) in HIV/HCV (hepatitis C virus) coinfected women was inversely associated with LDL-C levels and positively associated with 2 biomarkers of HIV progression (elevated HIV viral load and lower CD4$^+$ T-cell levels).[166]

Currently, the efficacy and safety of PCSK9 inhibitors in HIV patients have not been established. The BEIJERINCK trial (Evolocumab Effect on LDL-C Lowering on Background Statin Therapy; http://www.clinicaltrials.gov. Unique identifier: NCT02833844) will include 450 HIV positive subjects with hypercholesterolemia or mixed hyperlipidemia that will be randomized 2:1 to receive treatment with either evolocumab 420 mg subcutaneous injection or placebo every 4 weeks. The EPIC-HIV study (Effect of PCSK9 Inhibition on Cardiovascular Risk in Treated HIV Infection) is designed to investigate the of PCSK9 inhibition with alirocumab on arterial inflammation as assessed by FDG (fluorodeoxyglucose)–positron emission tomography/computed tomography and endothelial function as assessed by flow-mediated vasodilation (http://www.clinicaltrials.gov. Unique identifier:NCT03207945).

Statin Intolerance

Statin intolerance is characterized by the inability to take a statin because of patient-reported adverse events (symptoms or laboratory test abnormalities).[167] The intolerance to statin therapy may be complete (inability to tolerate any statin at any dose) or partial (inability to tolerate a statin dose that effectively lowers LDL-C or dosage used in a clinical outcomes trial in patients with similar characteristics). Statin-associated adverse muscle symptoms represent the most common reason that patients discontinue therapy.[168,169] Among patients who experience a statin-associated adverse event, 57% discontinue the statin.[170] Of the patients who discontinue statin because of an adverse event, 73% are unwilling to be rechallenged often owing to fear of recurrent side effects.

Statin intolerance in patients with a prior MI increases the risk of recurrent MI, hospitalizations for cardiovascular events,[170] and health care expenditures.[171] Nonstatin therapy is a secondary option to lower LDL-C in patients with statin adverse muscle symptoms.[172,173] Among participants enrolled in the GOULD registry (Getting to an Improved Understanding of Low-Density Lipoprotein Cholesterol and Dyslipidemia Management), 49% percent of patients prescribed PCSK9 inhibitors had complete statin intolerance.[174] Treatment with PCSK9 inhibitors has been well tolerated in patients with statin-associated adverse muscle symptoms in short-term trials and an open-label extension study.[175]

Cost-Effectiveness

The cost-effectiveness of these agents is complex and influenced by the efficiency of the therapy in certain subgroups and the medication cost in different countries and various insurance plans within a specific country.[176] Cost-effectiveness analysis of PCSK9 inhibitors is based on clinical and economic outcomes among patients with a prior history of ASCVD and the minimal qualifying LDL-C level ≥70 mg/dL. Cost/value methodology is based on incremental cost-effectiveness ratio and level of value in clinical guideline recommendations using quality-adjusted life year gained.[177] Based on the FOURIER trial,[9] the incremental cost-effectiveness ratio for statin and evolocumab (wholesale acquisition cost) is $1 336 221 and $799 596 using net price for evolocumab.[177] To achieve an incremental cost-effectiveness ratio of $150 000 per quality-adjusted life year, the net cost of evolocumab would have to be $9669 for the United States.[178] The cost-effectiveness of PCSK9 inhibitor therapy can be improved by selection of patients groups that achieved higher absolute benefit in FOURIER (recent MI, multivessel vascular disease, elevated Lp[a]). In ODYSSEY Outcomes,[13] treatment with alirocumab in the LDL-C subgroup ≥100 mg/dL achieved larger reductions in the primary outcome than subgroups with lower baseline LDL-C levels. After 12 months, HR for the primary outcome was 0.71 (95% CI, 0.58–0.87) and all-cause death 0.67 (95% CI, 0.50–0.89). Among patients with LDL-C, the value-based price benchmark range for alirocumab is $2306 to $3441, and for patients with LDL-C ≥41 mg/dL, the cost range is $4460 to $6578.[178] These analyses demonstrate that cost-effectiveness can be achieved in a higher risk subgroup at a lower medication cost. Among MI patients with statin discontinuation or downtitration is associated with a 51% higher risk of hospitalizations for CHD events in the ensuing 2 years, and higher cost expenditures in the year after MI of $40 776 compared with $26 728 in patients with high adherence (≥80%).[179] These higher medical care expenditures are equivalent to the costs of PCSK9 inhibitor therapy. In primary prevention, cost-benefit analyses suggest that in FH patients, a two-thirds reduction in cost of PCSK9 inhibitors will create a compelling argument for their use compared with other commonly reimbursed therapies.[180]

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Safety of PCSK9 Inhibitors and Very Low LDL-C Levels

To date, all phase 2 and 3 studies strongly suggest an excellent safety profile of both alirocumab and evolocumab, including among trial participants who attain LDL-C levels <15 mg/dL.[181] The most common treatment-emergent adverse reaction has been injection site reactions.[87,182] Potential adverse events of special interest included steroid hormone and vitamin levels, neurocognitive impairment and risk of infections and cancers.[93,182,183]

Neurocognitive events in the clinical trials with PCSK9 inhibitors were recorded as delirium (including confusion), cognitive and attention disorders and disturbances, dementia and amnestic conditions, disturbances in thinking and perception, and mental impairment disorders.[8,9,12] In a phase III clinical outcomes trials, differences in cognitive function were not different between treatment groups. In the EBBINGHAUS (Evaluating PCSK9 Binding Antibody Influence on Cognitive Health in High Cardiovascular Risk Subjects), a substudy of FOURIER, cognitive impairment was evaluated by assessing visual memory using the Cambridge Neuropsychology Test Automated Battery.[88] No group differences were observed in spatial working memory strategy index of executive function (primary end point), working memory, or psychomotor change (secondary end point). The safety of evolocumab on cognitive function was similar in older (>65 to <86 years) and younger participants (40 to 65 years). A natural randomization study supported the lack of association between cognitive impairment in people with loss-of-function variants in PCSK9.[74]

Fat-soluble vitamin concentrations (A, D, E, K) were measured as part of the prespecified safety analysis in a phase 2 trial with PCSK9 inhibitors.[182,183] In phase 2 trials, vitamin E levels were lower in both alirocumab-treated and evolocumab-treated participants; however, the results were nonsignificant after adjustment for LDL-C.[183] Data from 52-week double-blind, controlled trials did not show changes in levels of vitamin E, cortisol:corticotropin (ACTH) levels, or sex steroids. Vitamin E in erythrocyte membranes was unchanged from baseline to 52 weeks in both absolute and LDL-C normalized levels.

Current safety data with PCSK9 inhibitors extends to 52 to 78 weeks in open-label extension trials.[87,182] Long-term safety of very low LDL-C levels will continue to be monitored in open-label extension trials of FOURIER (http://www.clinicaltrials.gov. Unique identifier: NCT02867813).

Future Directions

The landscape of cardiovascular risk prevention in the PCSK9 era has already begun to shift. An imminent impact will likely be the reintroduction of LDL-C monitoring of efficacy in certain clinical practice guidelines and recommendations for even stricter target LDL-C levels ubiquitously. In many cases, new strict targets may be achievable with intensification of statin and ezetimibe treatment, but in some cases, PCSK9 inhibitors will also be required. However, given cost considerations and resource issues, health care providers and payers will need to carefully consider prioritizing those patient subgroups who may benefit the most from the use of these therapies. Additional considerations include the use of new therapeutic modalities to inhibit PCSK9. A promising agent in this regard is the small interfering RNA molecular inclisiran, which targets PCSK9 mRNA and is associated with sustained reductions of circulating PCSK9 and LDL-C of at least 6 months after a single subcutaneous injection.[184] Other atherogenic lipoproteins show similar sustained reductions. A planned larger scale RCT of ASCVD outcomes using this agent is essential, given its fundamentally different mechanism of action compared with the monoclonal antibodies. A clinical outcomes trial in patients with stable atherosclerotic vascular disease will examine the effects of inclisiran on cardiovascular events (HPS-4/TIMI 65 ORION-4 [Heart Protection Study-4/Thrombolysis in Myocardial Infarction [TIMI] 65 Trial to Evaluate the Effect of ALN-PCSSC Treatment on Low-Density Lipoprotein Cholesterol (ORION-4)]; www.timi.org accessed January 5, 2019). This trial has started in late 2018, and it is estimated to report results in 2024. Other modalities in development for delivering PCSK9 inhibition include other types of antibodies,[185] vaccines,[186] or gene editing.[187]

It is also essential to document whether PCSK9 inhibition can lead to unexpected effects in other tissues and organ systems; this is being examined by a range of preclinical model systems.[14] Such information can also be inferred by studying extended phenotypes of individuals with naturally occurring *PCSK9* loss-of-function variants in epidemiological scale databases, such as the UK Biobank or electronic health records collected from large health care system databases.

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References

1. Ference BA, Ginsberg HN, Graham I, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur Heart J*. 2017;38:2459–2472. doi: 10.1093/eurheartj/ehx144
2. Grundy SM, Stone NJ, Bailey AL, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the management of blood cholesterol: executive smmary: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines [published online ahead of print November 3, 2018]. *J Am Coll Cardiol*. doi: 10.1016/j.jacc.2018.11.002
3. Catapano AL, Graham I, De Backer G, et al; Additional Contributor, Cooney MT. 2016 ESC/EAS guidelines for the management of dyslipidaemias: the task force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS) Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *Atherosclerosis*. 2016;253:281–344.
4. Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, Bhala N, Peto R, Barnes EH, Keech A, Simes J, Collins R; Cholesterol Treatment Trialists' (CTT) Collaboration. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000

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participants in 26 randomised trials. *Lancet*. 2010;376:1670–1681. doi: 10.1016/S0140-6736(10)61350-5
5. Lloyd-Jones DM, Morris PB, Ballantyne CM, et al. 2016 ACC Expert Consensus Decision pathway on the role of non-statin therapies for LDL-cholesterol lowering in the management of atherosclerotic cardiovascular disease risk: a report of the American College of Cardiology Task Force on Clinical Expert Consensus Documents. *J Am Coll Cardiol*. 2016;68:92–125.
6. Cannon CP, Blazing MA, Giugliano RP, et al; IMPROVE-IT Investigators. Ezetimibe added to statin therapy after acute coronary syndromes. *N Engl J Med*. 2015;372:2387–2397. doi: 10.1056/NEJMoa1410489
7. Rosenson RS, Hegele RA, Fazio S, Cannon CP. The evolving future of PCSK9 inhibitors. *J Am Coll Cardiol*. 2018;72:314–329. doi: 10.1016/j.jacc.2018.04.054
8. Sabatine MS, Giugliano RP, Keech A, Honarpour N, Wang H, Liu T, Wasserman SM, Scott R, Sever PS, Pedersen TR. Rationale and design of the Further cardiovascular OUtcomes Research with PCSK9 Inhibition in subjects with Elevated Risk trial. *Am Heart J*. 2016;173:94–101. doi: 10.1016/j.ahj.2015.11.015
9. Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, Kuder JF, Wang H, Liu T, Wasserman SM, Sever PS, Pedersen TR; FOURIER Steering Committee and Investigators. Evolocumab and clinical outcomes in patients with cardiovascular disease. *N Engl J Med*. 2017;376:1713–1722. doi: 10.1056/NEJMoa1615664
10. Ridker PM, Amarenco P, Brunell R, Glynn RJ, Jukema JW, Kastelein JJ, Koenig W, Nissen S, Revkin J, Santos RD, Schwartz PF, Yunis C, Tardif JC; Studies of PCSK9 Inhibition and the Reduction of vascular Events (SPIRE) Investigators. Evaluating bococizumab, a monoclonal antibody to PCSK9, on lipid levels and clinical events in broad patient groups with and without prior cardiovascular events: rationale and design of the Studies of PCSK9 Inhibition and the Reduction of vascular Events (SPIRE) Lipid Lowering and SPIRE Cardiovascular Outcomes Trials. *Am Heart J*. 2016;178:135–144. doi: 10.1016/j.ahj.2016.05.010
11. Ridker PM, Revkin J, Amarenco P, et al; SPIRE Cardiovascular Outcome Investigators. Cardiovascular efficacy and safety of bococizumab in high-risk patients. *N Engl J Med*. 2017;376:1527–1539. doi: 10.1056/NEJMoa1701488
12. Schwartz GG, Bessac L, Berdan LG, et al. Effect of alirocumab, a monoclonal antibody to PCSK9, on long-term cardiovascular outcomes following acute coronary syndromes: rationale and design of the ODYSSEY outcomes trial. *Am Heart J*. 2014;168:682–689. doi: 10.1016/j.ahj.2014.07.028
13. Schwartz GG, Steg PG, Szarek M, et al; ODYSSEY OUTCOMES Committees and Investigators. Alirocumab and cardiovascular outcomes after acute coronary syndrome. *N Engl J Med*. 2018;379:2097–2107. doi: 10.1056/NEJMoa1801174
14. Seidah NG, Chrétien M, Mbikay M. The ever-expanding saga of the proprotein convertases and their roles in body homeostasis: emphasis on novel proprotein convertase subtilisin kexin number 9 functions and regulation. *Curr Opin Lipidol*. 2018;29:144–150. doi: 10.1097/MOL.0000000000000484
15. Seidah NG. New developments in proprotein convertase subtilisin-kexin 9's biology and clinical implications. *Curr Opin Lipidol*. 2016;27:274–281. doi: 10.1097/MOL.0000000000000295
16. Burke AC, Dron JS, Hegele RA, Huff MW. PCSK9: regulation and target for drug development for dyslipidemia. *Annu Rev Pharmacol Toxicol*. 2017;57:223–244. doi: 10.1146/annurev-pharmtox-010716-104944
17. Seidah NG, Abifadel M, Prost S, Boileau C, Prat A. The proprotein convertases in hypercholesterolemia and cardiovascular diseases: emphasis on proprotein convertase subtilisin/kexin 9. *Pharmacol Rev*. 2017;69:33–52. doi: 10.1124/pr.116.012989
18. Seidah NG, Benjannet S, Wickham L, Marcinkiewicz J, Jasmin SB, Stifani S, Basak A, Prat A, Chretien M. The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): liver regeneration and neuronal differentiation. *Proc Natl Acad Sci USA*. 2003;100:928–933. doi: 10.1073/pnas.0335507100
19. Traub O, Berk BC. Laminar shear stress: mechanisms by which endothelial cells transduce an atheroprotective force. *Arterioscler Thromb Vasc Biol*. 1998;18:677–685.
20. Ding Z, Liu S, Wang X, Deng X, Fan Y, Sun C, Wang Y, Mehta JL. Hemodynamic shear stress via ROS modulates PCSK9 expression in human vascular endothelial and smooth muscle cells and along the mouse aorta. *Antioxid Redox Signal*. 2015;22:760–771. doi: 10.1089/ars.2014.6054
21. Giunzioni I, Tavori H, Covarrubias R, Major AS, Ding L, Zhang Y, DeVay RM, Hong L, Fan D, Predazzi IM, Rashid S, Linton MF, Fazio S. Local effects of human PCSK9 on the atherosclerotic lesion. *J Pathol*. 2016;238:52–62. doi: 10.1002/path.4630
22. Swirski FK, Libby P, Aikawa E, Alcaide P, Luscinskas FW, Weissleder R, Pittet MJ. Ly-6Chi monocytes dominate hypercholesterolemia-associated monocytosis and give rise to macrophages in atheromata. *J Clin Invest*. 2007;117:195–205. doi: 10.1172/JCI29950
23. Tavori H, Giunzioni I, Predazzi IM, Plubell D, Shivinsky A, Miles J, Devay RM, Liang H, Rashid S, Linton MF, Fazio S. Human PCSK9 promotes hepatic lipogenesis and atherosclerosis development via apoE- and LDLR-mediated mechanisms. *Cardiovasc Res*. 2016;110:268–278. doi: 10.1093/cvr/cvw053
24. Ferri N, Tibolla G, Pirillo A, Cipollone F, Mezzetti A, Pacia S, Corsini A, Catapano AL. Proprotein convertase subtilisin kexin type 9 (PCSK9) secreted by cultured smooth muscle cells reduces macrophages LDLR levels. *Atherosclerosis*. 2012;220:381–386. doi: 10.1016/j.atherosclerosis.2011.11.026
25. Fan D, Yancey PG, Qiu S, Ding L, Weeber EJ, Linton MF, Fazio S. Self-association of human PCSK9 correlates with its LDLR-degrading activity. *Biochemistry*. 2008;47:1631–1639. doi: 10.1021/bi7016359
26. Linton MF, Babaev VR, Gleaves LA, Fazio S. A direct role for the macrophage low density lipoprotein receptor in atherosclerotic lesion formation. *J Biol Chem*. 1999;274:19204–19210.
27. Maxwell KN, Fisher EA, Breslow JL. Overexpression of PCSK9 accelerates the degradation of the LDLR in a post-endoplasmic reticulum compartment. *Proc Natl Acad Sci USA*. 2005;102:2069–2074. doi: 10.1073/pnas.0409736102
28. Zhang DW, Lagace TA, Garuti R, Zhao Z, McDonald M, Horton JD, Cohen JC, Hobbs HH. Binding of proprotein convertase subtilisin/kexin type 9 to epidermal growth factor-like repeat A of low density lipoprotein receptor decreases receptor recycling and increases degradation. *J Biol Chem*. 2007;282:18602–18612. doi: 10.1074/jbc.M702027200
29. Hussain MM, Strickland DK, Bakillah A. The mammalian low-density lipoprotein receptor family. *Annu Rev Nutr*. 1999;19:141–172. doi: 10.1146/annurev.nutr.19.1.141
30. Poirier S, Mayer G, Benjannet S, Bergeron E, Marcinkiewicz J, Nassoury N, Mayer H, Nimpf J, Prat A, Seidah NG. The proprotein convertase PCSK9 induces the degradation of low density lipoprotein receptor (LDLR) and its closest family members VLDLR and ApoER2. *J Biol Chem*. 2008;283:2363–2372. doi: 10.1074/jbc.M708098200
31. Canuel M, Sun X, Asselin MC, Paramithiotis E, Prat A, Seidah NG. Proprotein convertase subtilisin/kexin type 9 (PCSK9) can mediate degradation of the low density lipoprotein receptor-related protein 1 (LRP-1). *PLoS One*. 2013;8:e64145. doi: 10.1371/journal.pone.0064145
32. Yancey PG, Blakemore J, Ding L, Fan D, Overton CD, Zhang Y, Linton MF, Fazio S. Macrophage LRP-1 controls plaque cellularity by regulating efferocytosis and Akt activation. *Arterioscler Thromb Vasc Biol*. 2010;30:787–795. doi: 10.1161/ATVBAHA.109.202051
33. Yancey PG, Ding Y, Fan D, Blakemore JL, Zhang Y, Ding L, Zhang J, Linton MF, Fazio S. Low-density lipoprotein receptor-related protein 1 prevents early atherosclerosis by limiting lesional apoptosis and inflammatory Ly-6Chigh monocytosis: evidence that the effects are not apolipoprotein E dependent. *Circulation*. 2011;124:454–464. doi: 10.1161/CIRCULATIONAHA.111.032268
34. Leeb C, Eresheim C, Nimpf J. Clusterin is a ligand for apolipoprotein E receptor 2 (ApoER2) and very low density lipoprotein receptor (VLDLR) and signals via the Reelin-signaling pathway. *J Biol Chem*. 2014;289:4161–4172. doi: 10.1074/jbc.M113.529271
35. Denis M, Marcinkiewicz J, Zaid A, Gauthier D, Poirier S, Lazure C, Seidah NG, Prat A. Gene inactivation of proprotein convertase subtilisin/kexin type 9 reduces atherosclerosis in mice. *Circulation*. 2012;125:894–901. doi: 10.1161/CIRCULATIONAHA.111.057406
36. Waltmann MD, Basford JE, Konaniah ES, Weintraub NL, Hui DY. Apolipoprotein E receptor-2 deficiency enhances macrophage susceptibility to lipid accumulation and cell death to augment atherosclerotic plaque progression and necrosis. *Biochim Biophys Acta*. 2014;1842:1395–1405. doi: 10.1016/j.bbadis.2014.05.009
37. Ricci C, Ruscica M, Camera M, Rossetti L, Macchi C, Colciago A, Zanotti I, Lupo MG, Adorni MP, Cicero AFG, Fogacci F, Corsini A, Ferri N. PCSK9 induces a pro-inflammatory response in macrophages. *Sci Rep*. 2018;8:2267. doi: 10.1038/s41598-018-20425-x
38. Bai XQ, Peng J, Wang MM, Xiao J, Xiang Q, Ren Z, Wen HY, Jiang ZS, Tang ZH, Liu LS. PCSK9: a potential regulator of apoE/apoER2 against inflammation in atherosclerosis? *Clin Chim Acta*. 2018;483:192–196. doi: 10.1016/j.cca.2018.04.040

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39. Liu A, Frostegard J. PCSK9 plays a novel immunological role in oxidized LDL-induced dendritic cell maturation and activation of T cells from human blood and atherosclerotic plaque [published online ahead of print April 4, 2018]. *J Intern Med*. doi: 10.1111/joim.12758
40. Frostegard J, Zhang Y, Sun J, Yan K, Liu A. Oxidized low-density lipoprotein (OxLDL)-treated dendritic cells promote activation of T cells in human atherosclerotic plaque and blood, which is repressed by statins: microRNA let-7c is integral to the effect. *J Am Heart Assoc*. 2016;5:e003976.
41. Tang ZH, Peng J, Ren Z, Yang J, Li TT, Li TH, Wang Z, Wei DH, Liu LS, Zheng XL, Jiang ZS. New role of PCSK9 in atherosclerotic inflammation promotion involving the TLR4/NF-κB pathway. *Atherosclerosis*. 2017;262:113–122. doi: 10.1016/j.atherosclerosis.2017.04.023
42. Wu CY, Tang ZH, Jiang L, Li XF, Jiang ZS, Liu LS. PCSK9 siRNA inhibits HUVEC apoptosis induced by ox-LDL via Bcl/Bax-caspase9-caspase3 pathway. *Mol Cell Biochem*. 2012;359:347–358. doi: 10.1007/s11010-011-1028-6
43. Bernelot Moens SJ, Neele AE, Kroon J, van der Valk FM, Van den Bossche J, Hoeksema MA, Hoogeveen RM, Schnitzler JG, Baccara-Dinet MT, Manvelian G, de Winther MPJ, Stroes ESG. PCSK9 monoclonal antibodies reverse the pro-inflammatory profile of monocytes in familial hypercholesterolaemia. *Eur Heart J*. 2017;38:1584–1593. doi: 10.1093/eurheartj/ehx002
44. Cheng JM, Oemrawsingh RM, Garcia-Garcia HM, Boersma E, van Geuns RJ, Serruys PW, Kardys I, Akkerhuis KM. PCSK9 in relation to coronary plaque inflammation: results of the ATHEROREMO-IVUS study. *Atherosclerosis*. 2016;248:117–122. doi: 10.1016/j.atherosclerosis.2016.03.010
45. Varret M, Rabès JP, Saint-Jore B, et al. A third major locus for autosomal dominant hypercholesterolemia maps to 1p34.1-p32. *Am J Hum Genet*. 1999;64:1378–1387. doi: 10.1086/302370
46. Hunt SC, Hopkins PN, Bulka K, McDermott MT, Thorne TL, Wardell BB, Bowen BR, Ballinger DG, Skolnick MH, Samuels ME. Genetic localization to chromosome 1p32 of the third locus for familial hypercholesterolemia in a Utah kindred. *Arterioscler Thromb Vasc Biol*. 2000;20:1089–1093.
47. Abifadel M, Varret M, Rabès JP, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. *Nat Genet*. 2003;34:154–156. doi: 10.1038/ng1161
48. Timms KM, Wagner S, Samuels ME, Forbey K, Goldfine H, Jammulapati S, Skolnick MH, Hopkins PN, Hunt SC, Shattuck DM. A mutation in PCSK9 causing autosomal-dominant hypercholesterolemia in a Utah pedigree. *Hum Genet*. 2004;114:349–353. doi: 10.1007/s00439-003-1071-9
49. Nordestgaard BG, Chapman MJ, Humphries SE, et al; European Atherosclerosis Society Consensus Panel. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. *Eur Heart J*. 2013;34:3478–3390a. doi: 10.1093/eurheartj/eht273
50. Maxwell KN, Breslow JL. Adenoviral-mediated expression of Pcsk9 in mice results in a low-density lipoprotein receptor knockout phenotype. *Proc Natl Acad Sci USA*. 2004;101:7100–7105. doi: 10.1073/pnas.0402133101
51. Dron JS, Hegele RA. Complexity of mechanisms among human proprotein convertase subtilisin-kexin type 9 variants. *Curr Opin Lipidol*. 2017;28:161–169. doi: 10.1097/MOL.0000000000000386
52. Iacocca MA, Wang J, Sarkar S, Dron JS, Lagace T, McIntyre AD, Lau P, Robinson JF, Yang P, Knoll JH, Cao H, McPherson R, Hegele RA. Whole-gene duplication of PCSK9 as a novel gnetic mechanism for severe familial hypercholesterolemia. *Can J Cardiol*. 2018;34:1316–1324. doi: 10.1016/j.cjca.2018.07.479
53. Cohen J, Pertsemlidis A, Kotowski IK, Graham R, Garcia CK, Hobbs HH. Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in PCSK9. *Nat Genet*. 2005;37:161–165. doi: 10.1038/ng1509
54. Kotowski IK, Pertsemlidis A, Luke A, Cooper RS, Vega GL, Cohen JC, Hobbs HH. A spectrum of PCSK9 alleles contributes to plasma levels of low-density lipoprotein cholesterol. *Am J Hum Genet*. 2006;78:410–422. doi: 10.1086/500615
55. Cohen JC, Boerwinkle E, Mosley TH Jr, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N Engl J Med*. 2006;354:1264–1272. doi: 10.1056/NEJMoa054013
56. Kent ST, Rosenson RS, Avery CL, et al. PCSK9 loss-of-function variants, low-density lipoprotein cholesterol, and risk of coronary heart disease and stroke: data from 9 studies of blacks and whites. *Circ Cardiovasc Genet*. 2017;10:e001632. doi: 10.1161/CIRCGENETICS.116.001632
57. Benn M, Nordestgaard BG, Grande P, Schnohr P, Tybjaerg-Hansen A. PCSK9 R46L, low-density lipoprotein cholesterol levels, and risk of ischemic heart disease: 3 independent studies and meta-analyses. *J Am Coll Cardiol*. 2010;55:2833–2842. doi: 10.1016/j.jacc.2010.02.044
58. Cameron J, Holla ØL, Laerdahl JK, Kulseth MA, Berge KE, Leren TP. Mutation S462P in the PCSK9 gene reduces secretion of mutant PCSK9 without affecting the autocatalytic cleavage. *Atherosclerosis*. 2009;203:161–165. doi: 10.1016/j.atherosclerosis.2008.10.007
59. Dewpura T, Raymond A, Hamelin J, Seidah NG, Mbikay M, Chrétien M, Mayne J. PCSK9 is phosphorylated by a Golgi casein kinase-like kinase ex vivo and circulates as a phosphoprotein in humans. *FEBS J*. 2008;275:3480–3493. doi: 10.1111/j.1742-4658.2008.06495.x
60. Fisher TS, Lo Surdo P, Pandit S, et al. Effects of pH and low density lipoprotein (LDL) on PCSK9-dependent LDL receptor regulation. *J Biol Chem*. 2007;282:20502–20512. doi: 10.1074/jbc.M701634200
61. Lu X, Li J, Li H, et al. Coding-sequence variants are associated with blood lipid levels in 14,473 Chinese. *Hum Mol Genet*. 2016;25:4107–4116. doi: 10.1093/hmg/ddw261
62. Stitziel NO, Kathiresan S. Leveraging human genetics to guide drug target discovery. *Trends Cardiovasc Med*. 2017;27:352–359. doi: 10.1016/j.tcm.2016.08.008
63. Zhao Z, Tuakli-Wosornu Y, Lagace TA, Kinch L, Grishin NV, Horton JD, Cohen JC, Hobbs HH. Molecular characterization of loss-of-function mutations in PCSK9 and identification of a compound heterozygote. *Am J Hum Genet*. 2006;79:514–523. doi: 10.1086/507488
64. Hooper AJ, Marais AD, Tanyanyiwa DM, Burnett JR. The C679X mutation in PCSK9 is present and lowers blood cholesterol in a Southern African population. *Atherosclerosis*. 2007;193:445–448. doi: 10.1016/j.atherosclerosis.2006.08.039
65. Susan-Resiga D, Girard E, Kiss RS, Essalmani R, Hamelin J, Asselin MC, Awan Z, Butkinaree C, Fleury A, Soldera A, Dory YL, Baass A, Seidah NG. The proprotein convertase subtilisin/kexin type 9-resistant R410S low density lipoprotein receptor mutation: a novel mechanism causing familial hypercholesterolemia. *J Biol Chem*. 2017;292:1573–1590. doi: 10.1074/jbc.M116.769430
66. Lebeau P, Platko K, Al-Hashimi AA, Byun JH, Lhoták Š, Holzapfel N, Gyulay G, Igdoura SA, Cool DR, Trigatti B, Seidah NG, Austin RC. Loss-of-function PCSK9 mutants evade the unfolded protein response sensor GRP78 and fail to induce endoplasmic reticulum stress when retained. *J Biol Chem*. 2018;293:7329–7343. doi: 10.1074/jbc.RA117.001049
67. Lotta LA, Sharp SJ, Burgess S, et al. Association between low-density lipoprotein cholesterol-lowering genetic variants and risk of type 2 diabetes: a meta-analysis. *JAMA*. 2016;316:1383–1391. doi: 10.1001/jama.2016.14568
68. Schmidt AF, Swerdlow DI, Holmes MV, et al; LifeLines Cohort study group; UCLEB consortium. PCSK9 genetic variants and risk of type 2 diabetes: a mendelian randomisation study. *Lancet Diabetes Endocrinol*. 2017;5:97–105. doi: 10.1016/S2213-8587(16)30396-5
69. Ference BA, Robinson JG, Brook RD, Catapano AL, Chapman MJ, Neff DR, Voros S, Giugliano RP, Davey Smith G, Fazio S, Sabatine MS. Variation in PCSK9 and HMGCR and risk of cardiovascular disease and diabetes. *N Engl J Med*. 2016;375:2144–2153. doi: 10.1056/NEJMoa1604304
70. Preiss D, Sattar N. Statins and the risk of new-onset diabetes: a review of recent evidence. *Curr Opin Lipidol*. 2011;22:460–466. doi: 10.1097/MOL.0b013e32834b4994
71. Cao YX, Liu HH, Dong QT, Li S, Li JJ. Effect of the PCSK9-monoclonal antibodies on new-onset diabetes mellitus and glucose metabolism: a systematic review and meta-analysis. *Diabetes Obes Metab*. 2018;20:1391–1398. doi: 10.1111/dom.13235.
72. Hopewell JC, Malik R, Valdés-Márquez E, Worrall BB, Collins R; METASTROKE Collaboration of the ISGC. Differential effects of PCSK9 variants on risk of coronary disease and ischaemic stroke. *Eur Heart J*. 2018;39:354–359. doi: 10.1093/eurheartj/ehx373
73. Benn M, Nordestgaard BG, Frikke-Schmidt R, Tybjærg-Hansen A. Low LDL cholesterol, PCSK9 and HMGCR genetic variation, and risk of Alzheimer's disease and Parkinson's disease: Mendelian randomisation study. *BMJ*. 2017;357:j1648. doi: 10.1136/bmj.j1648
74. Mefford MT, Rosenson RS, Cushman M, Farkouh ME, McClure LA, Wadley VG, Irvin MR, Bittner V, Safford MM, Somaratne R, Monda KL, Muntner P, Levitan EB. PCSK9 variants, low-density lipoprotein cholesterol, and neurocognitive impairment: Reasons for Geographic and Racial Differences in Stroke Study (REGARDS). *Circulation*. 2018;137:1260–1269. doi: 10.1161/CIRCULATIONAHA.117.029785

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75. Harrison SC, Holmes MV, Burgess S, et al. Genetic association of lipids and lipid drug targets with abdominal aortic aneurysm: a meta-analysis. *JAMA Cardiol*. 2018;3:26–33. doi: 10.1001/jamacardio.2017.4293
76. Rao AS, Lindholm D, Rivas MA, Knowles JW, Montgomery SB, Ingelsson E. Large-scale phenome-wide association study of PCSK9 variants demonstrates protection against ischemic stroke. *Circ Genom Precis Med*. 2018;11:e002162. doi: 10.1161/CIRCGEN.118.002162
77. Mostaza JM, Lahoz C, Salinero-Fort MA, de Dios O, Castillo E, González-Alegre T, García-Iglesias F, Estirado E, Laguna F, Sabín C, López S, Cornejo V, de Burgos C, Sanchez V, Garcés C; Investigators of the SPREDIA-2 Group. R46L polymorphism in the PCSK9 gene: relationship to lipid levels, subclinical vascular disease, and erectile dysfunction. *J Clin Lipidol*. 2018;12:1039.e3–1046.e3. doi: 10.1016/j.jacl.2018.04.004
78. Jerome RN, Pulley JM, Roden DM, Shirey-Rice JK, Bastarache LA, R Bernard G, B Ekstrom L, Lancaster WJ, Denny JC. Using human 'experiments of nature' to predict drug safety issues: an example with PCSK9 inhibitors. *Drug Saf*. 2018;41:303–311. doi: 10.1007/s40264-017-0616-0
79. Walley KR, Thain KR, Russell JA, Reilly MP, Meyer NJ, Ferguson JF, Christie JD, Nakada TA, Fjell CD, Thair SA, Cirstea MS, Boyd JH. PCSK9 is a critical regulator of the innate immune response and septic shock outcome. *Sci Transl Med*. 2014;6:258ra143. doi: 10.1126/scitranslmed.3008782
80. Mitchell KA, Moore J-X, Rosenson RS, Irvin R, Guirgis FW, Shapiro N, Safford M, Wang HE. PCSK9 loss-of-function mutations and risk of infection and sepsis in the Reasons for Geographic And Racial Differences in Stroke (REGARDS) Cohort. *PLoS ONE*. In press.
81. Peters BJ, Pett H, Klungel OH, Stricker BH, Psaty BM, Glazer NL, Wiggins KL, Bis JC, de Boer A, Maitland-van der Zee AH. Genetic variability within the cholesterol lowering pathway and the effectiveness of statins in reducing the risk of MI. *Atherosclerosis*. 2011;217:458–464. doi: 10.1016/j.atherosclerosis.2011.06.023
82. Feng Q, Wei WQ, Chung CP, Levinson RT, Bastarache L, Denny JC, Stein CM. The effect of genetic variation in PCSK9 on the LDL-cholesterol response to statin therapy. *Pharmacogenomics J*. 2017;17:204–208. doi: 10.1038/tpj.2016.3
83. Hopkins PN, Defesche J, Fouchier SW, et al. Characterization of autosomal dominant hypercholesterolemia caused by PCSK9 gain of function mutations and its specific treatment with alirocumab, a PCSK9 monoclonal antibody. *Circ Cardiovasc Genet*. 2015;8:823–831. doi: 10.1161/CIRCGENETICS.115.001129
84. Defesche JC, Stefanutti C, Langslet G, Hopkins PN, Seiz W, Baccara-Dinet MT, Hamon SC, Banerjee P, Kastelein JJP. Efficacy of alirocumab in 1191 patients with a wide spectrum of mutations in genes causative for familial hypercholesterolemia. *J Clin Lipidol*. 2017;11:1338.e7–1346.e7. doi: 10.1016/j.jacl.2017.08.016
85. Raal FJ, Hovingh GK, Blom D, Santos RD, Harada-Shiba M, Bruckert E, Couture P, Soran H, Watts GF, Kurtz C, Honarpour N, Tang L, Kasichayanula S, Wasserman SM, Stein EA. Long-term treatment with evolocumab added to conventional drug therapy, with or without apheresis, in patients with homozygous familial hypercholesterolaemia: an interim subset analysis of the open-label TAUSSIG study. *Lancet Diabetes Endocrinol*. 2017;5:280–290. doi: 10.1016/S2213-8587(17)30044-X
86. Alves AC, Etxebarria A, Medeiros AM, Benito-Vicente A, Thedrez A, Passard M, Croyal M, Martin C, Lambert G, Bourbon M. Characterization of the first PCSK9 gain of function homozygote. *J Am Coll Cardiol*. 2015;66:2152–2154. doi: 10.1016/j.jacc.2015.08.871
87. Toth PP, Descamps O, Genest J, Sattar N, Preiss D, Dent R, Djedjos C, Wu Y, Geller M, Uhart M, Somaratne R, Wasserman SM; PROFICIO Investigators. Pooled safety analysis of evolocumab in over 6000 patients from double-blind and open-label extension studies. *Circulation*. 2017;135:1819–1831. doi: 10.1161/CIRCULATIONAHA.116.025233
88. Giugliano RP, Mach F, Zavitz K, et al; EBBINGHAUS Investigators. Cognitive function in a randomized trial of evolocumab. *N Engl J Med*. 2017;377:633–643. doi: 10.1056/NEJMoa1701131
89. Sabatine MS, De Ferrari GM, Giugliano RP, Huber K, Lewis BS, Ferreira J, Kuder JF, Murphy SA, Wiviott SD, Kurtz CE, Honarpour N, Keech AC, Sever PS, Pedersen TR. Clinical benefit of evolocumab by severity and extent of coronary artery disease: an analysis from FOURIER. *Circulation*. 2018;138:756–766.
90. Pedersen TR, RP Giugliano, PS Sever, AC Keech, M.S. Murphy, MS Sabatine, for the FOURIER Steering Committee & Investigators. Further cardiovascular Outcomes Research with PCSK9. Inhibition in subjects with elevated risk focus on cerebrovascular disease. Paper presented at: ESC; 2017; Barcelona, Spain.
91. Bonaca MP, Nault P, Giugliano RP, Keech AC, Pineda AL, Kanevsky E, Kuder J, Murphy SA, Jukema JW, Lewis BS, Tokgozoglu L, Somaratne R, Sever PS, Pedersen TR, Sabatine MS. Low-density lipoprotein cholesterol lowering with evolocumab and outcomes in patients with peripheral artery disease: insights from the FOURIER Trial (Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk). *Circulation*. 2018;137:338–350. doi: 10.1161/CIRCULATIONAHA.117.032235
92. Giugliano RP, Pedersen TR, Park JG, et al; FOURIER Investigators. Clinical efficacy and safety of achieving very low LDL-cholesterol concentrations with the PCSK9 inhibitor evolocumab: a prespecified secondary analysis of the FOURIER trial. *Lancet*. 2017;390:1962–1971. doi: 10.1016/S0140-6736(17)32290-0
93. Olsson AG, Angelin B, Assmann G, et al. Can LDL cholesterol be too low? Possible risks of extremely low levels. *J Intern Med*. 2017;281:534–553. doi: 10.1111/joim.12614
94. Tuñón J, Bäck M, Badimón L, et al; ESC Working Group on Atherosclerosis and Vascular Biology. Interplay between hypercholesterolaemia and inflammation in atherosclerosis: translating experimental targets into clinical practice. *Eur J Prev Cardiol*. 2018;25:948–955. doi: 10.1177/2047487318773384
95. Koenig W. High-sensitivity C-reactive protein and atherosclerotic disease: from improved risk prediction to risk-guided therapy. *Int J Cardiol*. 2013;168:5126–5134. doi: 10.1016/j.ijcard.2013.07.113
96. Ridker PM, Everett BM, Thuren T, et al; CANTOS Trial Group. Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med*. 2017;377:1119–1131. doi: 10.1056/NEJMoa1707914
97. Bohula EA, Giugliano RP, Leiter LA, Verma S, Park JG, Sever PS, Lira Pineda A, Honarpour N, Wang H, Murphy SA, Keech A, Pedersen TR, Sabatine MS. Inflammatory and cholesterol risk in the FOURIER trial. *Circulation*. 2018;138:131–140. doi: 10.1161/CIRCULATIONAHA.118.034032
98. Pradhan AD, Aday AW, Rose LM, Ridker PM. Residual inflammatory risk on treatment with PCSK9 inhibition and statin therapy. *Circulation*. 2018;138:141–149. doi: 10.1161/CIRCULATIONAHA.118.034645
99. Koenig W. Low-grade inflammation modifies cardiovascular risk even at very low LDL-C levels: are we aiming for a dual target concept? *Circulation*. 2018;138:150–153. doi: 10.1161/CIRCULATIONAHA.118.035107
100. Puri R, Nissen SE, Somaratne R, Cho L, Kastelein JJ, Ballantyne CM, Koenig W, Anderson TJ, Yang J, Kassahun H, Wasserman SM, Scott R, Borgman M, Nicholls SJ. Impact of PCSK9 inhibition on coronary atheroma progression: rationale and design of global assessment of plaque regression with a PCSK9 antibody as measured by intravascular ultrasound (GLAGOV). *Am Heart J*. 2016;176:83–92. doi: 10.1016/j.ahj.2016.01.019
101. Nicholls SJ, Puri R, Anderson T, et al. Effect of evolocumab on progression of coronary disease in statin-treated patients: the GLAGOV randomized clinical trial. *JAMA*. 2016;316:2373–2384. doi: 10.1001/jama.2016.16951
102. Defesche JC, Gidding SS, Harada-Shiba M, Hegele RA, Santos RD, Wierzbicki AS. Familial hypercholesterolaemia. *Nat Rev Dis Primers*. 2017;3:17093. doi: 10.1038/nrdp.2017.93
103. Akioyamen LE, Genest J, Shan SD, Reel RL, Albaum JM, Chu A, Tu JV. Estimating the prevalence of heterozygous familial hypercholesterolaemia: a systematic review and meta-analysis. *BMJ Open*. 2017;7:e016461. doi: 10.1136/bmjopen-2017-016461
104. Cuchel M, Bruckert E, Ginsberg HN, et al; European Atherosclerosis Society Consensus Panel on Familial Hypercholesterolaemia. Homozygous familial hypercholesterolaemia: new insights and guidance for clinicians to improve detection and clinical management. A position paper from the Consensus Panel on Familial Hypercholesterolaemia of the European Atherosclerosis Society. *Eur Heart J*. 2014;35:2146–2157. doi: 10.1093/eurheartj/ehu274
105. Paquette M, Genest J, Baass A. Familial hypercholesterolemia: experience from the French-Canadian population. *Curr Opin Lipidol*. 2018;29:59–64. doi: 10.1097/MOL.0000000000000487
106. Smyth N, Ramsay M, Raal FJ. Population specific genetic heterogeneity of familial hypercholesterolemia in South Africa. *Curr Opin Lipidol*. 2018;29:72–79. doi: 10.1097/MOL.0000000000000488
107. Berberich AJ, Hegele RA. The complex molecular genetics of familial hypercholesterolaemia. *Nat Rev Cardiol*. 2019;16:9–20. doi: 10.1038/s41569-018-0052-6

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108. Iacocca MA, Wang J, Dron JS, Robinson JF, McIntyre AD, Cao H, Hegele RA. Use of next-generation sequencing to detect LDLR gene copy number variation in familial hypercholesterolemia. *J Lipid Res*. 2017;58:2202–2209. doi: 10.1194/jlr.D079301
109. Wang J, Dron JS, Ban MR, et al. Polygenic versus monogenic causes of hypercholesterolemia ascertained clinically. *Arterioscler Thromb Vasc Biol*. 2016;36:2439–2445. doi: 10.1161/ATVBAHA.116.308027
110. Versmissen J, Oosterveer DM, Yazdanpanah M, et al. Efficacy of statins in familial hypercholesterolaemia: a long term cohort study. *BMJ*. 2008;337:a2423.
111. Santos RD, Gidding SS, Hegele RA, et al; International Atherosclerosis Society Severe Familial Hypercholesterolemia Panel. Defining severe familial hypercholesterolaemia and the implications for clinical management: a consensus statement from the International Atherosclerosis Society Severe Familial Hypercholesterolemia Panel. *Lancet Diabetes Endocrinol*. 2016;4:850–861. doi: 10.1016/S2213-8587(16)30041-9
112. Raal F, Scott R, Somaratne R, Bridges I, Li G, Wasserman SM, Stein EA. Low-density lipoprotein cholesterol-lowering effects of AMG 145, a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 serine protease in patients with heterozygous familial hypercholesterolemia: the Reduction of LDL-C with PCSK9 Inhibition in Heterozygous Familial Hypercholesterolemia Disorder (RUTHERFORD) randomized trial. *Circulation*. 2012;126:2408–2417. doi: 10.1161/CIRCULATIONAHA.112.144055
113. Raal FJ, Stein EA, Dufour R, et al; RUTHERFORD-2 Investigators. PCSK9 inhibition with evolocumab (AMG 145) in heterozygous familial hypercholesterolaemia (RUTHERFORD-2): a randomised, double-blind, placebo-controlled trial. *Lancet*. 2015;385:331–340. doi: 10.1016/S0140-6736(14)61399-4
114. Hovingh GK, Raal FJ, Dent R, Stefanutti C, Descamps O, Masana L, Lira A, Bridges I, Coll B, Sullivan D. Long-term safety, tolerability, and efficacy of evolocumab in patients with heterozygous familial hypercholesterolemia. *J Clin Lipidol*. 2017;11:1448–1457. doi: 10.1016/j.jacl.2017.09.003
115. Kastelein JJ, Ginsberg HN, Langslet G, et al. ODYSSEY FH I and FH II: 78 week results with alirocumab treatment in 735 patients with heterozygous familial hypercholesterolaemia. *Eur Heart J*. 2015;36:2996–3003. doi: 10.1093/eurheartj/ehv370
116. Kastelein JJ, Hovingh GK, Langslet G, Baccara-Dinet MT, Gipe DA, Chaudhari U, Zhao J, Minini P, Farnier M. Efficacy and safety of the proprotein convertase subtilisin/kexin type 9 monoclonal antibody alirocumab vs placebo in patients with heterozygous familial hypercholesterolemia. *J Clin Lipidol*. 2017;11:195.e4–203.e4. doi: 10.1016/j.jacl.2016.12.004
117. Ridker PM, Rose LM, Kastelein JJP, Santos RD, Wei C, Revkin J, Yunis C, Tardif JC, Shear CL; Studies of PCSK9 Inhibition and the Reduction of vascular Events (SPIRE) Investigators. Cardiovascular event reduction with PCSK9 inhibition among 1578 patients with familial hypercholesterolemia: results from the SPIRE randomized trials of bococizumab. *J Clin Lipidol*. 2018;12:958–965. doi: 10.1016/j.jacl.2018.03.088
118. Kindt I, Mata P, Knowles JW. The role of registries and genetic databases in familial hypercholesterolemia. *Curr Opin Lipidol*. 2017;28:152–160. doi: 10.1097/MOL.0000000000000398
119. Ng DM, Hooper AJ, Bellgard MI, Burnett JR. The role of patient registries for rare genetic lipid disorders. *Curr Opin Lipidol*. 2018;29:156–162. doi: 10.1097/MOL.0000000000000485
120. Razek O, Cermakova L, Armani H, Lee T, Francis GA, Mancini GBJ, Frohlich J, Brunham LR. Attainment of recommended lipid targets in patients with familial hypercholesterolemia: real-world experience with PCSK9 inhibitors. *Can J Cardiol*. 2018;34:1004–1009. doi: 10.1016/j.cjca.2018.04.014
121. Hartgers ML, Besseling J, Stroes ES, Wittekoek J, Rutten JHW, de Graaf J, Visseren FLJ, Imholz BPM, Roeters van Lennep JE, Huijgen R, Kastelein JJP, Hovingh GK. Achieved LDL cholesterol levels in patients with heterozygous familial hypercholesterolemia: a model that explores the efficacy of conventional and novel lipid-lowering therapy. *J Clin Lipidol*. 2018;12:972.e1–980.e1. doi: 10.1016/j.jacl.2018.04.002
122. Glueck CJ, Brown A, Goldberg AC, McKenney JM, Kantaros L, Stewart J, Elassal J, Koren A. Alirocumab in high-risk patients: observations from the open-label expanded use program. *J Clin Lipidol*. 2018;12:662–668. doi: 10.1016/j.jacl.2018.01.013
123. Andersen L, Davis T, Testa H, Andersen RL. PCSK9 inhibitor therapy in homozygous familial defective apolipoprotein B-100 due to APOB R3500Q: a case report. *J Clin Lipidol*. 2017;11:1471–1474. doi: 10.1016/j.jacl.2017.09.001
124. Raal FJ, Honarpour N, Blom DJ, Hovingh GK, Xu F, Scott R, Wasserman SM, Stein EA; TESLA Investigators. Inhibition of PCSK9 with evolocumab in homozygous familial hypercholesterolaemia (TESLA Part B): a randomised, double-blind, placebo-controlled trial. *Lancet*. 2015;385:341–350. doi: 10.1016/S0140-6736(14)61374-X
125. Hartgers ML, Defesche JC, Langslet G, Hopkins PN, Kastelein JJP, Baccara-Dinet MT, Seiz W, Hamon S, Banerjee P, Stefanutti C. Alirocumab efficacy in patients with double heterozygous, compound heterozygous, or homozygous familial hypercholesterolemia. *J Clin Lipidol*. 2018;12:390.e8–396.e8. doi: 10.1016/j.jacl.2017.12.008
126. Fahy EF, McCarthy E, Steinhagen-Thiessen E, Vaughan CJ. A case of autosomal recessive hypercholesterolemia responsive to proprotein convertase subtilisin/kexin 9 inhibition. *J Clin Lipidol*. 2017;11:287–288. doi: 10.1016/j.jacl.2016.10.002
127. Thedrez A, Blom DJ, Ramin-Mangata S, Blanchard V, Croyal M, Chemello K, Nativel B, Pichelin M, Cariou B, Bourane S, Tang L, Farnier M, Raal FJ, Lambert G. Homozygous familial hypercholesterolemia patients with identical mutations variably express the LDLR (Low-Density Lipoprotein Receptor): implications for the efficacy of evolocumab. *Arterioscler Thromb Vasc Biol*. 2018;38:592–598. doi: 10.1161/ATVBAHA.117.310217
128. Lambert G, Chatelais M, Petrides F, Passard M, Thedrez A, Rye KA, Schwahn U, Gusarova V, Blom DJ, Sasiela W, Marais AD. Normalization of low-density lipoprotein receptor expression in receptor defective homozygous familial hypercholesterolemia by inhibition of PCSK9 with alirocumab. *J Am Coll Cardiol*. 2014;64:2299–2300. doi: 10.1016/j.jacc.2014.07.995
129. Dubé JB, Boffa MB, Hegele RA, Koschinsky ML. Lipoprotein(a): more interesting than ever after 50 years. *Curr Opin Lipidol*. 2012;23:133–140. doi: 10.1097/MOL.0b013e32835111d8
130. Nordestgaard BG, Chapman MJ, Ray K, et al; European Atherosclerosis Society Consensus Panel. Lipoprotein(a) as a cardiovascular risk factor: current status. *Eur Heart J*. 2010;31:2844–2853. doi: 10.1093/eurheartj/ehq386
131. Nordestgaard BG, Langsted A. Lipoprotein (a) as a cause of cardiovascular disease: insights from epidemiology, genetics, and biology. *J Lipid Res*. 2016;57:1953–1975. doi: 10.1194/jlr.R071233
132. Zekavat SM, Ruotsalainen S, Handsaker RE, et al; NHLBI TOPMed Lipids Working Group. Deep coverage whole genome sequences and plasma lipoprotein(a) in individuals of European and African ancestries. *Nat Commun*. 2018;9:2606. doi: 10.1038/s41467-018-04668-w
133. Helgadottir A, Thorleifsson G, Gretarsdottir S, et al. Genome-wide analysis yields new loci associating with aortic valve stenosis. *Nat Commun*. 2018;9:987. doi: 10.1038/s41467-018-03252-6
134. Tsimikas S. A test in context: lipoprotein(a): diagnosis, prognosis, controversies, and emerging therapies. *J Am Coll Cardiol*. 2017;69:692–711. doi: 10.1016/j.jacc.2016.11.042
135. Lanktree MB, Rajakumar C, Brunt JH, Koschinsky ML, Connelly PW, Hegele RA. Determination of lipoprotein(a) kringle repeat number from genomic DNA: copy number variation genotyping using qPCR. *J Lipid Res*. 2009;50:768–772. doi: 10.1194/jlr.D800050-JLR200
136. Lanktree MB, Anand SS, Yusuf S, Hegele RA; SHARE Investigators. Comprehensive analysis of genomic variation in the LPA locus and its relationship to plasma lipoprotein(a) in South Asians, Chinese, and European Caucasians. *Circ Cardiovasc Genet*. 2010;3:39–46. doi: 10.1161/CIRCGENETICS.109.907642
137. Hopewell JC, Haynes R, Baigent C. The role of lipoprotein (a) in chronic kidney disease. *J Lipid Res*. 2018;59:577–585. doi: 10.1194/jlr.R083626
138. Pirillo A, Catapano AL. PCSK9 inhibition and Lp(a) reduction: another piece of the puzzle? *Eur Heart J*. 2018;39:2586–2588. doi: 10.1093/eurheartj/ehy311
139. Stein EA, Raal F. Future directions to establish lipoprotein(a) as a treatment for atherosclerotic cardiovascular disease. *Cardiovasc Drugs Ther*. 2016;30:101–108. doi: 10.1007/s10557-016-6654-5
140. Chan DC, Barrett PH, Watts GF. Recent explanatory trials of the mode of action of drug therapies on lipoprotein metabolism. *Curr Opin Lipidol*. 2016;27:550–556. doi: 10.1097/MOL.0000000000000348
141. Raal FJ, Giugliano RP, Sabatine MS, et al. PCSK9 inhibition-mediated reduction in Lp(a) with evolocumab: an analysis of 10 clinical trials and the LDL receptor's role. *J Lipid Res*. 2016;57:1086–1096. doi: 10.1194/jlr.P065334
142. Gaudet D, Watts GF, Robinson JG, Minini P, Sasiela WJ, Edelberg J, Louie MJ, Raal FJ. Effect of alirocumab on lipoprotein(a) Over ≥1.5 years (from the Phase 3 ODYSSEY Program). *Am J Cardiol*. 2017;119:40–46. doi: 10.1016/j.amjcard.2016.09.010

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143. Stein EA, Honarpour N, Wasserman SM, Xu F, Scott R, Raal FJ. Effect of the proprotein convertase subtilisin/kexin 9 monoclonal antibody, AMG 145, in homozygous familial hypercholesterolemia. *Circulation*. 2013;128:2113–2120. doi: 10.1161/CIRCULATIONAHA.113.004678
144. Romagnuolo R, Scipione CA, Boffa MB, Marcovina SM, Seidah NG, Koschinsky ML. Lipoprotein(a) catabolism is regulated by proprotein convertase subtilisin/kexin type 9 through the low density lipoprotein receptor. *J Biol Chem*. 2015;290:11649–11662. doi: 10.1074/jbc.M114.611988
145. Reyes-Soffer G, Pavlyha M, Ngai C, et al. Effects of PCSK9 inhibition with alirocumab on lipoprotein metabolism in healthy humans. *Circulation*. 2017;135:352–362. doi: 10.1161/CIRCULATIONAHA.116.025253
146. Watts GF, Chan DC, Somaratne R, Wasserman SM, Scott R, Marcovina SM, Barrett PHR. Controlled study of the effect of proprotein convertase subtilisin-kexin type 9 inhibition with evolocumab on lipoprotein(a) particle kinetics. *Eur Heart J*. 2018;39:2577–2585. doi: 10.1093/eurheartj/ehy122
147. Sabatine MS, Giugliano RP, Wiviott SD, Raal FJ, Blom DJ, Robinson J, Ballantyne CM, Somaratne R, Legg J, Wasserman SM, Scott R, Koren MJ, Stein EA; Open-Label Study of Long-Term Evaluation against LDL Cholesterol (OSLER) Investigators. Efficacy and safety of evolocumab in reducing lipids and cardiovascular events. *N Engl J Med*. 2015;372:1500–1509. doi: 10.1056/NEJMoa1500858
148. O'Donoghue ML, Fazio S, Giugliano RP, Stroes ESG, Kanevsky E, Gouni-Berthold I, Im K, Lira Pineda A, Wasserman SM, Češka R, Ezhov MV, Jukema JW, Jensen HK, Tokgözoğlu SL, Mach F, Huber K, Sever PS, Keech AC, Pedersen TR, Sabatine MS. Lipoprotein(a), PCSK9 inhibition and cardiovascular risk: Insights from the FOURIER Trial. *Circulation*. 2018. doi: 10.1161/CIRCULATIONAHA.118.037184.
149. Bittner V, Szarek M, Aylward PE, et al. Lp(a) and cardiovascular outcomes: an analysis from the ODYSSEY OUTCOMES Trial. *Atheroscler Suppl*. 2018;32:24–25 (C3-5.01).
150. Burgess S, Ference BA, Staley JR, et al; European Prospective Investigation Into Cancer and Nutrition–Cardiovascular Disease (EPIC-CVD) Consortium. Association of LPA variants with risk of coronary disease and the implications for lipoprotein(a)-lowering therapies: a Mendelian randomization analysis. *JAMA Cardiol*. 2018;3:619–627. doi: 10.1001/jamacardio.2018.1470
151. Verbeek R, Hoogeveen RM, Langsted A, Stiekema LCA, Verweij SL, Hovingh GK, Wareham NJ, Khaw KT, Boekholdt SM, Nordestgaard BG, Stroes ESG. Cardiovascular disease risk associated with elevated lipoprotein(a) attenuates at low low-density lipoprotein cholesterol levels in a primary prevention setting. *Eur Heart J*. 2018;39:2589–2596. doi: 10.1093/eurheartj/ehy334
152. McConnell JP, Guadagno PA, Dayspring TD, Hoefner DM, Thiselton DL, Warnick GR, Harris WS. Lipoprotein(a) mass: a massively misunderstood metric. *J Clin Lipidol*. 2014;8:550–553. doi: 10.1016/j.jacl.2014.08.003
153. Afshar M, Thanassoulis G. Lipoprotein(a): new insights from modern genomics. *Curr Opin Lipidol*. 2017;28:170–176. doi: 10.1097/MOL.0000000000000392
154. Leiter LA, Tinahones FJ, Karalis DG, Bujas-Bobanovic M, Letierce A, Mandel J, Samuel R, Jones PH. Alirocumab safety in people with and without diabetes mellitus: pooled data from 14 ODYSSEY trials. *Diabet Med*. 2018;35:1742–1751. doi: 10.1111/dme.13817
155. Rosenson RS, Daviglus ML, Reaven P, Pozzilli P, Bays H, Monsalvo ML, Elliott M, Somaratne R, Handelsman Y. Efficacy and safety of evolocumab in patients with type 2 diabetes mellitus and hypercholesterolemia or mixed dyslipidemia. *Diabetes*. 2018;67:suppl 1:A34:128–OR.
156. Su X, Zhang L, Lv J, Wang J, Hou W, Xie X, Zhang H. Effect of statins on kidney disease outcomes: a systematic review and meta-analysis. *Am J Kidney Dis*. 2016;67:881–892. doi: 10.1053/j.ajkd.2016.01.016
157. Baigent C, Landray MJ, Reith C, et al; SHARP Investigators. The effects of lowering LDL cholesterol with simvastatin plus ezetimibe in patients with chronic kidney disease (Study of Heart and Renal Protection): a randomised placebo-controlled trial. *Lancet*. 2011;377:2181–2192. doi: 10.1016/S0140-6736(11)60739-3
158. Charytan DM, Sabatine MS, Pedersen TR, Kyungah I, Peneda L, Wasserman SM, Sever P, Keech AC, Giugliano RP. Efficacy and safety of evolocumab in CKD: Data from the FOURIER trial. Towards better medication usage in patients with CKD. Paper Presented at: American Society of Nephrology Annual Conference: 2018; San Diego, CA.
159. Vachiat A, McCutcheon K, Tsabedze N, Zachariah D, Manga P. HIV and ischemic heart disease. *J Am Coll Cardiol*. 2017;69:73–82. doi: 10.1016/j.jacc.2016.09.979
160. Rosenson RS, Colantonio, Hubbard D, Monda KL, Reading SR, Chen L, Dluzniewski PJ, Burkholder GA, Muntner P. Risk of cardiovascular events among persons living with HIV in the current era. *Circulation*. 2018;138:A14410.
161. Rosenson RS, Colantonio LD, Burkholder GA, Chen L, Muntner P. Trends in utilization of statin therapy and contraindcisted statin use in human immunodeficiency virus-infected adults treated with antiretroviral therapy from 2007 through 2015. *J Am Heart Assoc* 2018;7:e010345. doi: 10.1161/JAHA.118.010345.
162. Burkholder GA, Muntner P, Zhao H, Mugavero MJ, Overton ET, Kilgore M, Drozd DR, Crane HM, Moore RD, Mathews WC, Geng E, Boswell S, Floris-Moore M, Rosenson RS. Low-density lipoprotein cholesterol response after statin initiation among persons living with human immunodeficiency virus. *J Clin Lipidol*. 2018;12:988.e5–998.e5. doi: 10.1016/j.jacl.2018.03.082
163. Gilbert JM, Fitch KV, Grinspoon SK. HIV-related cardiovascular disease, statins, and the REPRIEVE trial. *Top Antivir Med*. 2015;23:146–149.
164. Boccara F, Ghislain M, Meyer L, Goujard C, Le May C, Vigouroux C, Bastard JP, Fellahi S, Capeau J, Cohen A, Cariou B; ANRS-COPANA Study Group. Impact of protease inhibitors on circulating PCSK9 levels in HIV-infected antiretroviral-naive patients from an ongoing prospective cohort. *AIDS*. 2017;31:2367–2376. doi: 10.1097/QAD.0000000000001633
165. Zanni MV, Stone LA, Toribio M, Rimmelin DE, Robinson J, Burdo TH, Williams K, Fitch KV, Lo J, Grinspoon SK. Proprotein convertase subtilisin/kexin 9 levels in relation to systemic immune activation and subclinical coronary plaque in HIV. *Open Forum Infect Dis*. 2017;4:ofx227. doi: 10.1093/ofid/ofx227
166. Kuniholm MH, Liang H, Anastos K, Gustafson D, Kassaye S, Nowicki M, Sha BE, Pawlowski EJ, Gange SJ, Aouizerat BE, Pushkarsky T, Bukrinsky MI, Prasad VR. Association of a 3' untranslated region polymorphism in proprotein convertase subtilisin/kexin type 9 with HIV viral load and CD4+ levels in HIV/hepatitis C virus coinfected women. *AIDS*. 2017;31:2483–2492. doi: 10.1097/QAD.0000000000001648
167. Rosenson RS, Baker S, Banach M, et al. Optimizing cholesterol treatment in patients with muscle complaints. *J Am Coll Cardiol*. 2017;70:1290–1301. doi: 10.1016/j.jacc.2017.07.752
168. Tanner RM, Safford MM, Monda KL, Taylor B, O'Beirne R, Morris M, Colantonio LD, Dent R, Muntner P, Rosenson RS. Primary care physician perspectives on barriers to statin treatment. *Cardiovasc Drugs Ther*. 2017;31:303–309. doi: 10.1007/s10557-017-6738-x
169. Zhang H, Plutzky J, Shubina M, Turchin A. Continued statin prescriptions after adverse reactions and patient outcomes: a cohort study. *Ann Intern Med*. 2017;167:221–227. doi: 10.7326/M16-0838
170. Mefford MT, Tajeu GS, Tanner RM, Colantonio LD, Monda KL, Dent R, Farkouh ME, Rosenson RS, Safford MM, Muntner P. Willingness to be reinitiated on a statin (from the REasons for Geographic and Racial Differences in Stroke Study). *Am J Cardiol*. 2018;122:768–774. doi: 10.1016/j.amjcard.2018.05.016
171. Colantonio LD, Deng L, Chen L, Farkouh ME, Monda KL, Harrison DJ, Maya JF, Kilgore ML, Muntner P, Rosenson RS. Medical expenditures among Medicare beneficiaries with statin-associated adverse effects following myocardial infarction. *Cardiovasc Drugs Ther*. 2018;32:601–610. doi: 10.1007/s10557-018-6840-8
172. Nissen SE, Stroes E, Dent-Acosta RE, et al; GAUSS-3 Investigators. Efficacy and tolerability of evolocumab vs ezetimibe in patients with muscle-related statin intolerance: the GAUSS-3 randomized clinical trial. *JAMA*. 2016;315:1580–1590. doi: 10.1001/jama.2016.3608
173. Moriarty PM, Jacobson TA, Bruckert E, Thompson PD, Guyton JR, Baccara-Dinet MT, Gipe D. Efficacy and safety of alirocumab, a monoclonal antibody to PCSK9, in statin-intolerant patients: design and rationale of ODYSSEY ALTERNATIVE, a randomized phase 3 trial. *J Clin Lipidol*. 2014;8:554–561. doi: 10.1016/j.jacl.2014.09.007
174. Cannon CP, de Lemos J, Rosenson R, Ballantyne C, Boatman B, Yedigarova L, Mues K, Hsu H-C, Bhatt D, Kosiborod M, on behalf of thre GOULD investigators. Getting to an improved understanding of low-density lipoprotein cholesterol and dyslipidemia management (GOULD): A registry of high cardiovascular risk patients in the United States. Paper Presented at: American College of Cardiology Annual Scientific Sessions; March 2018. *J Am Coll Cardiol*. 2018;71:A1767.
175. Cho L, Dent R, Stroes ESG, Stein EA, Sullivan D, Ruzza A, Flower A, Somaratne R, Rosenson RS. Persistent safety and efficacy of evolocumab in patients with statin intolerance: a subset analysis of the OSLER open-label extension studies. *Cardiovasc Drugs Ther*. 2018;32:365–372. doi: 10.1007/s10557-018-6817-7

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176. Anderson JL, Heidenreich PA, Barnett PG, Creager MA, Fonarow GC, Gibbons RJ, Halperin JL, Hlatky MA, Jacobs AK, Mark DB, Masoudi FA, Peterson ED, Shaw LJ. ACC/AHA statement on cost/value methodology in clinical practice guidelines and performance measures: a report of the American College of Cardiology/American Heart Association Task Force on Performance Measures and Task Force on Practice Guidelines. *J Am Coll Cardiol*. 2014;63:2304–2322. doi: 10.1016/j.jacc.2014.03.016
177. Institute for Clinical and Economic Review Posts Updated Economic Analyses for PCSK9 Inhibition Evolocumab, Finds Less Favorable Cost-Effectiveness. https://icer-review.org/announcements/pcsk9-neu/. Accessed January 15, 2019.
178. Fonarow GC, Keech AC, Pedersen TR, Giugliano RP, Sever PS, Lindgren P, van Hout B, Villa G, Qian Y, Somaratne R, Sabatine MS. Cost-effectiveness of evolocumab therapy for reducing cardiovascular events in patients with atherosclerotic cardiovascular disease. *JAMA Cardiol*. 2017;2:1069–1078. doi: 10.1001/jamacardio.2017.2762
179. Institute for Clinical and Economic Review Updates Value-Based Price Benchmark for Alirocumab, a PCSK9 Inhibitor for Treatment of High Cholesterol. https://icer-review.org/announcements/alirocumab-prelim-evidence-update/. Accessed January 15, 2019.
180. Kazi DS, Moran AE, Coxson PG, Penko J, Ollendorf DA, Pearson SD, Tice JA, Guzman D, Bibbins-Domingo K. Cost-effectiveness of PCSK9 inhibitor therapy in patients with heterozygous familial hypercholesterolemia or atherosclerotic cardiovascular disease. *JAMA*. 2016;316:743–753. doi: 10.1001/jama.2016.11004
181. Robinson JG, Rosenson RS, Farnier M, Chaudhari U, Sasiela WJ, Merlet L, Miller K, Kastelein JJ. Safety of very low low-density lipoprotein cholesterol levels with alirocumab: pooled data from randomized trials. *J Am Coll Cardiol*. 2017;69:471–482. doi: 10.1016/j.jacc.2016.11.037
182. Robinson JG, Farnier M, Krempf M, et al; ODYSSEY LONG TERM Investigators. Efficacy and safety of alirocumab in reducing lipids and cardiovascular events. *N Engl J Med*. 2015;372:1489–1499. doi: 10.1056/NEJMoa1501031
183. Blom DJ, Djedjos CS, Monsalvo ML, Bridges I, Wasserman SM, Scott R, Roth E. Effects of evolocumab on vitamin E and steroid hormone levels: results from the 52-week, phase 3, double-blind, randomized, placebo-controlled DESCARTES study. *Circ Res*. 2015;117:731–741. doi: 10.1161/CIRCRESAHA.115.307071
184. Ray KK, Landmesser U, Leiter LA, Kallend D, Dufour R, Karakas M, Hall TY, Troquay RP, Turner T, Visseren FL, Wijngaard P, Wright RS, Kastelein JJ. Iclisiran in patients at high cardiovascular risk with elevated LDL cholesterol. *N Engl J Med*. 2017;376:1430–1440.
185. Weider E, Susan-Resiga D, Essalmani R, Hamelin J, Asselin MC, Nimesh S, Ashraf Y, Wycoff KL, Zhang J, Prat A, Seidah NG. Proprotein convertase subtilisin/Kexin type 9 (PCSK9) single domain antibodies are potent inhibitors of low density lipoprotein receptor degradation. *J Biol Chem*. 2016;291:26586. doi: 10.1074/jbc.A116.717736
186. Chackerian B, Remaley A. Vaccine strategies for lowering LDL by immunization against proprotein convertase subtilisin/kexin type 9. *Curr Opin Lipidol*. 2016;27:345–350. doi: 10.1097/MOL.0000000000000312
187. Chadwick AC, Wang X, Musunuru K. In vivo base editing of PCSK9 (Proprotein Convertase Subtilisin/Kexin Type 9) as a therapeutic alternative to genome editing. *Arterioscler Thromb Vasc Biol*. 2017;37:1741–1747. doi: 10.1161/ATVBAHA.117.309881

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