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Inclisiran: new era of lipid-lowering therapy?

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SOUHRN

Dyslipidemie je nejčastější metabolickou poruchou v západní společnosti a hlavním rizikovým faktorem pro rozvoj ischemické choroby srdeční (ICHS). Léčba dyslipidemie je stále nedostatečná a doporučené cíle LDL cholesterolu jsou dosahovány jen u malé části pacientů. Nejdůležitější skupinou léků pro léčbu dyslipidemie jsou statiny. Ačkoli jde zřejmě o jednu z nejbezpečnějších lékových skupin vůbec, terapie statiny může vést ke svalovým bolestem a ke zvýšenému riziku rozvoje diabetes mellitus (DM). Přerušení nebo ukončení léčby statiny je v klinické praxi časté, statiny jsou také často předmětem negativních sdělení v médiích. Inklisiran je modifikovaný oligonukleotid fungující na principu interference RNA. Inhibuje translaci mRNA pro proprotein konvertázu subtilisin/kexin typu 9 (PCSK9), což vede k dlouhodobému poklesu koncentrace LDL cholesterolu. Účinek inklisiranu se snižuje o přibližně 2 % měsíčně, takže efekt jediné dávky trvá zhruba dva roky. Při podávání jedenkrát za šest měsíců vede terapie tímto lékem k poklesu LDL cholesterolu o 50–55 %, u vybraných pacientů potom až o 65 %. Terapie inklisiranem není spojena se změnou počtu krevní destičky, lymfocytů, monocytů nebo neutrofilů, nevede ke změnám koncentrací cirkulujících cytokinů tumor nekrotizujícího faktoru alfa (TNFα) nebo interleukinu 6 (IL-6) a není spojena s indukcí tvorby protilátek proti tomuto léku. Nežádoucí reakce v místě vpichu tak zůstávají jediným relevantním nežádoucím účinkem této léčby. Inklisiran tak získává potenciál stát se široce preskribovaným lékem, který výrazně přispěje k léčbě dyslipidemie a v konečném důsledku povede ke snížení incidence a prevalence ICHS.

ABSTRACT

Dyslipidemia is the most frequent metabolic abnormality in western world population and a major risk factor for coronary artery disease (CAD) development. Dyslipidemia treatment is still insufficient and achieving target LDL-level infrequent. Statins are a cornerstone of dyslipidemia management. Although statins are one of the safest drugs on the market, statin therapy can be associated with muscle symptoms and increased risk of DM development. Statin discontinuation is frequent, often driven by negative media coverage. Inclisiran is a therapeutic oligonucleotide that employs RNA interference and inhibits the translation of PCSK9-mRNA leading to long-lasting LDL-cholesterol lowering. LDL-cholesterol-lowering effect of inclisiran is reversed at the rate of approximately 2% per month, so the effect of a single dose persists up to approximately two years. When administered once every six months, inclisiran therapy decreases the level circulating LDL-cholesterol by 50–55% and up to 65% in selected patient populations.
Inclisiran therapy is not associated with alterations in platelet count, blood lymphocyte, monocyte or neutrophil count, does not cause alterations in blood TNF-alpha or IL-6 concentrations and does not induce relevant anti-drug antibodies. The only relevant side effect is an adverse reaction at the injection site. Inclisiran thus has the potential to become widely prescribed drug that will greatly contribute to dyslipidemia management and further decrease of CAD incidence.

Keywords:
Atherosclerosis
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LDL-cholesterol

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Despite a significant decline in CAD incidence,[1,2] CAD still remains a major public health issue and was (until the pandemic of covid-19) the most frequent cause of death.

Dyslipidemia

Dyslipidemia is a major risk factor for CAD development.[3] Dyslipidemia (defined as total cholesterol ≥5 mmol/L or HDL-cholesterol <1.0 mmol/L for males or <1.2 mmol/L for females or LDL-cholesterol ≥3 mmol/L or triacylglyceroles ≥1.7 mmol/L) was present in 81.0% of males and 70.6% of females with the increase with advancing age.[4] In patients between 55–65 years, dyslipidemia was present in almost 90% of population. At the same time, only a small percentage of population (12.7% of males and 8.5 % of females) was treated with lipid-lowering agents. ESC Guidelines for the management of dyslipidemias published and ESC Guidelines on cardiovascular disease prevention propose that in patients with established atherosclerotic cardiovascular disease (ASCVD) or at a very high risk, LDL-C reduction ≥50% and a target level <1.4 mmol/L are to be achieved.[5,6] This is in sharp contrast with real-world evidence; according to latest EURASPIRE V study, 71% of patients after coronary event had LDL-C ≥1.8 mmol/L.[7]

These unsatisfactory results were further confirmed by recently published study showing that only 17% and 22% patients with a very high risk in primary and secondary prevention, respectively, met the new 2019 goals.[8]

Dyslipidemia treatment

Statins are cornerstone of dyslipidemia management. Statin therapy is extremely potent, a large meta-analysis of 26 statin trials showed that a reduction of 1 mmol/L in LDL-cholesterol over 5 years reduced major vascular events by 22%, CHD death by 20% and all-cause mortality by 10%.[9] When ezetimibe and PCSK9-inhibitors were taken into account as well, each 1 mmol/L reduction in LDL-cholesterol was associated with a 19% relative risk (RR) reduction for major vascular events.[10] Although statins are one of the safest drug groups, several issues remain. Statin-associated muscle symptoms (SAMS) are the most commonly reported adverse effects of statins (10–30% of patients in observational studies),[11] which is in sharp contrast with randomized trials that report only minimal differences in muscle symptoms occurrence.[12] Statin-induced necrotizing autoimmune myopathy (SINAM) is the most serious but fortunately extremely rare side effect of statin therapy that is independent of the type and duration of statin use.[13] High-dose statin use is associated with higher risk of diabetes development; a finding first observed in the landmark JUPITER study (+25% increased risk for newly diagnosed diabetes compared to placebo)[14] and further confirmed in other studies (12% increase in pooled risk).[13,15–19] Importantly, the benefit of statin therapy exceeded the risk of new-onset DM. Nevertheless, statin discontinuation is high and is substantially driven by a negative media coverage about statins.[20] However, even in the primary prevention, a statin discontinuation is associated with markedly increased risk of admission for cardiovascular events.[21]

RNA interference

The fact that double-strand RNA (dsRNA) is able to induce post-transcriptional gene silencing was discovered in 1998.[22] For this crucial discovery, Craig Mello and Andrew Fire received a Nobel Prize in 2006. DsRNA originate in the nucleus as primary microRNA (pri-miRNA). Pri-miRNA is cleaved by complex of two proteins (Drosha-DGCR8) to produced short hairpin RNAs (shRNAs) called pre-miRNA (about 30 bp long). Pre-miRNA is bound and transported into the cytoplasm by exportin-5. In cytopolasm, pre-miRNA binds with two other proteins Dicer and TAR RNA-binding protein (TRBP). Dicer cleaves the terminal loop of pre-miRNA and induces the formation of an RNA-induced silencing complex (RISC)-loading complex with other proteins called Argonaute (Ago1–Ago4) proteins. Double-strand RNA is disentangled and antisense guide strand is selected and loaded into Ago complex, passenger (sense) strand is discarded.

The mature RISC then regulates gene expression by inhibiting mRNA translation.

This naturally occurring process can be mimicked by artificially-delivered dsRNA – small interfering RNAs (siRNAs). They enter the cytosol by endocytosis and directly interact with cytosolic enzymes Dicer and TRBP, undergo strand selection, and produce mature RISC. The selective gene silencing is thus induced (Fig. 1). The loaded RISC has a remarkably long half-life and is extremely potent; as few as 100–200 loaded RISC complexes are able to sufficiently eliminate the expression of a target gene.

RNAi in clinical practice

First clinical trials with unmodified siRNAs resulted in immune-related toxicity, poor stability due to exonuclease, and endonuclease degradation and thus poor effect on RNA inhibition.[23] It took several years of further experiments before appropriate modifications were developed before siRNA became ready for clinical trials. RNAi drugs were introduced in routine clinical medicine on 10th August 2018 when US Food and Drug Administration (FDA) approved patisiran for the treatment of hereditary transthyretin amyloidosis (hATTR) with polyneuropathy.

Inclisiran

Inclisiran is a chemically modified dsRNA that targets 3′-UTR (untranslated region) of PCSK9 mRNA, which is degraded. This leads to lower amount of PCSK9 protein, an increase in the number of LDL-receptors and reduced LDL-cholesterol level (Fig. 2). Compared to native ds-m RNA, inclisiran underwent five modification of the molecule – three modifications are made in the guide strand (fosfothionate, 2′-fluoro-RNA, 2′-O-methyl-RNA), one modification of the passenger strand (2′-deoxythimidine) and the conjugation of 3′-end of the passenger strand to a triantennary N-acetylgalaktosamine (GalNAc, Fig. 3). The combination of the 2′-fluoro and 2′-O-methyl modification greatly improves dsRNA stability. Deoxythimidine

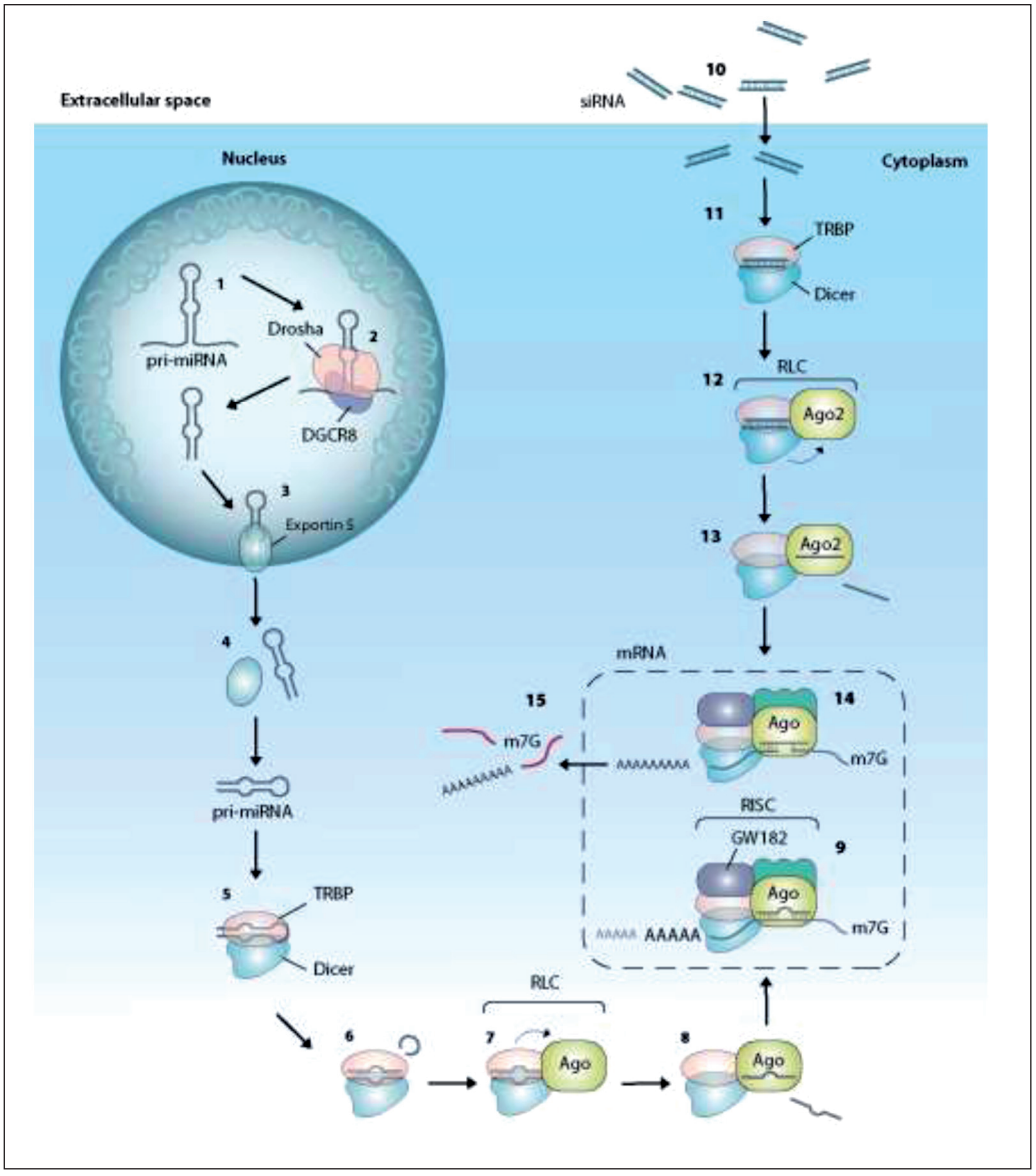

Fig. 1 – RNA silencing process. 1) Primary microRNA (miRNA) originate in the nucleus and (2) are cleaved by the Drosha-DGCR8 complex to produce pre-miRNA. (3) Pre-miRNA is bound by exportin-5 and transported into the cytoplasm (4) where it is released. (5) Subsequently, it is bound by proteins called Dicer and TAR RNA-binding protein (TRBP). (6) Dicer cleaves the terminal loop of pre-miRNA and (7) other protein (Ago1-Ago4) joins the pre-miRNA-Dicer-TRPB complex, which gives rise to RNA-induced silencing complex (RISC). (8) Antisense (guide) strain is selected and integrated within RISC, sense (passenger) strand is discarded. (9) The mature RISC regulates gene expression by inhibiting mRNA translation. Other proteins (GW182) are involved as well. The naturally occurring process can be mimicked by artificially delivered siRNAs. (10) Synthetic small interfering RNAs (siRNAs) enter the cell via endocytosis and (11) directly interacts with RNAi enzymes (Dicer and TRBP). (12) RISC-loading complex is created and (13) the proper (antisense) strand is selected giving rise to mature RISC. (14) siRNA guide strands have usually 100% complementarity to the target mRNA and thus induce long-lasting and selectively-targeted gene silencing. Adapted according to [37].

incorporated in the middle of the sense strand improves guide-strand loading and accelerates the release and degradation of the passenger strand. Triantennary GalNAc attached to the 3´-end of the inclisiran molecule targets inclisiran to hepatocytes as this conjugate is specifically recognized by the asialoglycoprotein receptor (ASGPR) that is abundantly expressed on the surface of liver hepatocytes.

The majority of applied inclisiran is accumulated in hepatocytes, but only a relatively small fraction becomes immediately loaded into RISC and thus becomes biologically active. However, loaded RISC has a half-life of many weeks and inclisiran trapped intracellularly in endosomes function as intracellular depo of the drug. LDL cholesterol-lowering effect of inclisiran is reversed at the rate of approximately 2% per month, so it can persist up to approximately two years.[24,25]

This mechanism of action is completely different than contemporarily used monoclonal antibodies (evolocumab, alirocumab). These drugs act extracellularly where they block existing and secreted PCSK9. On the contrary, inclisiran degrades mRNA for PCSK9

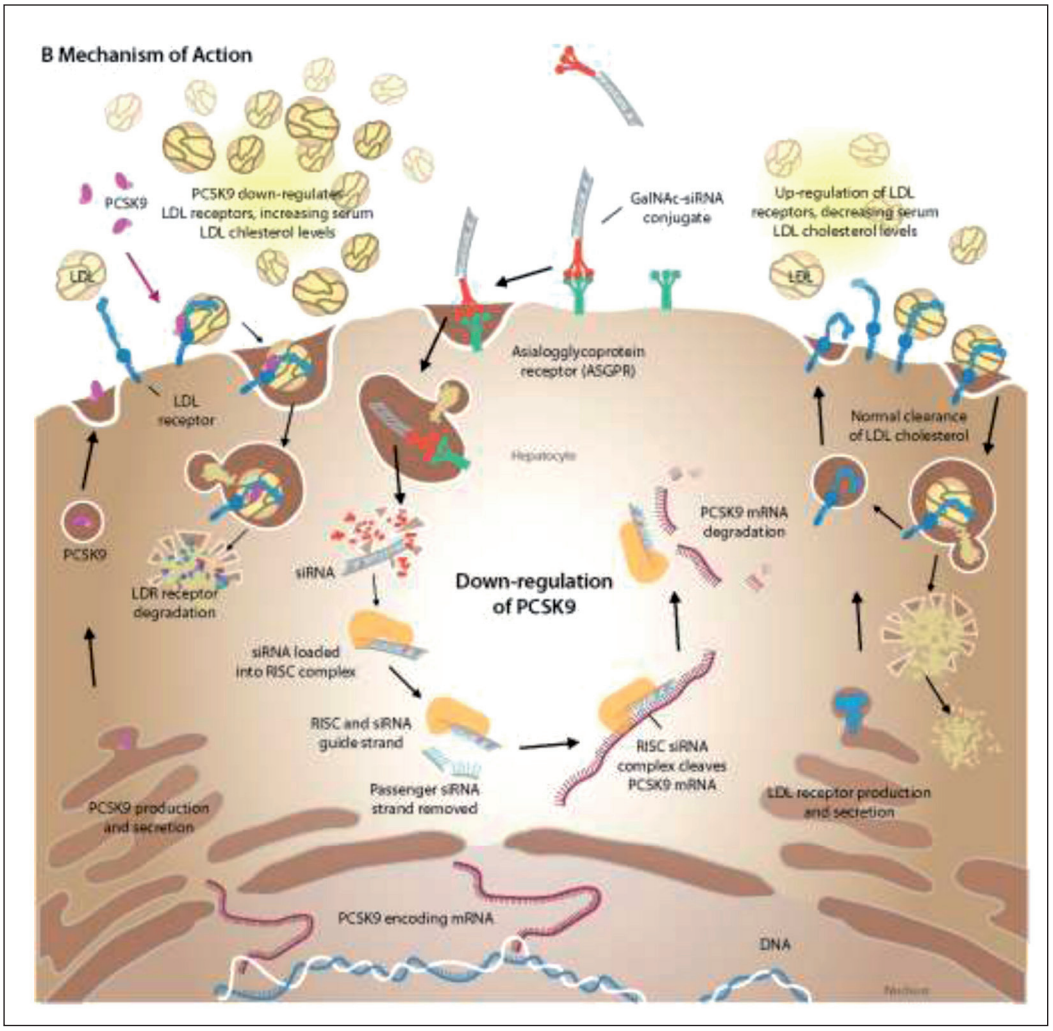

Fig- 2. – Inclisiran mechanism of action. Inclisiran targets 3′- untranslated region of PCSK9 mRNA resulting in its degradation. Lower amount of PCSK9 protein is produced leading to lower degradation of LDL-receptors. This ultimately leads to lower plasmatic LDL-cholesterol level.

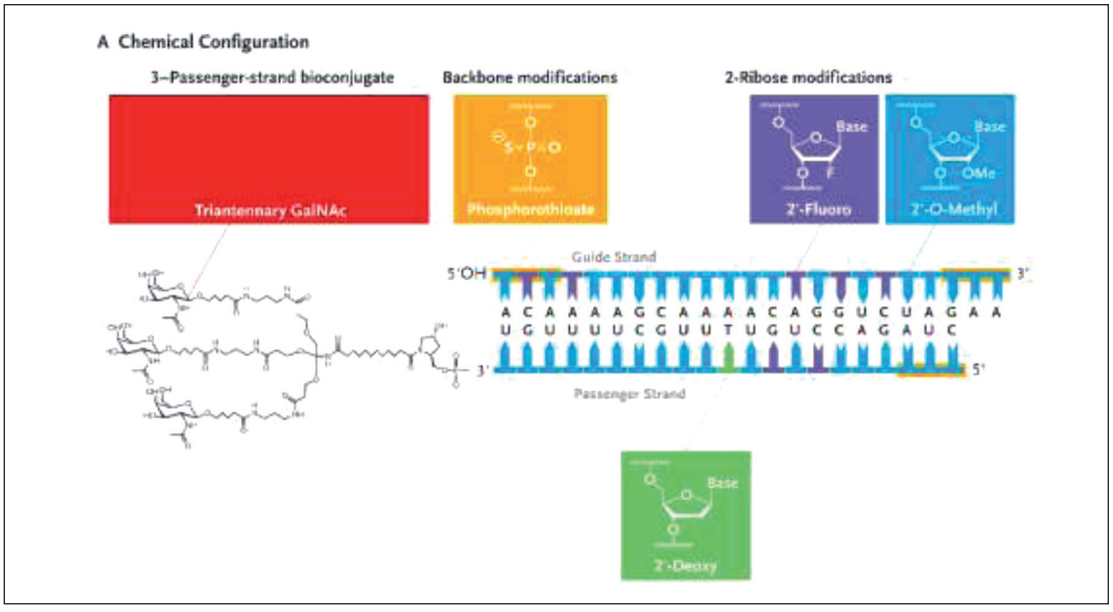

Fig. 3 – Inclisiran: modifications of native dsRNA molecule. Five modifications have been made in inclisiran molecule compared to the native ds-RNA. Three modifications have been made in the guide strand (fosfothionate, 2′-fluoro, 2′-O-methyl), one modification of the passenger strand (2′-deoxythimidine) and the conjugation of 3′-end of the passenger strand to a triantennary N-acetylgalaktosamine (GalNAc).

Table 1 – Comparison of inclisiran and PCSK9-inhibitors

	PCSK9-inhibitor antibodies (alirocumab, evolocumab)	RNAi (inclisiran)
Site of action	Extracellularly	Intracellularly
Mechanism	Binds to PCSK9 on the cell surface and thus inhibits PCSK9-mediated degradation of LDL receptor	Inhibits translation of PCSK9 mRNA
Molecular size	Large, monoclonal antibody	Small, oligonucleotide
Dosage	Every 2–4 weeks	Once in 6 months
Efficacy	Decrease in LDL-cholesterol by 55–75%	Decrease in LDL-cholesterol by 50–55% (up to 65% in selected patient populations)
Side-effects	Nasopharyngitis (7.4%), upper respiratory tract infections (4.6%), backache (4.4%), arthralgia (3.9%), flu-like symptoms (3.2%), adverse reactions at the injection site (2.2%)	Adverse reactions at the injection site (8.4%)
Storage	Refrigerator (2–8 °C)	Room temperature
Who applies	Patient himself	Physician

According to Repatha[38], Praluent[39] and Leqvio[36] product characteristics.

and thus prevents the PCSK9 synthesis. Main differences between inclisiran and PCSK9i are summarized in Table 1.

Clinical trials with inclisiran

Inclisiran reduces the level of LDL-cholesterol in healthy volunteers[26] as well as in patients with high risk of cardiovascular disease with elevated LDL cholesterol.[24] The effect on PCSK9 and LDL cholesterol levels were dose-dependent, two doses of inclisiran 300 mg s.c. (given at day 1 and day 90) lead to 52.6% decrease in LDL-cholesterol at day 180. Serious adverse events were rare and unrelated to inclisiran application; injection-site reactions occurred in 5% of patients that received inclisiran. ORION-10 and ORION-11[27] are two largest studies with inclisiran published so far, these studies enrolled patients with established atherosclerotic cardiovascular disease (100% of patients in ORION-10 and 87% or patients in ORION-11) or atherosclerotic cardiovascular disease equivalent (13% of patients in ORION-11). In both these studies, 300 mg of inclisiran was administered on day 1, 90 and every 6 months thereafter over a period of 540 days. In both trials, LDL cholesterol was reduced by approximately 50% from day 90 until the end of study. Together with LDL reduction, inclisiran also lowered levels of triglycerides, lipoprotein(a) and increased HDL-cholesterol. Inclisiran therapy was not associated with any laboratory abnormality concerning liver and kidney function, C-reactive protein level and platelet count. Anti-drug antibodies were detected in 2.0 and 2.5% of the samples from inclisiran-treated patients. The frequency of positive samples was similar between pretreatment and post-treatment samples, was in low titer and was not associated with changes in any significant clinical or pharmacologic variable. Inclisiran treatment was not found to induce an anti-inclisiran antibody production. Adverse events concerning injection-site were slightly more frequent in patients treated with inclisiran. Although the study was not powered to detect the difference in cardiovascular outcome, prespecified exploratory cardiovascular endpoints occurred in 7.4% and 7.8% of inclisiran-treated patients compared with 10.2% and 10.3% of patients treated with placebo. Similarly, inclisiran was found to reduce a LDL-cholesterol of about 50% in patients with familial hypercholesterolemia regardless of genotype.[28]

Safety

As the therapy with inclisiran is extremely long-lasting (an effect of the drug persists up to 2 years)[24,25] the safety issues are extremely important. Inclisiran does not exert any adverse effects on platelets count, blood lymphocyte, monocyte or neutrophil count, does not cause alterations in blood TNF-alpha or IL-6 concentrations and does not induce relevant anti-drug antibodies.[29] This is an important finding as treatment with single-strand antisense oligonucleotides (ASO) was associated with serious adverse events- treatment with both volanesorsen (apoC III inhibitor)[30] and inotersen (inhibitor of transthyretin protein production)[31] were associated with significant thrombocytopenia. Although the underlying mechanism of ASO-induced thrombocytopenia is poorly understood, it is hypothesized that distinct motifs of the second-generation ASO can bind to platelet proteins (platelet collagen receptor glycoprotein VI) resulting in platelet activation.[32]

However, inclisiran uses a different molecular principle (RNAi by double stranded siRNA) and is GalNAc-conjugated that specifically enhances hepatic uptake and plasma clearance.

The possibility of innate immune system activation with subsequent induction of inflammatory cytokines (TNF-α, IL-6) is another aspect of oligonucleotide therapeutic use.[33] The ability of oligonucleotide therapeutics to induce an immune responses seems to depend upon various factors including siRNA sequence as well as chemical modifications. Certain motifs (5´-UGU-3´, 5´- UGUGU-3´) may have potently activate immune system and induce the production of inflammatory cytokines.[34] Toll-like recep-

tor (7/8)-dependent cytokine production may be attenuated by either replacement of uridine with adenosine or by modification of 2´-hydroxyl uridines with 2´-ribose, 2´-fluoro, 2´-O-methyl, or 2´-deoxy moieties.[35] Importantly, these modifications were performed during inclisiran development and no increase in inflammatory biomarker concentration was observed after inclisiran administration.[29]

The summary of product characteristics of inclisiran (Leqvio) reports the only adverse side effect that is adverse reactions at the injection site occurring in 8.2% of patients.[36]

Conclusion

Inclisiran decreases the level of circulating LDL-cholesterol by 50–55% and up to 65% in selected patient populations even when applied once in six months; it is the first therapeutic agent employing RNA interference that has the potential of becoming widely used.

Conflict of interest
The author has received speaker honoraria from Novartis, AstraZeneca and Swixx Biopharma.

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