Published in final edited form as:

Curr Atheroscler Rep. 2015 April ; 17(4): 499. doi:10.1007/s11883-015-0499-4.

Targeting PCSK9 for therapeutic gains

Michael D. Shapiro, Sergio Fazio, and Hagai Tavori

The Knight Cardiovascular Institute, Center for Preventive Cardiology, Oregon Health and Science University, Portland, Oregon, USA

Abstract

Even though it is only a little over a decade from the discovery of proprotein convertase subtilisin/kexin type 9 (PCSK9) as a plasma protein that associates with both hypercholesterolemia and low cholesterol syndromes, a rich literature has developed describing its unique physiology and the impact of antagonism of this molecule on cholesterol metabolism for therapeutic purposes. Indeed, the PCSK9 story is unfolding rapidly, with many answers and more questions. This review summarizes the most recent data from phase II/III clinical trials of PCSK9 inhibition with the three leading antibodies, highlights the clinical significance of the ongoing studies, and suggests future areas of investigation based on recent basic science discoveries on the physiology of PCSK9.

Introduction

Accounts of the initial discovery and characterization of proprotein convertase subtilisin/kexin type 9 (PCSK9) have been well described [1]. The seminal discovery in 2003 by Abifadel *et al.* linked mutations in the gene encoding PCSK9 with autosomal dominant hypercholesterolemia (ADH) [2]. This finding uncovered a key new player in cholesterol homeostasis and set into motion intensive research in the field of PCSK9 biology. The basic construct places this extracellular secretory protein as a central regulator of plasma low-density lipoprotein (LDL)-cholesterol (LDL-C) concentration. PCSK9, by direct interaction with the LDL receptor (LDLR), enhances degradation of the hepatic LDLR by targeting it for destruction in the lysosome [3]. The fundamental observation that PCSK9 is intrinsically linked to LDLR recycling and familial hypercholesterolemia (FH) provided the basis for pursuing it as a therapeutic target. If PCSK9 causes the degradation of LDLR, then inhibiting PCSK9 would mean prolonging the lifespan of LDLR, which would lead to drastic reductions in plasma LDL-C levels. Major findings over the last decade have revealed the following:

- a. Gain-of-function mutations in PCSK9 are a cause of ADH [2]
- b. Loss-of-function mutations in PCSK9 are associated with low LDL-C levels and markedly reduced cardiovascular risk [4–7].

- c. Therapeutic antagonism of PCSK9 reduces LDL-C levels and, within the context of clinical trials, appears safe, efficacious, and well tolerated. [8]

In addition to these well-established findings, new lines of investigation have emerged, including effects of PCSK9 on the metabolism of intestinal apolipoprotein B (apoB)-containing triglyceride-rich lipoprotein (TRL) [9–11] a still undefined relationship between PCSK9 and lipoprotein(a) [Lp(a)] [12, 13] and potential efficacy of anti-PCSK9 treatment even in homozygous FH (HoFH) [14, 15]

Clinical Trials with PCSK9 inhibitors

The developing PCSK9 story is a striking example of how experiments of nature (human genetics) hold great potential to prioritize molecular targets and inform drug development. Numerous strategies have and are being attempted for therapeutic antagonism of PCSK9, including humanized monoclonal antibodies (mAbs), gene silencing, and use of small inhibitory peptides. Phase I/II clinical trial data with mAbs have been extensively reviewed. Interested readers are referred to a recent comprehensive appraisal of these trials [8]. A phase I trial using RNA inhibition (RNAi) technology was also recently reviewed [16] and will not be discussed in detail here. The purpose of this review is to summarize the findings of recent phase II/III clinical trials with humanized mAbs directed against PCSK9 (alirocumab, evolocumab – phase III; bococizumab – phase II).

Alirocumab

Alirocumab (SAR236553/REGN727 - Sanofi/Regeneron) is being evaluated in the ODYSSEY clinical program in over 23,000 participants in 12 phase III trials. With the exception of ODYSSEY CHOICE I and ODYSSEY OUTCOMES, all ODYSSEY trials are fully enrolled. ODYSSEY OUTCOMES is the large phase III cardiovascular outcome trial testing alirocumab plus statin vs. statin alone in subjects with recent acute coronary syndromes to determine its impact on major adverse cardiovascular events [17].

ODYSSEY MONO was the first phase III clinical trial testing the LDL-C lowering efficacy of alirocumab compared to ezetimibe at 24 weeks of treatment [18]. A total of 103 subjects with hypercholesterolemia and moderate atherosclerotic cardiovascular disease (ASCVD) risk were enrolled (with 88 subjects completing the 24 week treatment period). LDL-C was reduced by 47% with alirocumab vs 16% with ezetimibe ($p < 0.0001$). There was no significant difference in adverse event rates between the two groups.

Despite potent statin therapy, and the use of additional lipid-lowering therapy (LLT), most patients with FH fail to achieve LDL-C targets. The ODYSSEY FH I and FH II studies assessed the efficacy and safety of alirocumab in patients with heterozygous FH (HeFH) who were inadequately controlled on their current statin and other LLT [19]. Subjects with HeFH on maximum tolerated statin therapy were randomized to alirocumab 75 mg subcutaneous (SC) injection (up-titration possible to 150 mg) every 2 weeks (Q2W) ($n = 490$) versus placebo SC injection Q2W ($n = 245$). Overall, 735 patients were randomized (median age 52 years, mean LDL-C 145 mg/dL). In ODYSSEY FH I, alirocumab at 24 weeks reduced LDL-C by 49% (vs. 9% with placebo; $p < 0.0001$). The dose of alirocumab was uptitrated in 43.4% of participants. In ODYSSEY FH II, LDL-C at 24 weeks was

reduced by 49% with alirocumab vs. 3% with placebo ($p<0.0001$). In both studies, the LDL-C reduction was maintained for all the 52 weeks of the study. Treatment with alirocumab improved LDL-C goal attainment (72% vs. 2% in FH I, and 81% vs. 11% in FH II; both $p<0.0001$). The proportion of FH patients reaching these LDL-C goals is unprecedented. Furthermore, alirocumab appeared to be generally well tolerated and associated with a low rate of only innocuous adverse events.

The ODYSSEY HIGH FH study assessed the efficacy and safety of alirocumab in patients with severe HeFH [19, 20]. ODYSSEY High FH included 107 patients (half with prior history of coronary heart disease) with baseline LDL-C 160 mg/dL (mean: 200 mg/dL) despite maximally tolerated LLT (80% on high-dose statin therapy, 27% on ezetimibe). At week 24, there was a 46% reduction in LDL-C levels with alirocumab (versus 7% with placebo; $p<0.0001$). The LDL-C lowering response was maintained over 52 weeks. Importantly, 57% of participants on alirocumab (vs. 11% on placebo, $p<0.0001$) achieved an LDL-C level <100 mg/dL and 32% (vs. 3% on placebo, $p=0.0082$) achieved an LDL-C level <70 mg/dL. Again, alirocumab was well tolerated, with a similar adverse event rate compared with placebo. This study demonstrated that treatment with alirocumab on top of intensive LLT significantly increased the proportion of severe FH patients who achieved adequate LDL-C lowering.

The ODYSSEY LONG TERM study assessed the tolerability and efficacy of alirocumab vs. placebo in patients ($n=2,341$) at high risk for ASCVD [21]. Subjects were on maximally tolerated statin therapy with or without other LLT and had LDL-C >70 mg/dL at entry. All patients were randomized to double-blind treatment with alirocumab (150 mg Q2W, $n=1553$) or placebo ($n=788$) Q2W for up to 78 weeks. At 52 weeks, alirocumab resulted in a 61% reduction in LDL-C compared with placebo (from 123 to 53 mg/dL). Overall, 81% of patients achieved LDL-C goal. There was no signal for harm with alirocumab. A post-hoc analysis suggested a 54% reduction in major cardiovascular events over the 65 weeks (hazard ratio 0.46, 95% CI 0.26 – 0.82, $p<0.01$). A recent sub-analysis of this study was reported [22] which compared the efficacy by subgroup and safety when LDL-C <25 mg/dL. The LDL-C lowering response was consistent across all subgroups, irrespective of diagnosis (FH versus non-FH), gender, baseline LDL-C, age, body mass index, or presence of moderate chronic kidney disease, diabetes, or elevated triglycerides (TG). Over the study period, alirocumab was well tolerated. Importantly, subjects achieving an LDL-C <25 mg/dL did not experience a greater number of treatment-emergent adverse events.

ODYSSEY ALTERNATIVE aimed to evaluate the efficacy and safety of alirocumab vs. ezetimibe in patients with statin intolerance [23, 24]. Large, well-controlled randomized trials of cholesterol-lowering drugs in statin intolerant patients are lacking [25]. In order to qualify for this trial, participants had to have a history of intolerance to at least 2 statins (including one at the lowest dose). Perhaps surprising was the fact that 47 out of the 361 subjects dropped out during the placebo run in phase, 25 due to muscle related side effects. The remaining 314 subjects were randomized to either alirocumab 75/150 mg SC Q2W plus placebo orally, every day (po qd), ezetimibe 10 mg po qd plus placebo SC Q2W, or atorvastatin 20 mg po qd plus placebo SC Q2W. Remarkably, those subjects who were challenged with atorvastatin 20 mg po qd had a comparable rate of side effects to those in

the alirocumab and ezetimibe arms. The majority (60%) of “statin intolerant” subjects were actually able to tolerate atorvastatin. Importantly, all patients received alirocumab during the open label phase of the trial and the incidence of myalgias was significantly less common than in the double-blind treatment phase (4% vs 25%, respectively). The findings of this study suggest that alirocumab may help remedy an unmet clinical need; significant LDL-C lowering in patients intolerant to statin therapy.

ODYSSEY OPTIONS I and II evaluated the efficacy and safety of alirocumab as add-on therapy to either atorvastatin (20–40 mg, OPTIONS I) or rosuvastatin (10–20 mg, OPTIONS II) compared with either: 1) addition of ezetimibe 10 mg; 2) doubling the dose of statin; or 3) switching from atorvastatin 40 mg to rosuvastatin 40 mg (OPTIONS I only) in patients at high ASCVD risk [26, 27]. In OPTIONS I, 345 subjects were on atorvastatin 20–40 mg at baseline. The addition of alirocumab decreased LDL-C levels (44% in subjects on atorvastatin 20 mg and 54% in subjects on atorvastatin 40 mg at baseline), compared with either ezetimibe (21% and 23%), doubling the statin dose (5.0% and 4.8%) or switching from atorvastatin 40 mg to rosuvastatin 40 mg (21%) ($p=0.01$). In OPTIONS II, alirocumab significantly reduced LDL-C levels (51% in patients on rosuvastatin 10 mg and 36% in patients on rosuvastatin 20 mg) compared with adding ezetimibe (14% and 11.0%) ($p=0.0125$). The addition of alirocumab did not result in significantly greater LDL-C reduction than doubling rosuvastatin for patients on rosuvastatin 20 mg at baseline. The majority (60%) of subjects who received alirocumab achieved an LDL-C level <70 mg/dL at 24 weeks (vs. 43–44% of subjects who received ezetimibe and 30–31% of subjects who doubled the dose of rosuvastatin). Adverse event rates were similar across the treatment groups in each study. Once again, these studies demonstrate the potential value of a mAb targeted to PCSK9 in patients at high ASCVD risk who do not achieve adequate LDL-C reduction despite standard LLT.

ODYSSEY COMBO I and II evaluated the efficacy and safety of alirocumab on top of maximally tolerated daily statin, with or without other LLT in 966 subjects at high ASCVD risk [28]. ODYSSEY COMBO I included 316 high risk subjects (78% with a history of coronary heart disease and with median baseline LDL-C of 97 mg/dl) who were randomized to receive either alirocumab ($n=209$) 75–150 mg Q2W or placebo ($n=107$) for 52 weeks [29]. LDL-C was reduced by 48% (mean 51 mg/dL) with alirocumab vs. 2% (mean 98 mg/dL) with placebo at 24 weeks. This LDL-C lowering was maintained at 52 weeks (LDL-C reduced by 42%). Overall, 75% of patients on alirocumab versus 9% on placebo achieved the LDL-C goal of <70 mg/dL at week 24 ($p<0.0001$). ODYSSEY COMBO II enrolled 720 high-risk subjects who had residually elevated LDL-C despite maximally tolerated statin therapy. Patients were randomized to alirocumab 75–150 mg SC Q2W ($n=479$) or ezetimibe 10 mg po qd ($n=241$) for 104 weeks [30]. At week 24, there was a 51% reduction in LDL-C levels (30% reduction versus ezetimibe). On alirocumab, 77% of patients achieved an LDL-C goal of <70 mg/dl compared with 46% on ezetimibe (60% of alirocumab treated patients attained an LDL-C <50 mg/dL). Alirocumab was well tolerated with a similar adverse event rate as for placebo in both COMBO trials.

Evolocumab

Evolocumab (AMG 145 - Amgen) is being studied as part of the PROFICIO program in phase III clinical trials in over 28,000 participants. FOURIER is the large cardiovascular outcome trial that will assess whether the addition of evolocumab to a statin will reduce cardiovascular events more than statin alone in 22,500 subjects with ASCVD.

MENDEL-2 trial assessed the efficacy and safety of evolocumab monotherapy in a low-risk, treatment-naïve population (n=615) with hypercholesterolemia (mean LDL-C 140 mg/dL) [31]. Treatment with evolocumab lowered LDL-C by 57% with the Q2W regimen (vs. 18% with ezetimibe) and by 56% with the every 4 week (Q4W) regimen (vs. 19% with ezetimibe). Both twice monthly and once monthly treatment with evolocumab resulted in achieving an LDL-C <70 mg/dl in 69–72% of subjects (vs. 2% and 1% of the corresponding ezetimibe groups). There was no difference in adverse events, including muscle symptoms, amongst evolocumab, placebo, and ezetimibe-treated patients.

DESCARTES was a randomized, double-blind, placebo-controlled, multicenter phase 3 trial which compared evolocumab with placebo in patients with LDL-C ≥75 mg/dL [32]. Patients were randomized (2:1) to evolocumab (420 mg) or placebo Q4W. In total, 800 (88%) subjects completed 52 weeks of treatment. At 52 weeks, evolocumab treatment resulted in an overall 57% least-squares mean reduction in LDL-C versus placebo. Additionally, 82% of subjects achieved an LDL-C <70 mg/dL. Adverse event rates, including myalgias, were similar those treated with evolocumab and placebo.

The recent ACC/AHA guidelines have deemphasized LDL-C goals and have shifted the focus to the intensity of statin therapy for lowering ASCVD risk. With this in mind, the LAPLACE-2, a randomized, double-blind phase III trial, assessed the efficacy of evolocumab in patients with hypercholesterolemia randomly assigned to high- or moderate-intensity statin therapy [33]. Participants (n=1,899) were allocated to one of five statin regimens: high-intensity statins (atorvastatin 80 mg or rosuvastatin 40 mg) or moderate intensity statins (simvastatin 40 mg, atorvastatin 10 mg, or rosuvastatin 5 mg). Eligible subjects were then randomly assigned to evolocumab and placebo (n=1117), placebo and ezetimibe (n=221), or placebo only (n=558), on top of statin therapy. All evolocumab-treated groups demonstrated highly significant reductions in LDL-C versus placebo (63–75% for evolocumab vs. 19–32% for ezetimibe). Among the high-intensity statin groups, 93–95% of patients treated with evolocumab achieved an LDL-C <70 mg/dL. In other words, LAPLACE-2 demonstrated that the addition of evolocumab on top of statin therapy resulted in substantial and consistent LDL-C lowering irrespective of statin type or dose. Evolocumab was well tolerated with adverse event rates similar to those in placebo and ezetimibe-treated groups.

The GAUSS-2 study was a follow-up to the GAUSS trial, which evaluated the efficacy of different dose regimens of evolocumab in patients intolerant to statins [34]. GAUSS-2 included patients who were intolerant to at least 2 statins (50% of subjects were intolerant to 3 statins and 22% to 4 or more statins). In this trial, subjects were randomized to evolocumab (n=205) or ezetimibe (n=102). Not surprisingly, those treated with evolocumab received significantly greater LDL-C lowering, as compared to those who received ezetimibe

(mean percent LDL-C reductions were 56% with evolocumab vs. 18% with ezetimibe). Evolocumab was well tolerated and there was actually a lower incidence of myalgia with evolocumab than with ezetimibe (16 [8%] versus 18 [18%]). This finding is consistent with the idea that statin-induced myalgias are not related to low plasma LDL-C levels.

In RUTHERFORD-2, 331 HeFH patients with elevated LDL-C (> 100 mg/dL) despite statin $\pm$ ezetimibe were randomized in a 2:1 fashion to evolocumab 140 mg 2-weekly or matching placebo, or evolocumab 420 mg monthly or matching placebo for 12 weeks [35]. At 12 weeks, both dosing regimens resulted in similar and profound LDL-C lowering (-61% for Q2W regimen and -56% for Q4W regimen). The findings from RUTHERFORD-2 enhance the existing clinical trial results regarding the ability of PCSK9 inhibitors to effectively reduce LDL-C in HeFH patients with excellent tolerability.

TESLA Part B was a randomized, double-blind, placebo-controlled trial of evolocumab in 50 patients with HoFH (mean LDL-C at baseline 351 mg/dL) [14]. All subjects in this trial were on statins at baseline and 91% also received ezetimibe. The vast majority of participants (45 subjects, 92% of total) had identifiable LDL receptor mutations with 22 (45%) subjects being true homozygotes and 23 (47%) subjects being compound heterozygotes. Evolocumab lowered LDL-C by 94 mg/dL (31%) versus placebo. Not surprisingly, there was no significant response to treatment with evolocumab in 1 subject with 2 null LDLR mutations [10% reduction] and 1 subject with autosomal recessive hypercholesterolemia [4% reduction]. In addition, participants with defective mutations in one or both LDLR alleles responded better to treatment (mean LDL-C reduction 41%) compared with those with at least one null mutation (mean LDL-C reduction 21%). Despite these relatively positive results, the mean LDL-C level at the end of the trials was a very high 273 mg/dL. Whether combination therapy with a PCSK9 inhibitor in conjunction with other novel agent(s) and/or apheresis for patients with HoFH is safe and effective remains to be determined. Importantly, evolocumab was well tolerated with no adverse safety signal.

Bococizumab

Bococizumab (RN316 - Pfizer) is a humanized mAb directed against PCSK9. It has been investigated in several phase I and II clinical trials [36]. The SPIRE program consists of five ongoing phase III trials, including SPIRE-HF (HeFH), SPIRE-HR and SPIRE-LDL (hypercholesterolemia, mixed dyslipidemia), and SPIRE-1 and SPIRE-2 (large outcomes trials in patients at high risk for ASCVD events).

Although a full primary article reporting the effects of treatment with bococizumab has not yet been published, several abstracts have been presented. Gumbiner et al. reported on the results of a phase II randomized, placebo-controlled trial evaluating the efficacy and safety of bococizumab in subjects with residual hypercholesterolemia despite high-dose statin therapy [37]. Twelve weeks of treatment with bococizumab lowered LDL-C by 56% as compared with a 4% reduction in the placebo group. Bococizumab may possibly be more potent at LDL-C lowering compared to the other agents as dosing was interrupted at week 4 due to LDL-C levels dropping below 25 mg/dL in several patients in this trial. The study drug was well tolerated and no bococizumab-related adverse events were reported.

Ballantyne et al recently reported on the efficacy and safety of bococizumab from a randomized, placebo-controlled, dose ranging study [38]. Statin-treated subjects with an LDL-C 80 mg/dL were randomized to Q2W SC placebo, bococizumab 50 mg, 100 mg, or 150 mg; or Q4W placebo, bococizumab 200 mg or 300 mg. Bococizumab dosing was reduced if LDL-C dropped below 25 mg/dL. Bococizumab 150 mg Q2W lowered LDL-C by 53 points vs. placebo, and significantly reduced LDL-C across all doses. Up to 44% of subjects in the bococizumab groups had their dose reduced. There was no difference in the incidence of adverse events in any of the bococizumab groups and placebo.

In summary, the current evidence base for alirocumab, evolocumab, and bococizumab is more than promising. Interested readers are referred to a recent comprehensive summary tables of all completed Phase I and Phase II trials recently published by Ballantyne et al [38]. PCSK9 inhibitors by themselves reduce LDL-C by ~60% across a variety of patient subgroups, including those with residual hypercholesterolemia despite maximally tolerated statin therapy, statin intolerance, or genetic hypercholesterolemia. Additionally, patients with receptor defective HoFH treated with PCSK9 inhibitors can expect a ~30% reduction in LDL-C. These profound reductions in LDL-C are associated with excellent short-term safety and tolerability, and no signals in terms of myalgias or elevations in transaminases or creatine kinase levels. Two key outstanding clinical questions remain: 1) does the magnitude of LDL-C lowering seen with an inhibitor of PCSK9 on top of statin therapy translate to improved clinical outcomes; and 2) are these agents safe in the long-term? Results are awaited from the ongoing ODYSSEY OUTCOMES, FOURIER, and SPIRE trials to address both of these issues. Summary of all phase III trial with mAb against PCSK9 is shown in Table 1.

PCSK9 inhibitor effects on other lipid fractions

PCSK9 inhibitors have been developed for the purpose of blocking LDLR degradation and with the aim to lower plasma LDL-C levels. However, effects on non-LDL lipid fractions have been observed as well. In the clinical trials, apoB levels were reduced by 48–59%, TG levels by as much as 34%, and HDL-C levels rose by 2–13% [39–47]. Perhaps, the most significant non-LDL effect of PCSK9 inhibitors is the lowering of Lp(a) levels, as there are limited therapeutic options to lower this atherogenic lipoprotein. Although niacin has been traditionally used to address high Lp(a) levels, it is now unclear if niacin has an impact on cardiovascular events in subjects taking a statin [48–50]. Two pooled analyses have evaluated the magnitude of Lp(a) lowering with alirocumab and evolocumab [12, 13], and these are discussed in detail below. The mechanism by which PCSK9 inhibition affects lipoprotein not dependent on LDLR removal is not understood.

Emerging Issues

In addition to data from clinical trials, several new lines of investigation have emerged to suggest additional roles for PCSK9 in intestinal and hepatic TRL metabolism, Lp(a) biology, and in the absence of LDLR.

PCSK9 appears to target receptors other than LDLR, including the very low density lipoprotein receptor (VLDLR) and CD36, resulting in their degradation [51]. In vivo studies

revealed that PCSK9 deficiency was associated with a 2-fold decrease in postprandial TG levels, suggesting improved TG clearance and a role for PCSK9 in TG metabolism possibly via its ability to enhance the degradation of VLDLR and CD36. Indeed, PCSK9 secreted by the liver affects VLDLR levels in adipocytes [52]. Lack of PCSK9 increases surface level of VLDLR, which facilitates free fatty acid uptake in visceral adipocytes. Consequently, PCSK9-deficient mice demonstrate 80% more visceral adipose tissue as compared to wild-type mice visceral fat accumulation, but do not develop liver steatosis or obesity. Thus PCSK9 inhibitors are not anticipated to cause adverse effects on liver and adipose tissue metabolism [53]. Other recent pre-clinical studies suggest a direct role for PCSK9 in the regulation of intestinal TRL secretion and apoB48 production [9–11, 54]. Taken together, these results justify the design of clinical investigations to address the effect of PCSK9 mAbs in the context of post-prandial hypertriglyceridemia, an important additional therapeutic target.

With regard to Lp(a) levels, Gaudet et al, assessed the impact of alirocumab 150 mg Q2W in pooled data from 3 double-blind, randomized, placebo-controlled, phase II trials [13]. Alirocumab resulted in a significant reduction in Lp(a) from baseline compared with placebo (–30.3% vs. –0.3%, $p<0.0001$). Median percentage Lp(a) reductions in the alirocumab group were of similar magnitude across a range of baseline Lp(a) levels, resulting in greater absolute reductions in Lp(a) in patients with higher baseline levels. Similarly, Raal et al, performed a pooled analysis of 1,359 patients in 4 phase II trials with evolocumab to assess the effects on Lp(a) levels. Evolocumab treatment for 12 weeks resulted in significant ($p<0.001$) mean dose-related reductions in Lp(a) compared to control: 29.5% (23.3% – 35.7%) and 24.5% (20.4% – 28.7%) with 140 mg and 420 mg, dosed every 2 and 4 weeks, respectively, with no plateau of effect [12]. Given the consistency and similar magnitude of reduction of Lp(a) lowering effect, this finding is likely to be a class effect. The underlying mechanism of Lp(a) reduction is incompletely understood, since Lp(a) is assembled extracellularly and is not cleared by LDLR, and remains a major unanswered question in the field. Interestingly, reduction of Lp(a) was observed in all clinical trial using mAbs against PCSK9 but not in the single trial using RNAi (which reduces production of PCSK9), suggesting that this effect is specific to the inhibition of circulating PCSK9 action or to the binding of antibodies to the PCSK9 molecule [55]. Since PCSK9 physically interacts with LDL (but not with VLDL) particles [56–58], it can be assumed that PCSK9 also interacts with Lp(a). Thus, the injection of anti-PCSK9 mAbs may lead to the formation of immune complexes (mAb-PCSK9-Lp(a) and mAb-PCSK9-LDL) that can be cleared either via target-specific pathways or by the reticulo-endothelial system. A schematic illustration of this proposed mechanism for antibody-mediated lipoprotein clearance is shown in Figure 1. Clearance of these immune complexes may explain that effect of the mAb on Lp(a) reduction. Alternatively, the reduction in Lp(a) levels may be mediated by reduced apoB synthesis, as was recently shown in clinical data using the apoB synthesis inhibitor mipomersen [59]. Regardless, reduction of elevated Lp(a) levels in high-risk individuals represents an unmet clinical need and this application of PCSK9 inhibitors needs to be addressed mechanistically in human studies.

With currently available medications, the majority of patients with FH cannot adequately lower plasma LDL-C. This is a forgone conclusion particularly in those with HoFH. In view

of the putative mechanism of action of PCSK9 inhibitors, functional LDLR are thought to be essential to reduce LDL-C. In HoFH both LDLR alleles are usually abnormal [60]. Since most patients with HoFH have LDLR-defective mutations, PCSK9 inhibitors might be more efficacious in lowering LDL-C in these patients than in those with no residual LDLR function (<2% receptor activity) – so-called null mutations [61]. In TESLA Part B, 50 subjects with HoFH were randomly assigned to evolocumab vs. placebo, as reviewed earlier [14]. Patients with defective mutations in one or both LDLR alleles responded better to treatment (41% reduction in mean LDL-C vs placebo) than did those with at least one null mutation (25% reduction). As predicted, the HoFH patient with two null LDLR mutations and the patient with autosomal recessive hypercholesterolemia did not respond to evolocumab. In addition to medical therapy, LDL apheresis is commonly used in patients with HoFH. It was recently shown that more than 50% of plasma PCSK9 is removed during LDL apheresis in patients severe FH, suggesting that PCSK9 removal may facilitate a resetting of the rate of cholesterol-return to base line [62]. Hence, the findings from TESLA Part B and the apheresis study provide motivation to investigate the effect of PCSK9 mAbs in combination with apheresis, preferably with mAb injection immediately after the apheresis treatment in order to delay the rate of cholesterol-return to base line.

Conclusion

The first PCSK9 decade has seen the rapid translation of genetic and biological discoveries into a new clinical approach with mass market potential. Clinical trials of mAbs targeted to PCSK9 have demonstrated remarkable efficacy in LDL-C reduction with an excellent short-term safety and tolerability profile. Indeed, the ability to substantially lower LDL-C is an unmet clinical need in several patient populations, including those with FH, statin intolerance, and inadequate LDL-C lowering on conventional LLT. The second PCSK9 decade will determine the fate of this new pharmacological approach with the results of the large phase III cardiovascular outcome trials eagerly awaited. Despite widespread optimism for the validation of this new pharmacological target, many questions remain unanswered. The physiological roles of PCSK9 are not fully understood and thus the implications of long-term antagonism of this protein remain to be determined. This fascinating story continues to unfold and will potentially usher in a new era of cholesterol management and ASCVD risk reduction.

Acknowledgments

This study was supported by the National Institutes of Health (National Heart, Lung, and Blood Institute) through grant R01-HL106845 to Dr. Fazio.

References

1. Abifadel M, et al. Living the PCSK9 adventure: from the identification of a new gene in familial hypercholesterolemia towards a potential new class of anticholesterol drugs. *Curr Atheroscler Rep*. 2014; 16(9):439. [PubMed: 25052769]
2. Abifadel M, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. *Nat Genet*. 2003; 34(2):154–6. [PubMed: 12730697]

3. Zhang DW, et al. 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(25):18602–12. [PubMed: 17452316]
4. Cohen J, et al. Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in PCSK9. *Nat Genet.* 2005; 37(2):161–5. [PubMed: 15654334]
5. Cohen JC, et al. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N Engl J Med.* 2006; 354(12):1264–72. [PubMed: 16554528]
6. Hooper AJ, et al. The C679X mutation in PCSK9 is present and lowers blood cholesterol in a Southern African population. *Atherosclerosis.* 2007; 193(2):445–8. [PubMed: 16989838]
7. Fasano T, et al. A novel loss of function mutation of PCSK9 gene in white subjects with low-plasma low-density lipoprotein cholesterol. *Arterioscler Thromb Vasc Biol.* 2007; 27(3):677–81. [PubMed: 17170371]
8. Dadu RT, Ballantyne CM. Lipid lowering with PCSK9 inhibitors. *Nat Rev Cardiol.* 2014; 11(10):563–75. [PubMed: 24958078]
9. Rashid S, et al. PCSK9 Promotes Intestinal Overproduction of Triglyceride-Rich Apolipoprotein-B Lipoprotein Through Both LDL-Receptor Dependent and Independent Mechanisms. *Circulation.* 2014; 130(4):431–41. [PubMed: 25070550]
10. Levy E, et al. PCSK9 plays a significant role in cholesterol homeostasis and lipid transport in intestinal epithelial cells. *Atherosclerosis.* 2013; 227(2):297–306. [PubMed: 23422832]
11. Le May C, et al. Proprotein convertase subtilisin kexin type 9 null mice are protected from postprandial triglyceridemia. *Arterioscler Thromb Vasc Biol.* 2009; 29(5):684–90. [PubMed: 19265033]
12. Raal FJ, et al. Reduction in lipoprotein(a) with PCSK9 monoclonal antibody evolocumab (AMG 145): a pooled analysis of more than 1,300 patients in 4 phase II trials. *J Am Coll Cardiol.* 2014; 63(13):1278–88. [PubMed: 24509273]
13. Gaudet D, et al. Effect of Alirocumab, a Monoclonal Proprotein Convertase Subtilisin/Kexin 9 Antibody, on Lipoprotein(a) Concentrations (a Pooled Analysis of 150 mg Every Two Weeks Dosing from Phase 2 Trials). *The American Journal of Cardiology.* 2014; 114(5):711–715. [PubMed: 25060413]
14. Raal FJ, et al. Inhibition of PCSK9 with evolocumab in homozygous familial hypercholesterolaemia (TESLA Part B): a randomised, double-blind, placebo-controlled trial. *The Lancet.* (0)
15. Stein EA, et al. Effect of the proprotein convertase subtilisin/kexin 9 monoclonal antibody, AMG 145, in homozygous familial hypercholesterolemia. *Circulation.* 2013; 128(19):2113–20. [PubMed: 24014831]
16. Cully M. Dyslipidaemia: RNAi targeting PCSK9 decreases lipid levels in a human trial. *Nat Rev Cardiol.* 2013; 10(12):682.
17. Schwartz GG, 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. *American Heart Journal.* 2014; 168(5):682–689.e1. [PubMed: 25440796]
18. Roth EM, et al. A 24-WEEK STUDY OF ALIROCUMAB AS MONOTHERAPY VERSUS EZETIMIBE: THE FIRST PHASE 3 DATA OF A PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9 INHIBITOR. *Journal of the American College of Cardiology.* 2014; 63(12_S)
19. Kastelein JP, et al. Efficacy and Safety of Alirocumab in Patients with Heterozygous Familial Hypercholesterolemia not Adequately Controlled with Current Lipid-Lowering Therapy: Design and Rationale of the ODYSSEY FH Studies. *Cardiovascular Drugs and Therapy.* 2014; 28(3):281–289. [PubMed: 24842558]
20. Ginsberg HN, et al. ODYSSEY HIGH FH: Efficacy and Safety of Alirocumab in Patients With Severe Heterozygous Familial Hypercholesterolemia. *Circulation.* 2014
21. Robinson J, et al. Long-term safety, tolerability and efficacy of alirocumab versus placebo in high cardiovascular risk patients: first results from the ODYSSEY LONG TERM study in 2,341 patients. *Eur Heart J.* 2014:35.

22. Robinson J, et al. Long-term Safety, Tolerability and Efficacy of Alirocumab versus Placebo in 2,341 High Cardiovascular Risk Patients: ODYSSEY LONG TERM. *Circulation*. 2014;130.
23. Moriarty PM, et al. ODYSSEY ALTERNATIVE: Efficacy And Safety of the Proprotein Convertase Subtilisin/kexin Type 9 Monoclonal Antibody, Alirocumab, versus Ezetimibe, in Patients With Statin Intolerance as Defined by a Placebo Run-in and Statin Rechallenge Arm. *Circulation*. 2014;130.
24. Moriarty PM, et al. 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. *Journal of Clinical Lipidology*. 2014; 8(6):554–561. [PubMed: 25499937]
25. Guyton JR, et al. An assessment by the Statin Intolerance Panel: 2014 update. *J Clin Lipidol*. 2014; 8(3 Suppl):S72–81. [PubMed: 24793444]
26. Bays H, et al. Efficacy and Safety of Combining Alirocumab With Atorvastatin or Rosuvastatin versus Statin Intensification or Adding Ezetimibe in High Cardiovascular Risk Patients: ODYSSEY OPTIONS I and II. *Circulation*. 2014;130.
27. Robinson JG, et al. Efficacy and Safety of Alirocumab as Add-on Therapy in High-Cardiovascular-Risk Patients With Hypercholesterolemia Not Adequately Controlled With Atorvastatin (20 or 40 mg) or Rosuvastatin (10 or 20 mg): Design and Rationale of the ODYSSEY OPTIONS Studies. *Clinical Cardiology*. 2014; 37(10):597–604. [PubMed: 25269777]
28. Colhoun H, et al. Efficacy and safety of alirocumab, a fully human PCSK9 monoclonal antibody, in high cardiovascular risk patients with poorly controlled hypercholesterolemia on maximally tolerated doses of statins: rationale and design of the ODYSSEY COMBO I and II trials. *BMC Cardiovascular Disorders*. 2014; 14(1):121. [PubMed: 25240705]
29. Kereiakes DJ, et al. Efficacy and Safety of Alirocumab in High Cardiovascular Risk Patients With Suboptimally Controlled Hypercholesterolemia on Maximally Tolerated Doses of Statins: The ODYSSEY COMBO I Study. *Circulation*. 2014;130.
30. Cannon CP, et al. Efficacy and safety of alirocumab in high cardiovascular risk patients with inadequately controlled hypercholesterolaemia on maximally tolerated daily statin: results from the ODYSSEY COMBO II study. *Eur Heart J*. 2014;35.
31. Koren MJ, et al. Anti-PCSK9 Monotherapy for Hypercholesterolemia The MENDEL-2 Randomized, Controlled Phase III Clinical Trial of Evolocumab. *Journal of the American College of Cardiology*. 2014; 63(23):2531–2540. [PubMed: 24691094]
32. Blom DJ, et al. A 52-Week Placebo-Controlled Trial of Evolocumab in Hyperlipidemia. *New England Journal of Medicine*. 2014; 370(19):1809–1819. [PubMed: 24678979]
33. Robinson JG, et al. Effect of evolocumab or ezetimibe added to moderate- or high-intensity statin therapy on ldl-c lowering in patients with hypercholesterolemia: The laplace-2 randomized clinical trial. *JAMA*. 2014; 311(18):1870–1883. [PubMed: 24825642]
34. Stroes E, et al. Anti-PCSK9 Antibody Effectively Lowers Cholesterol in Patients With Statin Intolerance: The GAUSS-2 Randomized, Placebo-Controlled Phase 3 Clinical Trial of Evolocumab. *Journal of the American College of Cardiology*. 2014; 63(23):2541–2548. [PubMed: 24694531]
35. Raal FJ, et al. PCSK9 inhibition with evolocumab (AMG 145) in heterozygous familial hypercholesterolaemia (RUTHERFORD-2): a randomised, double-blind, placebo-controlled trial. *The Lancet*. (0)
36. Weinreich M, Frishman WH. Antihyperlipidemic therapies targeting PCSK9. *Cardiol Rev*. 2014; 22(3):140–6. [PubMed: 24407047]
37. Gumbiner BM, et al. Effects of 12 Weeks of Treatment With RN316 (PF-04950615), a Humanized IgG2 a Monoclonal Antibody Binding Proprotein Convertase Subtilisin Kexin Type 9, in Hypercholesterolemic Subjects on High and Maximal Dose Statins. *Circulation*. 2012;126.
38. Ballantyne CM, et al. =EFFICACY AND SAFETY OF BOCOCIZUMAB (RN316/PF-04950615), A MONOCLONAL ANTIBODY AGAINST PROPROTEIN CONVERTASE SUBTILISIN/ KEXIN TYPE 9 IN STATIN-TREATED HYPERCHOLESTEROLEMIC SUBJECTS: RESULTS FROM A RANDOMIZED, PLACEBO-CONTROLLED, DOSE-RANGING STUDY (NCT: 01592240). *Journal of the American College of Cardiology*. 2014; 63(12_S)

39. McKenney JM, et al. Safety and efficacy of a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 serine protease, SAR236553/REGN727, in patients with primary hypercholesterolemia receiving ongoing stable atorvastatin therapy. *J Am Coll Cardiol.* 2012; 59(25):2344–53. [PubMed: 22463922]
40. Roth EM, et al. Atorvastatin with or without an Antibody to PCSK9 in Primary Hypercholesterolemia. *New England Journal of Medicine.* 2012; 367(20):1891–1900. [PubMed: 23113833]
41. Stein E, et al. Effect of a monoclonal antibody to PCSK9, REGN727/SAR236553, to reduce low-density lipoprotein cholesterol in patients with heterozygous familial hypercholesterolaemia on stable statin dose with or without ezetimibe therapy: a phase 2 randomised controlled trial. *Lancet.* 2012; 380:29–36. [PubMed: 22633824]
42. Giugliano RP, et al. Efficacy, safety, and tolerability of a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 in combination with a statin in patients with hypercholesterolaemia (LAPLACE-TIMI 57): a randomised, placebo-controlled, dose-ranging, phase 2 study. *The Lancet.* 380(9858):2007–2017.
43. Koren MJ, et al. Efficacy, safety, and tolerability of a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 as monotherapy in patients with hypercholesterolaemia (MENDEL): a randomised, double-blind, placebo-controlled, phase 2 study. *The Lancet.* 2012; 380(9858):1995–2006.
44. Raal F, et al. 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(20):2408–2417. [PubMed: 23129602]
45. Sullivan D, et al. Effect of a monoclonal antibody to pcsk9 on low-density lipoprotein cholesterol levels in statin-intolerant patients: The gauss randomized trial. *JAMA.* 2012; 308(23):2497–2506. [PubMed: 23128163]
46. Stein EA, et al. Effect of a Monoclonal Antibody to PCSK9 on LDL Cholesterol. *New England Journal of Medicine.* 2012; 366(12):1108–1118. [PubMed: 22435370]
47. Dias CS, et al. Effects of AMG 145 on Low-Density Lipoprotein Cholesterol Levels: Results From 2 Randomized, Double-Blind, Placebo-Controlled, Ascending-Dose Phase 1 Studies in Healthy Volunteers and Hypercholesterolemic Subjects on Statins. *Journal of the American College of Cardiology.* 2012; 60(19):1888–1898. [PubMed: 23083772]
48. Group HTC. HPS2-THRIVE randomized placebo-controlled trial in 25 673 high-risk patients of ER niacin/laropiprant: trial design, pre-specified muscle and liver outcomes, and reasons for stopping study treatment. *Eur Heart J.* 2013; 34(17):1279–91. [PubMed: 23444397]
49. Boden W, et al. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. *N Engl J Med.* 2011; 365:2255–2267. [PubMed: 22085343]
50. Albers JJ, et al. Relationship of apolipoproteins A-1 and B, and lipoprotein(a) to cardiovascular outcomes: the AIM-HIGH trial (Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglyceride and Impact on Global Health Outcomes). *J Am Coll Cardiol.* 2013; 62(17):1575–9. [PubMed: 23973688]
51. Poirier S, et al. The Proprotein Convertase PCSK9 Induces the Degradation of Low Density Lipoprotein Receptor (LDLR) and Its Closest Family Members VLDLR and ApoER2. *Journal of Biological Chemistry.* 2008; 283(4):2363–2372. [PubMed: 18039658]
52. Roubtsova A, et al. Circulating Proprotein Convertase Subtilisin/Kexin 9 (PCSK9) Regulates VLDLR Protein and Triglyceride Accumulation in Visceral Adipose Tissue. *Arteriosclerosis, Thrombosis, and Vascular Biology.* 2011; 31(4):785–791.
53. Zaid A, et al. Proprotein convertase subtilisin/kexin type 9 (PCSK9): Hepatocyte-specific low-density lipoprotein receptor degradation and critical role in mouse liver regeneration. *Hepatology.* 2008; 48(2):646–654. [PubMed: 18666258]
54. Rashid S, et al. Decreased plasma cholesterol and hypersensitivity to statins in mice lacking Pcsk9. *Proc Natl Acad Sci U S A.* 2005; 102(15):5374–9. [PubMed: 15805190]

55. Fitzgerald K, et al. Effect of an RNA interference drug on the synthesis of proprotein convertase subtilisin/kexin type 9 (PCSK9) and the concentration of serum LDL cholesterol in healthy volunteers: a randomised, single-blind, placebo-controlled, phase 1 trial. *The Lancet*. 383(9911):60–68.
56. Tavori H, et al. Serum proprotein convertase subtilisin/kexin type 9 and cell surface low-density lipoprotein receptor: evidence for a reciprocal regulation. *Circulation*. 2013; 127(24):2403–13. [PubMed: 23690465]
57. Kosenko T, et al. Low density lipoprotein binds to proprotein convertase subtilisin/kexin type-9 (PCSK9) in human plasma and inhibits PCSK9-mediated low density lipoprotein receptor degradation. *J Biol Chem*. 2013; 288(12):8279–88. [PubMed: 23400816]
58. Sun H, et al. Proprotein convertase subtilisin/kexin type 9 interacts with apolipoprotein B and prevents its intracellular degradation, irrespective of the low-density lipoprotein receptor. *Arterioscler Thromb Vasc Biol*. 2012; 32(7):1585–95. [PubMed: 22580899]
59. Stein EA, et al. Apolipoprotein B Synthesis Inhibition With Mipomersen in Heterozygous Familial Hypercholesterolemia: Results of a Randomized, Double-Blind, Placebo-Controlled Trial to Assess Efficacy and Safety as Add-On Therapy in Patients With Coronary Artery Disease. *Circulation*. 2012; 126(19):2283–2292. [PubMed: 23060426]
60. Raal FJ, Santos RD. Homozygous familial hypercholesterolemia: Current perspectives on diagnosis and treatment. *Atherosclerosis*. 2012; 223(2):262–268. [PubMed: 22398274]
61. Stein EA, et al. Effect of the Proprotein Convertase Subtilisin/Kexin 9 Monoclonal Antibody, AMG 145, in Homozygous Familial Hypercholesterolemia. *Circulation*. 2013; 128(19):2113–2120. [PubMed: 24014831]
62. Tavori H, et al. Loss of Plasma Proprotein Convertase Subtilisin/Kexin 9 (PCSK9) After Lipoprotein Apheresis. *Circulation Research*. 2013; 113(12):1290–1295. [PubMed: 24122718]

Figure 1.

Table 1

Summary of Phase III Clinical Trials with Alirocumab (ODYSEY Program)

Study	Population	Study Duration	% LDL-C reduction		P value
			Alirocumab	Comparator	
MONO	Hypercholesterolemia and moderate ASCVD risk	24 weeks	47%	16% (ezetimibe)	p<0.0001
FH I	HeFH	24 weeks	49%	9% (placebo)	p<0.0001
FH II	HeFH	24 weeks	49%	3% (placebo)	p<0.0001
HIGH FH	HeFH	24 weeks	46%	7% (placebo)	p<0.0001
LONG TERM	High ASCVD risk/HeFH	52 weeks	57%	+4% increase (placebo)	p<0.0001
ALTERNATIVE	High ASCVD risk and history of intolerance to at least 2 statins	24 weeks	45%	15% (ezetimibe)	p<0.0001
OPTIONS I	High ASCVD risk	24 weeks	44–54%	21–23% (ezetimibe) 5% (double dose statin) 21% (statin switch)	p=0.01
OPTIONS II	High ASCVD risk	24 weeks	36–51%	11–14% (ezetimibe) 16% (double statin dose)	p=0.0125
COMBO I	High ASCVD risk	24 weeks	48%	2% (placebo)	p<0.0001
COMBO II	High ASCVD risk	24 weeks	51%	21% (ezetimibe)	p<0.0001

LDL-C, low density lipoprotein-cholesterol; FH, familial hypercholesterolemia; HeFH, heterozygous familial hypercholesterolemia; ASCVD, atherosclerotic cardiovascular disease

Table 2

Summary of Phase III Clinical Trials with Evolocumab (Proficio Program)

Study	Population	Study Duration	% LDL-C reduction		P value
			Evolocumab	Comparator	
MENDEL-2	Hypercholesterolemia and low ASCVD risk	12 weeks	56–57%	18–19% (ezetimibe) 0.1 – 1% (placebo)	p<0.01
DESCARTES	Hypercholesterolemia with varying levels of ASCVD risk	52 weeks	47–55%	2–10 (placebo)	p<0.001
LAPLACE-2	Hypercholesterolemia and mixed dyslipidemia	Mean of weeks 10 and 12	63–75%	19–32% (ezetimibe)	p<0.001
GAUSS-2	Hypercholesterolemia and history of intolerance to at least 2 statins	Mean of weeks 10 and 12	55–56%	37–39% (ezetimibe)	p<0.001
RUTHERFORD-2	HeFH	12 weeks	56–61%	1% reduction to +5% increase (ezetimibe)	p<0.001
TESLA Part B	HoFH	12 weeks	23%	+8% increase (placebo)	p<0.0001

LDL-C, low density lipoprotein-cholesterol; ASCVD, atherosclerotic cardiovascular disease; HeFH, heterozygous familial hypercholesterolemia; HoFH, homozygous familial hypercholesterolemia