Chapter

Novel Therapies for Dyslipidemia

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Abstract

Multiple studies have shown a strong correlation between low-density lipoprotein cholesterol (LDL-C) concentration and development as well as progression of atherosclerosis and cardiovascular disorders. Thus, the decrease of the LDL-C burden through lifestyle modification and/or pharmacological interventions unanimously demonstrated a decrease in cardiovascular events and mortality. To date, statins are considered the cornerstone of lipid-lowering therapy. The Cholesterol Treatment Trialists' (CTT) Collaboration has shown consistency of treatment benefits across a wide patient population. However, new data are now revealing that a considerate patient population failed to achieve lipid goals solely on statins and a significant percentage cannot tolerate treatment. Therefore, extensive work has recently been done in generating novel LDL-C-lowering agents that would act through mechanisms different from statins. Among others, monoclonal antibodies to protein convertase subtilisin/kexin type 9 (PCSK9) and ezetimibe seem particularly promising. Both PCSK9 monoclonal antibodies and ezetimibe have shown to be well tolerated and very effective at lowering LDL-C.

Keywords: dyslipidemia, PCSK9 inhibitors, ezetimibe, LDL-C

1. Introduction

Cardiovascular disease (CVD), which includes coronary heart disease (CHD) and strokes, is considered as a number one cause of morbidity and mortality worldwide. Together with hypertension, dyslipidemia is among the most prevalent risk factors leading to CVD. Thus, treatment of dyslipidemia is crucial in reducing CVD events and the morbidity and mortality associated with them. Studies in the last decade have confirmed a causal relationship between low-density lipoprotein cholesterol (LDL-C) and the risk of atherosclerotic cardiovascular diseases (ASCVD) [1]. LDL-C can be lowered by diet restriction and lifestyle changes or by various lipid-lowering therapies, among which statins (3-hydroxy-3-methylglutaryl-coenzyme A [HMG-CoA] reductase inhibitors) are currently considered the cornerstone medication. Based on the 2018 guidelines published by the American College of Cardiology (ACC) and the American Heart Association (AHA), multiple recommendations have been made for patients with or at risk of developing cardiovascular disease [2].

Under certain circumstances, non-statin medications like ezetimibe and PCSK9 inhibitors are found to be useful particularly in combination with statin therapy. Clinical trials pertaining to these novel therapies have shown great benefits with significant LDL-C-lowering potential and decrease in cardiovascular risk. These agents are generally well tolerated, but long-term safety and cost remain to be proven.

2. PCSK9 inhibitors as cholesterol-lowering therapy

2.1 Genetics behind the discovery of PCSK9 inhibitors

PCSK9 gene was initially discovered as part of a search for members of the protein convertase family of serine proteases [3]. It was initially named as neural apoptosis-regulated convertase-1 (NARC1) due to its upregulation in apoptosis of primary cultures of cerebellar neurons. Subsequent studies classified it as a member of the proteinase K family of subtilases, and it was renamed as (proprotein convertase subtilisin/kexin type 9 (PCSK9)). The gene mapped to chromosome 1p32 comprises 12 exons, and it is translated into a protein of 692 amino acids (NM-174936.3) and is mainly expressed in the liver [3]. Interestingly, the gene coding for PCSK9 coincides with a region linked to dominant inheritance of hypercholesterolemia in American and French families who had no mutations in either LDL-R or APOB genes [4, 5]. Loss-of-function mutations in the later are the two main culprits that comprise 99% of cases with familial hypercholesterolemia (FH). Further genetic analysis of these index families identified a few rare heterozygous missense mutations in PCSK9 gene, all coinciding with hypercholesterolemia [6]. Thus, PCSK9 is now recognized as the third gene causing FH and currently accounts for <1% of FH cases [7].

A crucial step in further understanding the role of this gene in cholesterol metabolism was achieved by Maxwell and Breslow, who showed that transgenic overexpression of PCSK9 in mice caused a FH-like phenotype due to increased intracellular degradation of the LDL-receptor (LDL-R) [8, 9]. This observation shed light into the physiological implication of PCSK9 gene in the lipid metabolism and dyslipidemia. It became clear that gain-of-function rather than loss-of-function mutations in PCSK9 lead to hypercholesterolemia. In the last decade, many disease-causing mutations, occurring in various domains of the protein and leading to either increased transcription or impaired autocatalysis, have confirmed the above findings [10–13].

It was not until 2005 when studies in individuals with extremely low LDL-C levels revealed some PCSK9 coding variants that were not seen in individuals with high levels of LDL-C. Some of these variants encoded truncated version of the protein that was clearly predicted to cause loss-of-function of PCSK9 [14, 15]. Mendelian randomization experiments clearly revealed that individuals with PCSK9 loss-of-function variants had lifelong depressed LDL-C levels as well as reduced ASCVD risk [16]. In these initial studies, of 3363 blacks, 2.6% carried heterozygous PCSK9 nonsense variants and were associated with 28 and 88% reductions in LDL-C and ASCVD risk, respectively. Of the 9524 white subjects examined, 3.2% had a PCSK9 mutation and were associated with 15 and 47% reductions in LDL-C and ASCVD risk, respectively. The above results were then confirmed in subsequent larger cohort studies [17, 18].

Taken together, the findings in either gain-of-function or loss-of-function mutations in PCSK9 gene have provided very strong evidence for the potential pharmacological use of targeted reduction of PCSK9 protein. Additionally, studies of complete human knockouts of PCSK9 (biallelic loss-of-function mutations) revealed only isolated decreased LDL-C levels with no deleterious health complications [19, 20]. This supports the potential safety of pharmacologically targeting PCSK9. However, it is worth mentioning that carriers of PCSK9 loss-of-function mutations showed an increased risk of developing type 2 diabetes mellitus (T2DM) [21, 22]. This finding would suggest that a similar side effect may be encountered in potential pharmacological inhibitors of PCSK9 (an increase of T2DM has been

reported in statin drugs) [23]. However, no such association was found in clinical trials with human monoclonal anti-PCSK9 [24]. An increased risk of T2DM was also observed in a phenome-wide association study performed in >337,000 individuals with PCSK9 p.R46L mutation [25]. The same mutation was found to have a protective effect on hyperlipidemia, coronary heart disease (CHD), ischemic stroke, and cerebral infarction. No association with cataracts, heart failure, atrial fibrillation, or cognitive dysfunction was reported.

2.2 Mechanism of action of PCSK9 protein

PCSK9 is a serine protease involved in cholesterol metabolism. In the liver, it binds to the LDL-receptor (LDL-R), inducing intracellular degradation, thus reducing serum LDL clearance. Generally, PCSK9 molecule is absent allowing repeated recycling of the LDL-R receptor. One study also suggests that intracellular PCSK9 may be recycled so that a single molecule might contribute multiple times to receptor degradation [26]. Under physiological conditions, PCSK9 expression is very low compared to LDL-R, thus allowing continuous recycling of the receptor (**Figure 1A**). In the case of low intracellular levels of cholesterol, both LDL-R and PCSK9 are transcribed (**Figure 1B**). Once secreted in the plasma, PCSK9 serves as one of the many potential ligands for LDL-R. After endocytosis, LDL-R, LDL particle, and PCSK9 enter the lysosome. Once inside the lysosome, the LDL particle is degraded, whereas the LDL-R attached to PCSK9 fails to exit the lysosome, where it gets degraded and can no longer be recycled (**Figure 1B**) [10, 27, 28]. Using this intricate mechanism, PCSK9 would prevent overexpression of LDL-R and thus increase the intracellular cholesterol levels. On the other hand, in patients with elevated cholesterol levels, administering PCSK9 monoclonal antibodies would neutralize the PCSK9 molecules floating in the plasma, thus increasing LDL-R recycling and surface LDL-R (**Figure 1C**).

To better understand the action of PCSK9 inhibitors, we can correlate it with the pharmacological action of HMG-CoA reductase inhibitors (statins). Cholesterol is mainly synthetized in the liver via the mevalonate pathway, with HMG-CoA reductase being the rate-limiting enzyme in the process. The decrease in the intracellular cholesterol in the liver is sensed by sterol regulatory element-binding protein 2 (SREBP2) which then increases the production of HMG-CoA reductase to promote the intracellular synthesis of cholesterol as well as increase LDL-R and PCSK9 levels. As a result, statins will (1) decrease intracellular cholesterol production, (2) increase LDL-R expression on the hepatocytes, and (3) increase PCSK9 levels. This

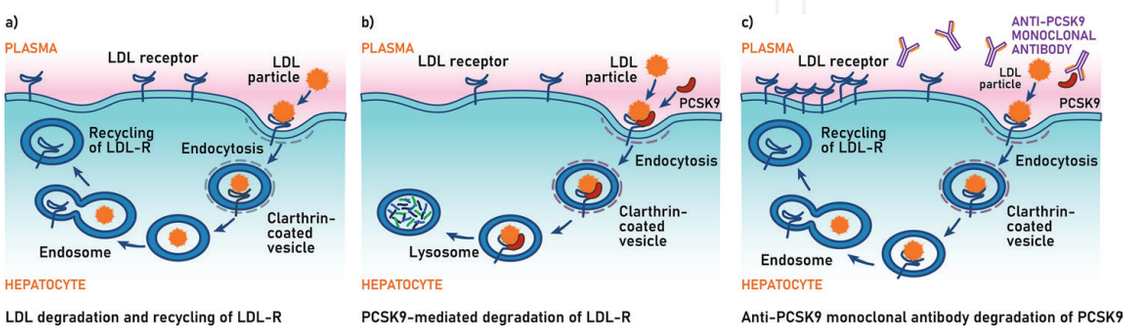

Figure 1.
Under physiological conditions, PCSK9 expression is very low compared to LDL-R, thus allowing continuous recycling of the receptor (a). In the case of low intracellular levels of cholesterol, both LDL-R and PCSK9 are transcribed (b). In patients with elevated cholesterol levels, administering PCSK9 monoclonal antibodies would neutralize the PCSK9 molecules floating in the plasma, thus increasing LDL-R recycling and surface LDL-R (c).

can explain why addition of anti-PCSK9 monoclonal antibodies can accentuate the lipid-lowering effect of statins.

2.3 Therapeutic rationale for PCSK9 inhibitors

Currently, statins are the first-choice agents to reduce high blood cholesterol which is considered one of the main risk factors for cardiovascular disease (CVD). Cholesterol Treatment Trialists' (CTT) Collaboration as well as other clinical trials showed that primary prevention with statins reduced all-cause mortality, reduced combined fatal and nonfatal stroke, reduced revascularization rate, and improved patient quality of life [29, 30]. Statin prescription is not associated with serious harm and is considered to be cost-effective [31]. That said, in the last decade, many patients have reported more and more severe side effects particularly with high-intensity statin therapy [32]. Additionally, certain patient populations (familial hypercholesterolemia) are at particular high risk for cardiovascular events. The risk can be attributed to the complexity of the underlying disease, and sole treatment with statins may be insufficient.

Familial hypercholesterolemia: Familial hypercholesterolemia (FH) is a complex genetic disorder characterized by high LDL-C levels and early incidence of ASCVD [33]. There are two forms of FH: a heterozygous and homozygous one with a prevalence of 1 in 250 and 1 in 250,000, respectively [34]. The disorder is characterized by high-serum LDL-C concentration, xanthomas including Achilles tendon thickening, and premature coronary artery disease (CAD). In FH patients, the prevalence of CAD is extremely high, and its age of onset is 15–20 years earlier than usual. Thus, early diagnosis and appropriate treatment are crucial.

Statin intolerance: Statin intolerance is defined as an inability to take statin because of reported side effects. It is classified as complete (inability to tolerate any statin at any dose) or partial (inability to tolerate high doses of statin). The most common reported adverse effect is muscle pain due to myopathy and rhabdomyolysis in severe cases. Though rare, rhabdomyolysis can be serious if not detected and treated early. An increased risk of type 2 diabetes has also been reported, particularly with high-dose statins [35]. Two recent meta-analyses observed a 9% increased risk for incident diabetes associated with statin therapy, with little heterogeneity between studies. Hemorrhagic stroke also appears to be increased by statin therapy, although estimates are imprecise. However, overall stroke events were reduced, indicating a net benefit.

2.4 Anti-PCSK9 monoclonal antibodies and the cardiovascular outcome studies

Multiple studies were performed to investigate the cardiovascular effect of the anti-PCSK9 monoclonal antibodies. The Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk (FOURIER) trial, a randomized, double-blinded, placebo-controlled trial published in 2017, is currently considered one of the landmark studies in the efficacy of anti-PCSK9 monoclonal antibodies [36]. The aim of the study was to understand whether evolocumab (anti-PCSK9 monoclonal antibody) which lowers LDL-C levels by 69% can prevent cardiovascular events. This trial included 27,564 patients with atherosclerotic cardiovascular disease and LDL-C levels of ≥70 mg/dl while on maximally tolerated statin therapy. Patients were then randomly assigned to either evolocumab (140 mg every 2 weeks or 429 mg monthly) or placebo. The primary end point of the study was the composite of cardiovascular death, myocardial infarction, stroke, hospitalization for unstable angina, or coronary revascularization. The secondary end point was the composite of cardiovascular death, myocardial infarction, or

stroke. The median duration of follow-up was 2.2 years. Inhibition of PCSK9 with evolocumab on a background of statin therapy lowered LDL-C levels to a median of 30 mg/dl. Addition of evolocumab to statin therapy significantly reduces the risk of cardiovascular events, with a 15% reduction risk of primary composite and a 20% reduction risk of the secondary composite outcomes. Based on this study, PCSK9 inhibitor therapy should be considered for ASCVD risk reduction in patients with stable ASCVD, particularly in those with additional risk factors, on maximally tolerated statin therapy, with on-treatment LDL-C ≥70 mg/dl or non-HDL-C ≥100 mg/dl. In the same FOURIER trial, similar recommendations but with a lower level of evidence can also be applicable to patients with progressive ASCVD (additional diagnosis of MI or nonhemorrhagic stroke).

2.5 Anti-PCSK9 monoclonal antibodies in patients with statin intolerance

There is currently no universally accepted definition for statin intolerance. Previous studies have confirmed statin-related muscle side effects; however more needs to be done to accurately address the issue. That said, statin intolerance in individuals with a history of CVD is a challenge to a clinician. According to the latest National Lipid Association (NLA) recommendations, PCSK9 inhibitors may be considered to reduce LDL-C in selected very-high-risk patients who are classified as statin-intolerant, in the presence of additional LDL-C-lowering therapies [37]. The quality of evidence for such a recommendation is low.

2.6 Anti-PCSK9 monoclonal antibodies in patients with severe hypercholesterolemia

Severe hypercholesterolemia is defined as LDL-C ≥190 mg/dl. The majority of these patients have polygenic hypercholesterolemia attributed to multiple, undefined genetic factors. Familial hypercholesterolemia (both heterozygous and homozygous) is due to defined mutations yet less commonly encountered. Regardless of the etiology, long-term risk for cardiovascular diseases in all these patients is very high. However, studies have shown that particularly patients with clinically defined FH have a greater risk for cardiovascular events despite being on maximum dose of statins. Studies in patients with heterozygous familial hypercholesterolemia who received PCSK9 inhibitors (either alirocumab or evolocumab) revealed significant additional LDL-C reduction.

Based on the current data, the National Lipid Association is recommending addition of PCSK9 inhibitors in patients with LDL-C ≥190 mg/dl with additional risk factors or genetic confirmation of FH on maximally tolerated statin ± ezetimibe.

2.7 Intravascular ultrasound trial

In order to determine the effects of PCSK9 inhibitors (evolocumab) on progression of coronary atherosclerosis in statin-treated patients, the Global Assessment of Plaque Regression with a PCSK9 Antibody as Measured by Intravascular Ultrasound (GLAGOV) multicenter, double-blind, placebo-controlled, randomized clinical trial was conducted [38]. In this trial 968 patients with angiographic coronary disease were studied. These patients were randomized to receive monthly 420 mg evolocumab or placebo via subcutaneous injection for 76 weeks, in addition to moderate- or high-intensity statins. In this trial, the primary efficacy was the change in percent atheroma volume from baseline to week 78, measured by serial intravascular ultrasonography (IVUS) imaging. Secondary efficacy was measured

in normalized total atheroma volume and patients demonstrating plaque regression. Interestingly, IVUS showed atherosclerosis regression during 18 months of therapy in patients treated with the combination of evolocumab and statins and absence of regression in patients treated with statin alone. Additionally, the evolocumab group showed a significant reduction in percent atheroma volume as well as total atheroma volume (−0.95%, $p < 0.01$, between group difference, −4.9 mm^3, $p < 0.01$, respectively). Taken together, PCSK9 inhibitors produce additional benefits on coronary disease progression in statin-treated patients.

2.8 Efficacy and safety of anti-PCSK9 monoclonal antibodies

The most recent study to evaluate the effects of alirocumab on the occurrence of cardiovascular events in patients who have experienced an acute coronary syndrome (ODYSSEY OUTCOMES) suggested a reduction in mortality with alirocumab in patients after acute coronary syndrome [39].Based on the results from multiple trials, alirocumab reduced the risk of all-cause mortality: 6 fewer deaths per 1000 patients treated (RR, 0.82; 95% CI 0.72–0.95). No reduction in cardiovascular mortality was observed (RR, 0.87; 95% CI, 0.73–1.02) [40]. On the other hand, trials of evolocumab did not show a reduction in either all-cause or cardiovascular mortality (RR, 0.91; 95% CI, 0.57–1.44 and RR, 1.04; 95% CI, 0.88–1.24, respectively). Taken together, PCSK9 inhibitors did not reduce the risk of all-cause or cardiovascular mortality.

Both alirocumab and evolocumab reduced the risk of myocardial infarction, stroke, and coronary revascularization. Alirocumab, but not evolocumab, reduced stable angina hospitalization, and neither drug reduced heart failure.

Regarding the safety of anti-PCSK9 monoclonal antibodies (alirocumab and evolocumab), all phase 2 and 3 studies have demonstrated excellent safety profile [41]. The most commonly reported adverse effect has been injection site reaction [42]. As part of the safety analysis, fat-soluble vitamin concentrations (A, D, E, K) were measured. Compared to placebo, no change in the levels of any of these vitamins was reported. Additionally, no increase in neurocognitive events, new-onset or worsening diabetes, muscle-related events, or myalgia has been noted [43].

Bococizumab has been excluded from most of these studies as the drug is no longer available after the cessation of development by the manufacturer due to high rates of neutralizing antibody formation and subsequent loss of therapeutic efficacy (SPIRE trial) [44].

3. Ezetimibe as cholesterol-lowering therapy

Since the early 1990s when statins were initially introduced, several large clinical trials have highlighted the benefits of their use with beneficial effects above and beyond lipid lowering [45]. Statins are currently the cornerstone of hyperlipidemia treatment. However, due to safety concerning high-dose therapy as well as residual risk of CVD especially in high-risk patients, additional lipid-modifying therapies have emerged in the last decades.

Ezetimibe reduces absorption of cholesterol from the brush border of the small intestine by targeting the Niemann-Pick C1-like 1 (NPC1L1) protein (**Figure 2**) [46, 47]. Genetic studies have shown that polymorphisms affecting NPC1L1 are associated with lower levels of LDL cholesterol and a lower risk of cardiovascular diseases [48]. A decrease in cholesterol absorption results in a decrease of total cholesterol, triglycerides, and LDL cholesterol and an increase in HDL cholesterol.

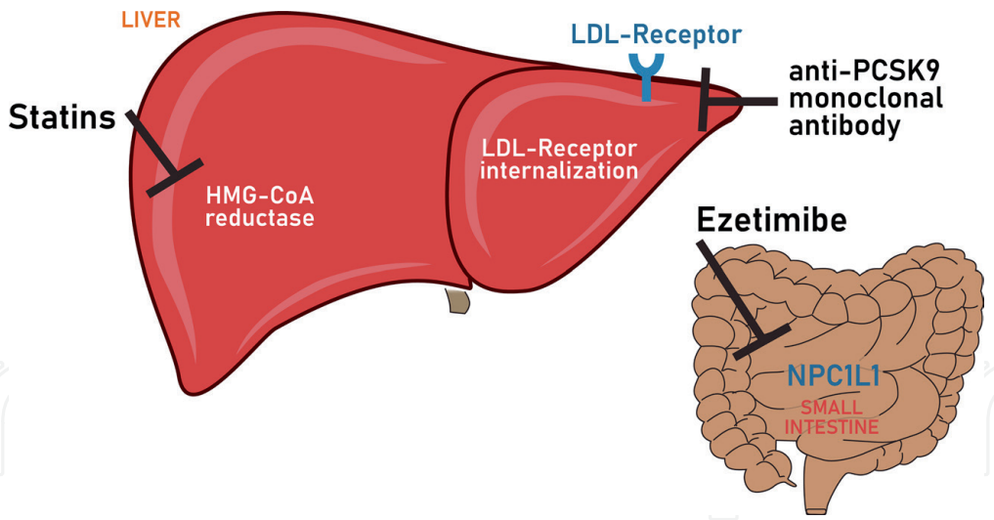

Figure 2.
Ezetimibe reduces absorption of cholesterol from the brush border of the small intestine by targeting the Niemann-Pick C1-like 1 (NPC1L1) protein. In the liver, HMG-CoA reductase (statins) promotes the intracellular synthesis of cholesterol as well as increases LDL-R and PCKS9 levels. PCSK9 monoclonal antibodies would neutralize the PCSK9 molecules floating in the plasma, thus increasing LDL-R recycling and surface LDL-R in the liver.

It was FDA-approved in 2002 as an agent to treat people with hyperlipidemia. Ezetimibe given as monotherapy leads to an LDL reduction of approximately 20%. When added to statins, ezetimibe reduces LDL cholesterol levels by an additional 23–24% on average [49, 50]. The Improved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT) was then conducted to understand if further lowering of the LDL-C levels by statin-ezetimibe dual therapy leads to clinical benefit [51]. In this trial they studied the effect of ezetimibe combined with simvastatin, as compared with that of simvastatin alone, in stable patients who had an acute coronary syndrome and whose LDL cholesterol values were within the guideline recommendations. The primary end points were death from cardiovascular disease, a major coronary event, or nonfatal stroke, assessed from the time of randomization until the first occurrence of one of the events. The combination of simvastatin and ezetimibe resulted in additional lowering of LDL-C levels as well as lower risk of cardiovascular event. A reduction of cholesterol levels of 12.8 mg per deciliter correlated with a proportionally 7.2% lower rate of major vascular events, providing further evidence for a relationship between lower lipid and improved outcomes.

Multiple statin trials including the CTT collaboration have shown clinical benefits when LDL cholesterol was lowered to progressively lower levels. It was on the basis of these trials that a LDL-C of <70 mg per deciliter has been recommended for patients with acute coronary syndrome. Data from the IMPROVE-IT trial support the previous finding and show a direct evidence between further lipid lowering and improved outcomes [51]. Additionally, this trial provides evidence, for the first time, that a non-statin lipid-lowering agent can reduce cardiovascular risk by lowering LDL-C and that statins are not the only beneficiary drugs in hyperlipidemia. The American College of Cardiology recommends consideration of ezetimibe therapy in addition to maximally tolerated statin therapy for both primary and secondary prevention in patients who have not achieved target reduction in their LDL-C by maximally tolerated statin therapy alone.

3.1 Administration, side effects, and contraindications of ezetimibe

Ezetimibe is currently marketed as a monotherapy under the trade name of Zetia, as well as in combination with a statin such as Vytorin and Liptruzet. It has a

half-life of 22 hours, and it can be administered at 10 mg daily. It can be taken at the same time as fenofibrates or statins, but it is recommended to take it at least 2 hours before or 4 hours after taking bile acid sequestrants.

Based on current studies, very few side effects to ezetimibe have been reported, most common being headache, runny nose, and sore throat [52]. Rhabdomyolysis has been reported only in combination with statin therapy and rarely with monotherapy. Due to its daily dosing and limited side effects, ezetimibe is considered a safer drug with no compliance issues. Contraindications to its use include hypersensitivity to possible ingredients of the formulation. It is not recommended in patients with moderate to severe hepatic impairment but can be administered in patients with renal impairment without any need for dose adjustment. Liver function tests need to be performed only if it is administered with a statin. It is worth mentioning that patients taking ezetimibe with cyclosporine are at an increased risk of ezetimibe toxicity as it can result in 2.3–12-fold increase in exposure [53]. In these cases, cyclosporine concentrations should be closely monitored.

3.2 Ezetimibe versus anti-PCSK9 monoclonal inhibitor (evolocumab) as add-on therapy for secondary prevention of cardiovascular events

When results from trials of ezetimibe (IMPROVE-IT) and PCSK9 inhibitors (FOURIER RCT) are compared in regard to secondary prevention of cardiovascular events in patients with ASCVD and type 2 diabetes, evolocumab (PCSK9 inhibitor) seems to be more effective [54]. Interestingly, as of December 2018, the annual cost of evolocumab is $6540 and $88 for ezetimibe. From a healthcare cost standpoint, ezetimibe has a significantly lower cost and may be considered a preferred add-on therapy for these patients. The difference in avoiding cardiovascular events between the two therapies is negligible compared with the significant difference in the drug costs. However, a randomized controlled trial (RCT) comparing evolocumab and ezetimibe would be necessary to evaluate and compare the effectiveness of these two drugs. Additionally, long-term effects of these novel drugs in reducing the cardiovascular events in patients with ASCVD and type 2 diabetes will be needed.

4. Conclusions

Dyslipidemia is a major risk factor for both fatal and nonfatal CVD. Lowering cholesterol levels particularly LDL-C, through lifestyle modifications as well as pharmacological interventions, is crucial for CVD risk reduction. Currently, statins are the cornerstone medication for treatment and primary prevention of hypercholesterolemia. Studies have shown that benefits from statins outweigh any possible adverse effects. However, as the intensity of statin therapy increases, intolerance to their use becomes more prominent. Additionally, high-risk patients on maximally tolerated statins may benefit from novel LDL-C-lowering therapies. Clinical trials have provided evidence that these novel drugs such as PCSK9 inhibitors or ezetimibe can successfully lower LDL-C in levels and contribute in lowering cardiovascular events in high-risk patients with elevated LDL-C on maximally tolerated statins. Both ezetimibe and PCSK9 inhibitors have demonstrated a modest absolute ASCVD risk reduction and good safety profiles. However, given cost considerations particularly for PCSK9 inhibitors, healthcare providers will need to carefully consider the subgroup of patients benefiting the most from their use.

Conflict of interest

The author declares no conflict of interest.

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