

CARDIOVASCULAR THERAPEUTICS

Proprotein Convertase Subtilisin/Kexin Type 9 Inhibitors: Ready to Target Atherosclerotic Cardiovascular Disease beyond Statins

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ABSTRACT

Dyslipidemia contributes to 50% of atherogenic cardiovascular events (CVEs). Statins decrease low-density lipoprotein cholesterol (LDL-C) on an average of 1 mmol and this is transformed into 20 to 25% reduction in CVE. Proprotein convertase subtilisin/kexin type 9 (PCSK9) fully humanized monoclonal antibodies (MoAbs) decrease LDL-C by another 1 to 1½ mmol on top of statins and this decreases CVE by another 20%. Therefore, the era has come when we are able to minimize the dyslipidemia-related atherogenic risk to a very great extent. The PCSK9 MoAbs require 12 to 26 injections per year. Inclisiran, which is a small interfering ribonucleic acid (siRNA), has shown to decrease LDL-C consistently for 6 months after a single injection. It is therefore emerging as a very important competitor to PCSK9 MoAbs. The future ongoing trials will tell us more about this molecule.

Keywords: Alirocumab, Proprotein convertase subtilisin/kexin type 9 inhibitors evolocumab, Small interfering ribonucleic acid.

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INTRODUCTION

Dyslipidemia is a major modifiable risk factor for atherosclerotic CVD (ASCVD). As per the World Health Organization, 50% of CVEs are attributed to dyslipidemia (Graph 1) and the rest 50% is related to nonlipid atherogenic risk factors.

Figure 1 shows the evolution of lipid guidelines during the last 29 years ever since Adult Treatment Panel-1 (ATP-1) came into existence in 1987.

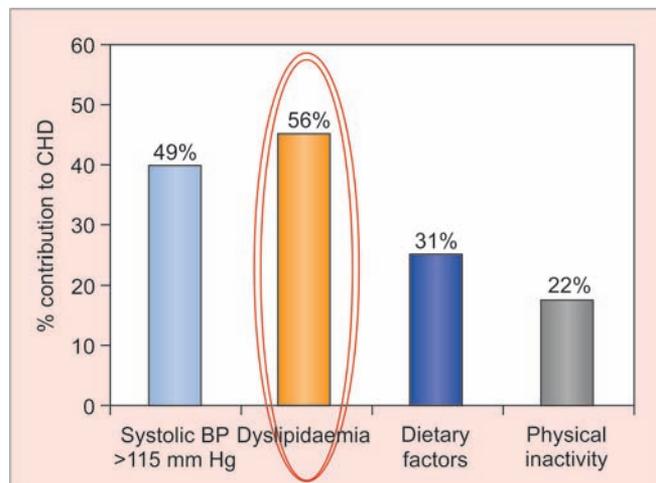
Among lipids, LDL-C and nonhigh-density lipoprotein cholesterol (HDL-C) are the principal targets.

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Graph 1: Relation of lipid to coronary heart disease

Apolipoprotein B (ApoB) is also a target but usually it is not utilized due to lack of facilities for estimating it, and an idea about its level is obtained from non-HDL-C. All trials of HDL-C elevation on top of statins have been negative and there is no evidence-based target for it. In fact, HDL-C is fallen angel.

For several years LDL-C goal <70 has been recommended for the very high risk group. The Lipid Association of India has slashed down this goal to <50¹ and the American Association of Clinical Endocrinologists (AAACE) guidelines 2017² to <55 mL/dL (Tables 1 and 2). The goals for non-HDL-C are 30 mg/dL more than the LDL-C goal.

TRIGLYCERIDES

Triglycerides (TGs) may be playing an important role in Indian dyslipidemia but this has not been properly investigated in the Indian context. Data on TGs show that low TG levels with loss of function mutation ApoC3³ and angiopoietin-like protein 4 (ANGPTL4)⁴ are associated with decreased coronary heart disease, so increased TG levels are associated with increased cardiovascular and all-cause mortality. But we do not have any randomized controlled trial which has shown benefit of lowering TG on top of statins. The Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial,⁵ which tested efficacy of fibrates on top of statins in diabetic patients, failed to

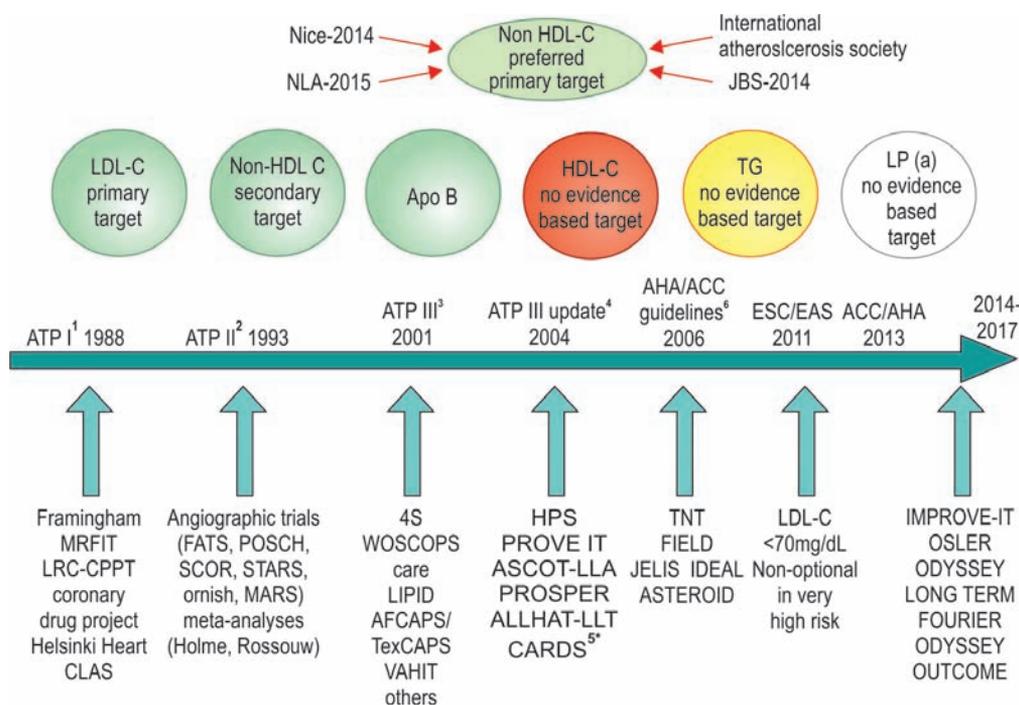


Fig. 1: Evolution of lipid treatment guidelines during last 29 years after ATP-1

Table 1: Treatment goals for LDL-C and non-LDL-C as per Lipid Association of India

Risk category	Treatment Goal		Consider drug therapy	
	LDL-C (mg/dL)	Non-HDL-C (mg/dL)	LDL-C (mg/dL)	Non-HDL-C (mg/dL)
Very high risk	<50	<80	≥50 (preferably in all)	≥80 (preferably in all)
High risk	<70	<100	≥70 (preferably in all)	≥100 (preferably in all)
Moderate risk	<100	<130	≥100	≥130
Low risk	<100	<130	≥130*	≥160*

*After an initial adequate nonpharmacological intervention for at least 3 months

Table 2: American association of clinical endocrinologists guidelines 2017

Risk levels	High desirable levels*	Very high desirable levels**	Extreme desirable levels***
LDL-C (mg/dL)	<100	<70	<55
Non-HDL-C (mg/dL)	<130	<100	<80
TG (mg/dL)	<150	<150	<150
ApoB (mg/dL)	<90	<80	<70

DM: Diabetes mellitus; *High: DM but no other major risk and/or age <40; **Very high: DM + major ASCVD risk(s) (HTN, Fam Hx, low HDL-C, smoking, CKD3,4); ***Extreme: DM plus established clinical CVD

show any benefit. Diabetologists often use fibrates based on subgroup analysis of the ACCORD trial⁵ and meta-analysis of fibrate trials but the problem with this data is that subgroup analysis of ACCORD trial in patients with raised TG >204 mg/dL and low HDL-C <34 mg/dL did show a benefit but subgroup analysis is only hypothesis generating and the finding needs to be confirmed in a large randomized trial which is nonexistent at the present state of time. Likewise, in the meta-analysis of fibrate trials,⁶ these drugs were not used on top of statins in all trials. It is important to realize that it is ApoB particles which enter the vessel wall and not TG *per se*. The risk of atherosclerosis is related to the number of atherogenic

particles and each atherogenic lipoprotein particle contains a single molecule of ApoB. Therefore, the concentration of ApoB provides a direct measure of the number of circulating atherogenic particles. In most hypertriglyceridemic (HyperTG) patients including diabetics, LDL particles make up 85 to 90% of total ApoB particles and the contribution of very low density lipoprotein is only 10 to 15%. Fibrates lower LDL particles by only about 10% and therefore fibrates at most may only play a minor role. The Quebec cardiovascular study also showed that if there is HyperTG with normal ApoB levels, there is no increase in odds ratio of ischemic heart disease (IHD) but if there is HyperTG with increased ApoB levels, the



Table 3: Drugs for treatment of dyslipidemia

<i>LDL-C</i>	<i>Non-HDL-C</i>	<i>TG</i>	<i>HDL</i>
Statins	Intensify statin	Fibrate	No drug recommended
Ezetimibe	Fibrates	Omega 3 fatty acid	
PCSK9 inhibitors	Omega 3 fatty acid	Saroglitazar [#]	
• Evolocumab			
• Alirocumab			
Colesevelam			
Lomitapide (for FH)			
Mipomersen (for FH)			
siRNA inclisiran ⁺			

[#]Available only in India, not approved by the FDA; ⁺Under evaluation

odds ratio of IHD are significantly increased. Dietary intake of fish and omega-3 fish oil is associated with reductions in the risks of total mortality, sudden death, and CAD through various mechanisms of action other than lowering of LDL-C.

Besides statins, several other drugs are used for the treatment of dyslipidemia. The drugs utilized for modulating dyslipidemia are outlined in Table 3.

Statins are the first line agents for targeting dyslipidemia. The Cholesterol Treatment Trialists (CTT) Meta-analysis Collaboration has shown that high-intensity statins on an average decrease LDL-C by 1 mmol and this reduction is seen across the range of LDL-C and this translates into reduction of CVE by 20 to 25% approximately irrespective of baseline LDL-C.

We have already witnessed the revolutionary era of statins for last 30 years and indeed statins have emerged as uncontested king for lipid management. We have voluminous data on statins and they are class IA recommendation for primary as well as secondary prevention of coronary artery disease. They are highly effective drugs and have minimal side effects. The year 2015 came with breaking news when two PCSK9 inhibitors, evolocumab and alirocumab, were approved for clinical use by the European Medicines Agency (EMA) and United States Food and Drug Administration (USFDA). With this a new era has started in lipid management. The PCSK9 inhibitors decrease LDL-C by another 1 to 1½ mmol and this is expected to decrease CVE by another 20 to 25% as per the analysis of Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk (FOURIER), Studies of PCSK9 Inhibition and the Reduction of Vascular Events (SPIRE), and Mendelian randomization study. Therefore, with combination of both drugs the dyslipidemia-related atherogenicity can be minimized to a great extent. The effect of reduction in LDL-C on CVEs is shown in Table 4.

Table 4: Effect of LDL-C reduction on atherogenic CVEs

<i>Atherogenic risk</i>				
<i>Nonlipid related</i>	<i>Lipid related</i>	<i>Reduction in LDL-C</i>	<i>Drug utilized</i>	<i>Decrease in CVEs</i>
50%	50%	1 mmol	High-intensity statins	25%
		2 mmol	Statins + PCSK9 inhibitors	45%

NONSTATIN DRUGS

In selected high-risk patients, such as those with existing ASCVD or patients with familial hypercholesterolemia (FH) not achieving LDL goals, use of nonstatins may be considered if maximally tolerated statin therapy has not shown >50% reduction in LDL-C from baseline.

Ezetimibe is the first nonstatin medication that should be considered in most of such patient scenarios, given its safety and tolerability, though modest efficacy, when added to moderate-dose statin as shown in the IMPROVE IT Trial.⁷

Bile acid sequestrants (BASs) may be considered as second-line therapy for patients in whom ezetimibe is not tolerated, but they should be avoided in patients with TGs >300 mg/dL.

Familial Hypercholesterolemia

For patients with homozygous hypercholesterolemia the goals are difficult to achieve. Statins, and nonstatins drugs, including ezetimibe, BASs, are utilized. The new drugs like lomitapide⁸ and mipomersen⁹ are used if necessary. Low-density lipoprotein-apheresis is approved for heterozygous FH.

The PCSK9 inhibitors, like evolocumab and alirocumab, may be considered if the goals of therapy have not been achieved on maximally tolerated statin and ezetimibe in FH. The PCSK9 inhibitors are also used in high-risk patients with clinical ASCVD.

What is PCSK9?

Proprotein convertase subtilisin/kexin type 9 is a protein that regulates LDL-C levels in the blood by regulating low-density lipoprotein receptors (LDL-R). It is secreted from the liver, goes into the blood, circulates back to liver, and directly binds to LDL-R increasing their degradation and thereby reducing the rate at which LDL-C is removed from circulation¹⁰ and thus increasing LDL-C levels in blood. Thus, PCSK9 is an important regulator of LDL metabolism. The PCSK9 is also affected by genetic mutation. There are two types of mutation: Loss of function mutation which results in decrease in LDL-C and provides atheroprotection; the gain of function mutation¹¹ causes FH and predisposes to ASCVD.

Both statins and ezetimibe¹² increase secretion of PCSK9, which could attenuate their efficacy by reducing the amount of cholesterol cleared from circulation. This explains the limitation of statin therapy and may be the best explanation regarding the classic rule of 6 observed with statin therapy. This rule refers to the fact that every time the statin dose is doubled, there is only an approximately 6% complementary decrease in LDL-C levels. Therefore, the combination of PCSK9 inhibition with a statin would be a sensible and logical approach and will cause a dramatic decrease in LDL-C levels.

Proprotein Convertase Subtilisin/Kexin Type 9 Inhibition

PCSK9 inhibition is now a validated therapeutic option for modulating LDL-C after the results of the FOURIER trial in 2017. Its inhibition can be achieved by MoAbs, gene silencing, and small peptides.¹³ The MoAbs are commonly used to inhibit PCSK9. Both evolocumab and alirocumab have been approved for clinical use in 2015 by EMA and USFDA.

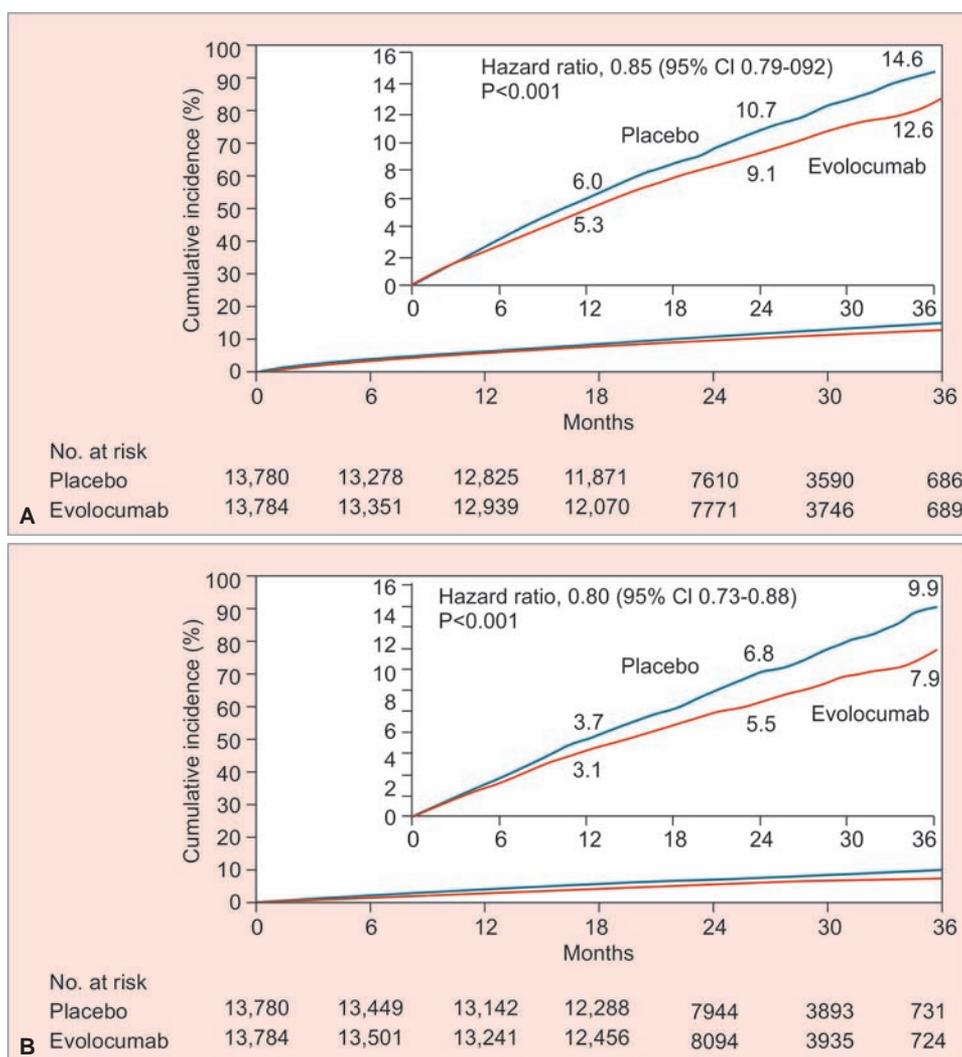
Evolocumab

This has already been approved for clinical use and is available commercially as 1 mL pen containing 140 mg. It is given in doses of 140 mg biweekly/420 mg monthly subcutaneously. It was tested in the Program to Reduce LDL-C and Cardiovascular Outcomes (PROFICIO) Global Program, which had 14 trials and roughly 30,000 patients. Most of the trials have been completed and the results of FOURIER were presented in 2017. Most of these trials have shown significant reduction in LDL-C by 40 to 60% on top of statins. There is also consistent and robust reduction in other lipoproteins. The lipoprotein Lp(a) is reduced by approximately 25%, TG and non-HDL-C are also decreased. The HDL-C and Apo-A1 are increased. The drug is used in the doses of 120 mg biweekly or 420 mg monthly subcutaneously.

Interestingly, when both statins and PCSK9 MoAbs are combined together, they decrease LDL to very low levels, as low as 25 mg/dL in a substantial number of cases. Two questions automatically erupt out of this. Is very low LDL-C safe and is it powered to produce incremental benefits on top of statins? The answer to both questions is yes because the FOURIER trial¹⁴ has proved both safety and efficacy of evolocumab. We had safety data from earlier trials also, like Open-Label Study of Long-Term Evaluation against LDL Cholesterol (OSLER), Evaluation of Cardiovascular Outcomes after an Acute Coronary Syndrome during Treatment with Alirocumab (ODYSSEY) long-term trials. In FOURIER study, LDL-C decreased from median baseline value 92 to 30 mg/dL, i.e., approximately by 60% ($p < 0.001$), 42% had LDL-C < 25 mg/dL. Despite such low levels of LDL-C, there was no evidence of muscle or liver toxicity, diabetogenicity or neurocognitive decline. The dedicated substudy, Evaluating PCSK9 Binding antiBody Influence on Cognitive Health in High cardiovascular Risk Subjects (EBBINGHAUS), also confirmed lack of neurocognitive decline with its use. The side effects which were more common with evolocumab were injection site reactions, 2.1% (evolocumab) vs 1.6% (placebo). Upper respiratory tract infections, nasopharyngitis, flu, back pain were observed in small number of patients. However, long-term use of these agents in future will further clarify regarding their safety.

We had the initial efficacy data from the OSLER and ODDYSEY long-term, and recently from the FOURIER trial. The OSLER trial showed 53% relative risk reduction (RRR) in the composite endpoint of death, myocardial infarction (MI), unstable angina hospitalization, coronary revascularization, stroke, transient ischemic attack, or congestive heart failure hospitalization [heart rate (HR) = 0.47; 95% confidence interval (CI) 0.28–0.78, $p = 0.003$] and the *post hoc* analysis of long-term ODYSSEY showed a 48% RRR reduction in mean adverse cardiac events (HR = 0.52; CI 0.31–0.90; $p = 0.02$). This year the FOURIER trial showed a statistically significant 15% risk reduction in the primary endpoint of CV death, MI, stroke, hospitalization for unstable angina, and revascularization (HR = 0.85; 95% CI, 0.79–0.92, $p < 0.001$). The secondary endpoint of CV death and stroke showed a statistically significant reduction of 20% reduction (HR = 0.80; 95% CI, 0.73–0.88, $p < 0.001$) (Graph 2).

The curves are divergent so that the risk reduction at 2 years translated to number needed to treat of 74 to prevent CVD, MI, stroke but at 3 years it decreased to 50. It seems that the future is brighter than the present, and long-term follow-up may lead to more fruitful results. The target of < 70 for the high-risk patients has been scrapped by the FOURIER trial. There was no J curve, so lower is



Graphs 2A and B: Primary and secondary efficacy endpoint in the FOURIER trial

better is also validated for very low LDL. The reduction in primary and key secondary endpoint was consistent across all key subgroups, including age, sex, different types of CVD, intensity of statin therapy, dosing regimen of evolocumab, and baseline LDL levels, including those with the lowest quartile of LDL-C starting at 74 mg/dL in whom evolocumab reduced LDL down to 22 mg/dL. The FOURIER trial showed reduction in MI by 27%, $p \leq 0.001$, stroke by 21%, $p = 0.01$, and coronary revascularization by 22%, $p \leq 0.001$. There was no significant decrease in all-cause or CV mortality.

Alirocumab

This is approved for clinical use in the United States and is commercially available as 1 mL pen containing 75 or 150 mg. It is given in doses of 75 mg biweekly or 150 mg subcutaneously. It was evaluated in ODYSSEY Global Program, which included 11 trials and roughly has 22,000 patients. Most of the trials have been completed except ODYSSEY outcome trial in post ACS patients, the results of which are keenly awaited.

Bococizumab

The SPIRE 1 and 2 with bococizumab was prematurely terminated because of the presence of neutralizing antibody in 29% and antidrug antibodies in 48% of patients taking the drug. This is because bococizumab is a partially murine MoAb unlike evolocumab and alirocumab, which are fully humanized MoAbs. The incidence of antidrug antibody and neutralizing antibody to various PCSK9 MoAbs is mentioned in Table 5.

Although both SPIRE I and SPIRE II trials were prematurely terminated, the SPIRE II which had LDL-C > 100 mg/dL showed a reduction in CVEs by 21% at 12 months, hinting that the drug is also useful for primary

Table 5: Incidence of neutralizing antibodies to PCSK9 MoAbs

Drug	Antidrug antibody (%)	Neutralizing antibody (%)
Bococizumab	48	29
Evolocumab	0	0.3
Alirocumab	5	1.3

Table 6: Indications of PCSK9

Failure to achieve LDL-C goals despite optimal doses of statins in patients with ASCVD
Statin intolerance
FH

prevention. The SPIRE I trial which enrolled cooperatively lower risk population (LDL-C >70) did not show any benefit.

The current indications of PCSK9 MoAbs are outlined in Table 6.

Given the lack of long-term safety and efficacy data on these agents, they are not recommended for use for primary prevention except in patients with FH. The data of PCSK9 for primary prevention like high-risk diabetics are yet to evolve out.

Future Developments in PCSK9 Inhibition

Despite exciting results of FOURIER trials, the trialists realized two problems with PCSK9 MoAbs, i.e., the drug requires 12 to 26 injections per year and adherence data with PCSK9 MoAbs showed no substantial improvement over statins. Due to this, attempts were made to explore other ways of inhibiting PCSK9. Inhibiting the synthesis of PCSK9 by siRNA (inclisiran) targeted to PCSK9 showed sustained reduction of LDL-C >50% (Graph 3) lasting for 6 months (mean LDL reduction of 52.6% at 180 days and 48% of patients had LDL-C below 50 mg/dL).¹⁵ The side effects are seen only in minority of patients and include cough, musculoskeletal pain, nasopharyngitis, headache, backache, and diarrhea.

After the exciting results of this molecule, the FDA has cleared Phase III trials for inclisiran. It seems that

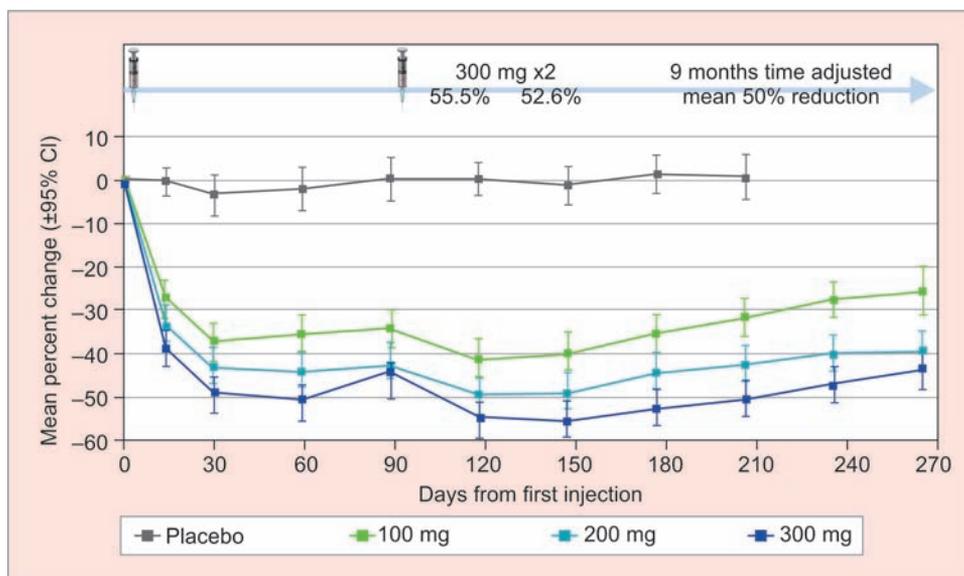
inclisiran may emerge as a strong competitor for evolocumab and alirocumab in future.

CONCLUSION

Dyslipidemia is a major modifiable risk factor for ASCVD. Statin trials have validated LDL-C; the lower is better and they decrease lipid-related CVEs by 20 to 25%. The new drug PCSK9 MoAbs has validated that further lowering of LDL-C as low as 25 mg/dL produces incremental benefit with incredible safety as shown in the FOURIER trial. It therefore seems that the goal of LDL-C will be further slashed down in future.

REFERENCES

1. Iyengar SS, Puri R, Narasingan SM, Wangnoo SK, Mohan V, Mohan JC, Misra A, Sriram U, Dalal JJ, Gupta R, et al. LAI Expert Consensus Statement on Management of Dyslipidemia in Indians 2016. JAPI 2016 Mar;64:7-52.
2. Garber AJ, Abrahamson MJ, Barzilay JI, Blonde L, Bloomgarden ZT, Bush MA, Dagogo-Jack S, DeFronzo RA, Einhorn D, Fonseca VA, et al. Consensus statement by the American association of clinical endocrinologists and American college of endocrinology of the comprehensive type 2 diabetes management algorithm – 2017 executive summary. Endocr Pract 2017 Feb;23(2):207-227.
3. TG and HDL Working Group of the Exome Sequencing Project, National Heart, Lung, and Blood Institute; Crosby J, Peloso GM, Auer PL, Crosslin DR, Stitzel NO, Lange LA, Lu Y, Tang ZZ, Zhang H, et al. Loss-of-function mutations in APOC3, triglycerides, and coronary disease. N Engl J Med 2014 Jul;371(1):22-31.
4. Folsom AR, Peacock JM, Demerath E, Boerwinkle E. Variation in ANGPTL4 and risk of coronary heart disease: the atherosclerosis risk in communities study. Metabolism 2008 Nov;57(11):1591-1596.



Graph 3: ORION-1 trial showing effect of inclisiran on LDL-C



5. The ACCORD Study Group. Effects of combination lipid therapy in type 2 diabetes mellitus. *N Engl J Med* 2010 Apr;362(17):1563-1574.
6. Lee M, Saver JL, Towfighi A, Chow J, Ovbiagele B. Efficacy of fibrates for cardiovascular risk reduction in persons with atherogenic dyslipidemia: a meta-analysis. *Atherosclerosis* 2011 Aug;217(2):492-498.
7. Cannon CP, Blazing MA, Giugliano RP, McCagg A, White JA, Theroux P, Darius H, Lewis BS, Ophuis TO, Wouter Jukema J, et al; for the IMPROVE-IT Investigators. Ezetimibe added to statin therapy after acute coronary syndromes. *N Engl J Med* 2015 Jun;372:2387-2397.
8. Neef D, Berthold HK, Gouni-Berthold I. Lomitapide for use in patients with homozygous familial hypercholesterolemia: a narrative review. *Expert Rev Clin Pharmacol* 2016 Mar;9(5):655-663.
9. Stein EA, Dufour R, Gagne C, Gaudet D, East C, Donovan JM, Chin W, Tribble DL, McGowan M. 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 Nov;126(19):2283-2292.
10. Yamamoto T, Lu C, Ryan RO. A two-step binding mode of PCSK9 interaction with the low density lipoprotein receptor. *J Biol Chem* 2011 Feb;286(7):5464-5470.
11. Abifadel M, Varret M, Rabes JP, Allard D, Ouguerram K, Devillers M, Cruaud C, Benjannet S, Wickham L, Erlich D, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. *Nat Genet* 2003 Jun;34(2):154-156.
12. Ason B, Tep S, Davis HR Jr, Xu Y, Tetzloff G, Galinski B, Soriano F, Dubinina N, Zhu L, Stefanni A, et al. Improved efficacy for ezetimibe and rosuvastatin by attenuating the induction of PCSK9. *J Lipid Res* 2011 Apr;52(4):679-687.
13. Marais DA, Blom DJ, Petrides F, Goueffic Y, Lambert G. Proprotein convertase subtilisin/kexin type 9 inhibition. *Curr Opin Lipidol* 2012 Dec;23(6):511-517.
14. Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, Kuder JF, Wang H, Liu T, Wasserman SM, et al; for the FOURIER Steering Committee and Investigators. Evolocumab and clinical outcomes in patients with cardiovascular disease. *N Engl J Med* 2017 May;376:1713-1722.
15. Ray KK, Landmesser U, Leiter AL, Kallend D, Dufour R, Karakas M, Hall T, Troquay RP, Turner T, Visseren FL, et al. Inclisiran in patients at high cardiovascular risk with elevated LDL cholesterol. *N Engl J Med* 2017 Apr;376:1430-1440.