

The Newest Frontier in Cholesterol Management; PCSK 9

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Abstract

Cardiovascular disease from atherosclerosis remains a major cause of death in Australia. Epidemiological studies have demonstrated that reducing low-density lipoprotein cholesterol (LDL-C) strongly correlates with improved cardiovascular risk in. Although statin based therapy remains our most effective and first line therapy, certain cohorts remain refractory to their benefits. Proprotein convertase subtilisin/kexin type 9 (PCSK9), a monoclonal antibody, is currently under research as a novel non-statin based therapy in multiple clinical trials. PCSK9 targets low-density lipoprotein receptors for internalisation and lysosomal degradation. This affects cholesterol homeostasis by reducing the cells ability to internalise circulating apolipoprotein B. Early trials have shown clear benefits in LDL-C reduction without major adverse incidence in populations intolerant to statins, those with familial hypercholesterolaemia and patients requiring additional treatment to meet cholesterol targets despite statin based therapy. This article is a clinical focus reviewing the current phase I, II and III trials being conducted and the potential benefits a monoclonal antibody to PCSK9 may hold for cholesterol regulation in the future.

Keywords: Hypercholesterolemia; LDL cholesterol; Coronary vascular disease; PCSK9

Background

It has now emerged that by blocking low-density lipoprotein receptor degradation by proprotein convertase subtilisin/kexin type 9, low-density lipoprotein cholesterol (LDL-C) can be reduced very effectively and significantly. These monoclonal antibodies are likely to provide a robust and reasonable alternative for certain populations; those intolerant to statins, those who do not attain recommended LDL-C levels despite maximal therapy and finally individuals with Familial Hypercholesterolemia.

Introduction

Cardiovascular disease, developing from atherosclerosis, continues to be a leading cause of death in industrialized countries. Control of cholesterol and specifically reduction of low-density lipoprotein cholesterol (LDL-C) has been demonstrated to strongly correlate with improved cardiovascular risk in epidemiological studies. Subsequently, all clinical guidelines have adopted specific LDL-C goals based on cardiovascular outcome trials [1]. In Australia, high-risk individuals for coronary vascular disease (CVD) and those with pre-existing CVD have a target LDL-C of less than 2.5mmol/L and 1.8mmol/L respectively. First line therapy remains statins; hydroxyl-methylglutaryl co-enzyme A reductase inhibitors. They are currently recognized as our most effective agent with up to 63% reduction in LDL-C from baseline [2]. However, despite their success, significant gaps remain in the treatment of hypercholesterolemia [3]. 10-20% of patients are unable to tolerate statins due to muscle related side effects and therefore do not achieve recommended LDL-C target levels. Other individuals continue to experience complications of atherosclerosis despite being on an adequate regime. Unfortunately our second line therapies; ezetimibe, fibrates and bile acid sequestrates have shown only limited clinical benefit. Ezetimibe, the most favoured second line treatment, reduces LDL-C levels by up to 18%, which is unlikely to meet the LDL-C goals our guidelines strive for unless used as dual treatment with statins. For these populations an alternative non-statin cholesterol agent is required. It has now emerged that by blocking the degradation of the low-density lipoprotein receptor (LDLR) by proprotein convertase subtilisin/kexin type 9 (PCSK9), LDL-C can be reduced very effectively and significantly.

What is PCSK9?

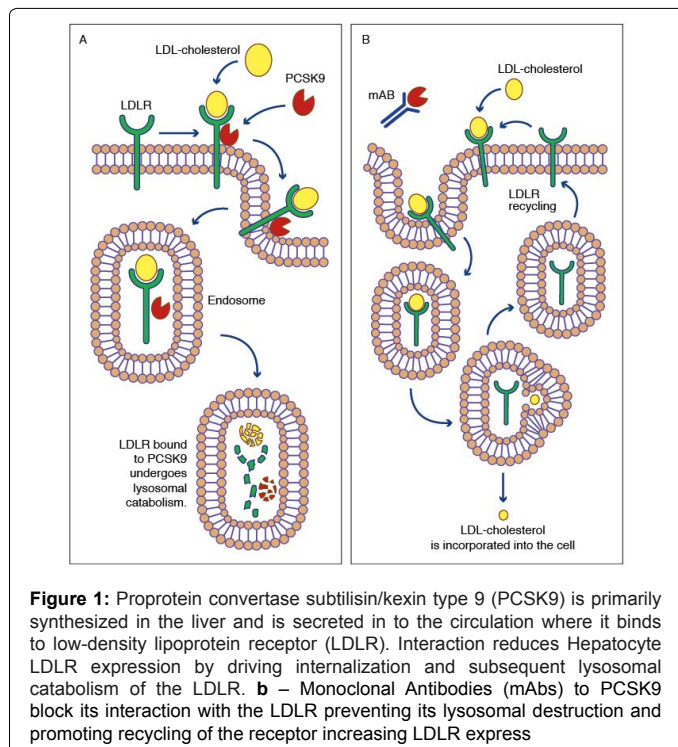
Central to cellular cholesterol homeostasis is the hepatic LDLR. PCSK9 plays a pivotal role in regulating LDLR recycling which is fundamental to the hepatocyte cells ability to efficiently internalize circulating apolipoprotein B (Apo B) [4]. LDLR binds Apo B resulting in internalization of the complex, allowing Apo B to undergo lysosomal degradation and release of cholesterol. The free LDLR is then recycled back to the hepatocyte membrane to once again bind circulating Apo B [5]. PCSK9, secreted by hepatocytes, binds the LDLR and limits their surface expression by targeting them for lysosomal degradation following internalization into the cell (Figure 1). In 2003 Abifadel et al mapped a mutation to chromosomal locus 1p32, the site of the PCSK9 gene, in patients with autosomal dominant hypercholesterolemia. The Mutation enhanced the function of PCSK9 resulting in reduced LDLR expression and concomitant hypercholesterolemia [6]. Cohen reported a nonsense mutation with loss of function correlating with a 28% reduction in mean LDL-C levels. Clinical benefit was also reported with a reduced incidence of ischemic heart disease of 47% to 88% depending on the different sequence variations leading to loss of function mutations of PCSK9 [7]. Importantly this did not appear to affect other aspects of health. As our understanding of the interplay between PCSK9 and the LDLR has developed it has been an interesting discovery to find that statins produce PCSK9 up-regulation. Statins and ezetimibe decrease the amount of cholesterol in hepatocyte cells thereby stimulating LDLR up regulation. As a result this compensatory up regulation of PCSK9 may counteract the LDL-cholesterol lowering effectiveness of statins and ezetimibe [8]. As a result of these findings PCSK9 has become a novel target of clinical trials. These trials have

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aimed at exploring the efficacy of a monoclonal antibody targeting PCSK9, preventing LDLR degradation and improving circulating levels of LDL-C.

Current Therapies and Clinical Trials

Evolocumab (AMG145)

Evolocumab is a fully humanised monoclonal PCSK9 antibody administered subcutaneously. The initial phase 1 trials evaluated the effect of evolocumab on heterozygous familial hypercholesterolemia (FH) and non-FH patients who required statin therapy as well as healthy patients. Subcutaneous doses of 7, 21, 70, 140, 210 and 420 mg of evolocumab were used. Compared to placebo, evolocumab reduced LDL-C levels by 64% after 1 dose of 21mg or more. This improved to 81% with repeated dosing of 35 mg or more [9]. Evolocumab was well tolerated with no serious adverse events. Subsequently the Goal Achievement after Utilizing an Anti PCSK9 Antibody in Statin Intolerant Subjects (GAUSS) trial was created to determine the efficacy of evolocumab in statin intolerant patients. 5 groups of patients who had a history of muscle related statin side effects received 12 weeks of evolocumab alone at 280, 350 or 420 mg or evolocumab 420 mg plus ezetimibe 10 mg or ezetimibe 10mg and placebo. Evolocumab and placebo was given subcutaneously at 4-week intervals. Treatment with evolocumab produced reductions in LDL-C levels from 41% to 63%. Maximal effect was evident within 2 weeks of therapy and maintained throughout the 12 weeks of treatment. In the Evolocumab alone groups LDL-C levels of less than 2.5 mmol/L and less than 1.8 mmol/L were achieved in 54% and 18% respectively compared to ezetimibe alone which achieved 7% and 0% respectively. Importantly 94% of subjects completed the trial with favourable short-term tolerability. Myalgia remained the most common reported side effect with 7 patients (7.4%) taking evolocumab only and 6 patients (20%) taking evolocumab/ezetimibe. Nasopharyngitis and fatigue were the other common reported effects [10]. The GAUSS-2 trial

sought to evaluate evolocumab in patients of higher cardiovascular risk with at least 2 statin intolerances. It was a 12 week double blind study in which 307 patients were randomized to evolocumab, 140 mg every two weeks (Q2W) or 420 mg once a month (Q4W) or 10mg oral ezetimibe. Evolocumab reduced LDL-C from baseline by 53% to 56%, corresponding to treatment differences versus ezetimibe of 37% to 39%. 96% of patients completed treatment. Myalgia occurred in 18%, 7% and 9% of patients receiving ezetimibe, evolocumab Q2W and evolocumab Q4W respectively [11].

The Phase II and III Monoclonal Antibody against PCSK9 to Reduce Elevated LDL-C in Subjects Currently Not Receiving Drug Therapy for Easy Lipid Levels (MENDEL) trials sought to establish the efficacy of evolocumab as a potential monotherapy in those not receiving statin therapy. The initial MENDEL trial reduced placebo corrected LDL-C levels by 37.3% to 52.5% from baseline. Patients had LDL-C levels between 2.5 mmol/L and 4.9 mmol/L and received 70, 105, 140 mg of evolocumab or placebo Q2W or 280, 350, 420 mg of evolocumab or placebo Q4W or 10 mg/day of ezetimibe over 12 weeks. 120 mg Q2W and 420 mg Q4W produced the greatest effect with no evidence of dose limiting adverse effects [12]. Subsequently the Phase III MENDEL-2 trial conducted by Koren compared biweekly 140 mg and monthly 420mg evolocumab, doses anticipated for clinical practise, with placebo and oral ezetimibe. 614 patients with fasting LDL-C levels of ≥ 2.5 mmol/L and < 4.9 mmol/L and a Framingham risk score of $\leq 10\%$ were randomised. Evolocumab had a mean reduction in LDL-C from baseline of 55% to 57% more than placebo and 38% to 40% more than ezetimibe. 97% of patients completed the study with comparable muscle related side effects across all treatment groups at 4% [13]. Most recently Blom completed a 52-week phase III trial evaluating the safety and efficacy of 420 mg of evolocumab every 4 weeks. Depending on their ATP-III defined cardiovascular risk patients had a run in period of varying lipid-lowering regimes, ranging from diet alone to 80 mg of atorvastatin and 10 mg of ezetimibe. The overall mean reduction in LDL-C when compared to placebo was 57.0%. Specifically, when compared to the run in therapy, evolocumab had a mean reduction in LDL-C of 55.7% in those who received diet control alone, 61.6% who took 10mg of atorvastatin, 56.8% amongst those who received 80mg of atorvastatin and 48.5% who received 80mg of atorvastatin and 10mg of ezetimibe. Additionally they found no decrement in efficacy from 12 weeks though to 52 weeks. Interestingly, unbound PCSK9 levels were measured at baseline and then compared at 13 and 37 weeks revealing a mean reduction of 91.1% and 86.9% respectively. Consistent with other studies the most common reported adverse events included nasopharyngitis, upper respiratory tract infections, influenza and back pain. Creatine kinase levels of five times above normal occurred in 7 patients (1.2%) receiving evolocumab compared to 1 patient (0.3%) in the placebo group [14].

Alircumab (REGN727/SAR236553)

Alircumab can be administered subcutaneously or intravenously. Initial phase 1 trials administered a single intravenous dose of up to 12 mg/kg to 40 healthy subjects and 250 mg of subcutaneous alircumab to 32 healthy patients. LDL-C was reduced by 65% with the intravenous administration showing greater efficacy. These trials were extended to patients with heterozygous FH with LDL-C ≥ 2.5 mmol/L receiving atorvastatin and non-FH with LDL-C ≥ 2.5 mmol/L and LDL-C ≥ 3.4 mmol/L who received atorvastatin or a modified diet respectively. Alircumab was administered subcutaneously on days 1, 29, and 43 at doses of 50, 100, or 150 mg with a reduction from baseline of 39.2%, 53.7% and 61% respectively. The study size was small but showed

promise for alircumab as potential monotherapy [15]. In a phase II placebo controlled trial, the efficacy of alircumab to lower LDL-C levels in primary hypercholesterolemia was compared to placebo. LDL-C levels at 12 weeks were reduced by 64.2% and 72.4% from baseline for 100 mg and 150 mg Q2W respectively whilst 200 mg and 300 mg Q4W produced 43.2% and 47.7% reductions respectively. The placebo group had a 5% reduction [16]. Roth et al designed a study to measure the efficacy of alircumab when co-administered with 80mg or 10mg of atorvastatin, as compared with high-dose atorvastatin alone, in patients with LDL cholesterol levels of ≥ 2.5 mmol/L. 92 patients had a run in period of 10 mg of atorvastatin for at least 7 weeks. Subjects were randomly assigned to receive 8 weeks of treatment with 80 mg of atorvastatin daily plus 150mg off subcutaneous alircumab Q2W, 10 mg of atorvastatin daily plus alircumab Q2W, or 80 mg of atorvastatin daily plus placebo Q2W. Reductions in LDL-C levels from baseline were 66.2%, 73.2% and 17.3% respectively for each group. Importantly all study drug group patients achieved LDL-C levels less than 2.5 mmol/L. The study suggested that administration of alircumab with statins might benefit patients in whom LDL cholesterol has not been reduced to recommended levels. There was also no significant difference between 80 mg or 10 mg atorvastatin plus the study drug. This suggested a limit to the up-regulation of LDL-receptor activity and that effects of alircumab are not synergistic when added to statins [17].

RN316

RN316 is a humanized PCSK9 monoclonal antibody administered intravenously that has recently been tested for its efficacy and safety in clinical trials. In the initial phase I studies, subjects with primary hypercholesterolemia were administered multiple doses of RN316 (0.25-1.5 mg/kg/week) for a period of 4 weeks. LDL-C levels were reduced by up to 90% from baseline with additional improvements in triglyceride and high-density lipoprotein cholesterol (HDL-C) concentrations. Gumbiner et al followed this up with a Phase II trial where hypercholesterolemic subjects receiving high dose statins were assigned to RN316 (dose range: 0.25–6.0 mg/kg; IV) every 4 weeks for a period of 12 weeks. 90 participants with an LDL-C ≥ 2.5 mmol/L were given the study drug or placebo while on concurrent high doses of atorvastatin (40 or 80 mg), rosuvastatin (20 or 40 mg), or simvastatin (40 or 80 mg). In the second arm 45 subjects with LDL-C ≥ 2.0 mmol/L was administered study drug or placebo while on atorvastatin 80 mg or rosuvastatin 40 mg. After 12 weeks the pooled mean reduction in LDL-C for those receiving the study drug was 56% compared with 4% in the placebo group. Again HDL-C concentrations increased but triglycerides showed no significant improvement. RN316 was well tolerated with no study drug related adverse events [18]. Currently a large multicentre phase III trial aims to recruit 6300 subjects with the aim to evaluate the efficacy, safety and tolerability of RN316 compared to placebo. Its primary objective is to demonstrate the superior efficacy to reduce major cardiovascular events in subjects with high risk of major cardiovascular events who are on maximally tolerated lipid-lowering therapy but have an LDL-C ≥ 2.5 mmol/L or non-HDL-C ≥ 3.4 mmol/L.

Safety issues related to PCSK9 inhibition

Whilst the discussed trials have shown encouraging results for pharmacological inhibition of PCSK9, the long-term effects upon human health remain unknown. Mice animal models lacking PCSK9 develop normally and have no gross neurological defects [19]. However, inactivation of PCSK9 in embryos of zebra fish results in disordered neuronal development and death [20]. Encouragingly we have identified individuals who are heterozygous for loss-of-function

mutations in PCSK9 and appear to be healthy with a normal lifespan [21]. Zhao et al. identified a healthy fertile college graduate who was a compound heterozygote for two inactivating mutations in PCSK9 who had a strikingly low plasma level of LDL-C (0.4 mmol/L). Recently Jasmin SB found that at autopsy, individuals with Alzheimer's disease had a 2-fold increased expression of PCSK9. Its role remains unclear but it may play a role in the homeostatic regulation of neuronal repair [22].

Discussion

There has been a rapid progress in our understanding of PCSK9 and its role in LDL metabolism. It provides a clear example of our current technological abilities in translational sciences to produce novel therapeutic targets. Monoclonal antibody therapy has been shown to achieve a reduction in LDL-C levels of 50% to 75%. Whilst it is unlikely these monoclonal antibodies will replace HMG-CoA reductase inhibitors in the near future due to production costs, health care economics and mode of delivery they are likely to provide a robust and reasonable alternative for certain populations; those intolerant to statins, those who do not attain recommended LDL-C levels despite maximal therapy and finally individuals with FH. In the context of the American College of Cardiology/American Heart Association [23] guidelines, PCSK9 monoclonal antibodies provide a realistic alternative, however long-term antibody use remains unevaluated.

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