Inclisiran - new hope in the management of lipid disorders?

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HIGHLIGHTS:

-Inclisiran is a siRNA inhibiting synthesis of PCSK9 protein in hepatocytes.

-The effects of the drug persist longer than of other approved lipid-lowering drugs.

-In clinical trials it maximally reduced LDL-C concentration by 52.6% at day 180

ABSTRACT:

Drugs reducing plasma concentrations of apolipoprotein-B (ApoB) containing lipoproteins have been demonstrated to reduce the risk of cardiovascular disease (CVD) in both primary and secondary prevention. Despite the demonstrated efficacy of statins and ezetimibe on low-density lipoprotein (LDL) concentration and long-term CVD risk, a large number of patients do not achieve their therapeutic goals. The introduction of monoclonal antibodies against proprotein convertase subtilisin/kexin type 9 **(**PCSK9) protein was a milestone in the treatment of lipid disorders, as their administration leads to unprecedentedly low LDL-C concentrations. Inclisiran represents an entirely new mechanism of PSCK9 protein inhibition in hepatocytes, targeting the messenger RNA (mRNA) for PCSK9. Its administration is necessary only every 3-6 months, which is an essential advantage over statin and monoclonal antibody therapy. The infrequent administration regimen can increase the number of patients who maintain their therapeutic goals, especially in patients struggling to comply with daily or biweekly pharmacotherapy.

Preclinical studies and Phase I and Phase II clinical trials of inclisiran have demonstrated its tolerability and efficacy in promoting long-term reduction of both PCSK9 protein and LDL-C. The efficacy and safety of inclisiran will continue to be assessed in ongoing and forthcoming trials on larger patient groups. If the results of these trials reflect previously published data, they will add further evidence that inclisiran might be a revolutionary new tool in the pharmacological management of plasma lipids. This review summarizes the currently available literature data on inclisiran with respect to its mechanism of action, effectiveness and safety as a lipid-lowering drug for CVD prevention.

Keywords: Inclisiran, dyslipidaemias, cardiovascular disease, atherosclerosis, lipoproteins.

ABBREVIATIONS:

ALT	Alanine aminotransferase
ASCVD	Atherosclerotic cardiovascular disease
CHD	Coronary heart disease
CRP	C-reactive protein
CVD	Cardiovascular disease
FH	Familial hypercholesterolaemia
HeFH	Heterozygous familial hypercholesterolaemia
HoFH	Homozygous familial hypercholesterolaemia
FOURIER	Further Cardiovascular Outcomes With PCSK9 Inhibition in Subjects with Elevated Risk
GGTP	γ-glutamyltransferase
HDL	High-density lipoprotein
HDL-C	High-density lipoprotein cholesterol
LDL	Low-density lipoprotein
LDL-C	Low-density lipoprotein cholesterol
MI	Myocardial infarction
RISC	RNA-induced silencing complex
RNA	Ribonucleic acid
ULN	Upper limit of normal
VLDL	Very low-density lipoprotein

INTRODUCTION

Among the risk factors for cardiovascular diseases (CVD), lipid disorders are one of the most common and perilous [1]. According to the results of Multi-centre National Population Health Examination Survey (WOBASZ II), the prevalence of dyslipidaemias in the Polish population above 20 years old is 70.3% and 64.3% in men and women respectively [2]. The current European Society of Cardiology guidelines for dyslipidaemia [3] along with the joint guidelines of Polish Lipid Association, College of Family Physicians in Poland and Polish Cardiac Society [4] emphasise the role of elevated low-density lipoprotein cholesterol (LDL-C) concentrations in the development of atherosclerosis. Moreover, both 2018 American Guidelines and 2016 Canadian Cardiovascular Society Guidelines for the management of lipid disorders also stress the benefits of CV risk reduction derived from effective treatment of hyperlipidaemia [5, 6].

High LDL-C levels significantly increase the risk of major adverse cardiovascular events (MACE) and contribute to premature morbidity and mortality. Therefore, the guidelines promote appropriate control and management of dyslipidaemias. The gold standard in the pharmacological treatment of hypercholesterolaemia is therapy with statins. The results of a large meta-analysis of 26 randomised clinical trials indicate that reduction of LDL-C concentration by 39 mg/dL reduces the risk of all-cause mortality by 10%, the risk of cardiovascular (CV) mortality by 20% and the risk of coronary revascularisation by 19% [7]. However, data derived from large prospective studies and clinical registries indicate that a sizeable proportion of patients taking statins do not reach their therapeutic target, even when the highest tolerated doses of statins are administered [8, 9]. Furthermore, chronic treatment with statins is associated with the occurrence of adverse effects, predominantly myalgia and myopathies [10, 11]. Real or perceived adverse effects are a major cause of treatment discontinuation.

As lipid disorders are becoming increasingly widespread and statin therapy has some major limitations, new approaches to lipid-lowering therapy are being investigated. Inclisiran, which is currently being evaluated in clinical trials, is one of the most highly promising new agents.

Inclisiran is a small interfering ribonucleic acid (siRNA). It inhibits synthesis of proprotein convertase subtilisin/kexin type 9 (PCSK9), one of the most important proteins in LDL-C metabolism. The purpose of this article is to review the currently available literature data on inclisiran concerning its mechanism of action, effectiveness and safety as a lipid-lowering drug for the prevention of CVD.

The role of PCSK9 in LDL-C metabolism

PCSK9 is one of the most important regulators of LDL-C metabolism. It is synthesized and modified in the endoplasmic reticulum of hepatocytes and promotes the lysosomal degradation of cell-membrane LDL receptors (Figure 1) [12]. After secretion into the plasma, PCSK9 is attached to the EGF-A domain of the LDL receptor, and such complex is internalized into the hepatocyte [13]. Moreover, there is evidence that PCSK9 protein chaperones the LDL receptor through the intracellular pathway [14, 15]. Via both aforementioned mechanisms of PCSK9 functioning, the LDL receptor does not follow its physiological pathway of return to the hepatocytic membrane, but undergoes transfer to the lysosome, where it is degraded. Hence, decreased presence of LDL receptors on the hepatocytes causes a reduced clearance of plasma LDL-C, and consequently increased concentration of circulating LDL-C. Furthermore, PCSK9 inhibits the intracellular degradation of apolipoprotein B, a protein constituent of LDL and very low-density lipoprotein (VLDL) particles [16, 17]. Although PCSK9 gain-of-function mutation is not the most prevalent genetic mutation responsible for development of familial hypercholesterolaemia (FH), it constitutes almost 1% of the mutations in this population of patients [18]. FH can exist in both homozygous and heterozygous forms. Homozygous FH (HoFH) is associated with LDL-C concentrations substantially higher then the reference ranges and results in very high CV risk, even in the very early years of life. HoFH is extremely rare, with prevalence in the majority of populations of around 1:300.000 [19]. In contrast, heterozygous FH (HeFH) is much more prevalent, and although in the past it was estimated to affect around 1 in 500 adult citizens, the more recent data derived from

unselected populations provide a stronger support for the prevalence of around 1:200 adults [20, 21]. The patients with FH are facing a significantly higher risk of coronary heart disease (CHD) than members of the general population. The authors of consensus statement of the European Atherosclerosis Society indicate that in average, the 12-year old patient with HoFH has the similar risk of developing CHD as 35-year old patient with HeFH and as a 55-year old patient without FH [22]. According to the results of Simon Broome Registry, the risk of developing a major adverse CV event in the population of 60-year-old patients with HeFH is 50% for men and 30% for women [23]. However, an early introduction of statin therapy in patients with HeFH can almost equalize their risk of CVD with the general population [22]. Moreover, in our recent study, we have shown that an introduction of a high-intensity statin treatment after an acute coronary syndrome (ACS) can almost equalize the long-term outcomes in patients with and without clinically diagnosed FH [24]

In contrast to the poor outcomes observed in patients with FH, loss-of-function mutations of *PCSK9* significantly reduce LDL-C concentration, and CV risk without notable adverse effects in other organs and systems [25, 26]. Therefore, PCSK9 inhibition has emerged as an interesting new therapeutic approach to the management of patients with lipid disorders.

Currently available anti-PCSK9 agents

Soon after the role of PCSK9 in the lipid metabolism had been established, attempts were made to suppress its biological activity. Approaches included the inhibition of specific messenger RNA (mRNA) translation, reduction of PCSK9 hepatocyte secretion and reduction of serum PCSK9 concentrations using monoclonal antibodies binding to this protein [27, 28]. Of these approaches, only monoclonal antibodies have so far gained regulatory approval for regular administration in the patients with hypercholesterolaemia. Two antibodies, alirocumab and evolocumab, have been approved. Both are fully human, specific, monoclonal antibodies that bind to the PCSK9 protein. Their primary mechanism of action is based on the capturing of the circulating fraction of this protein, which blocks binding to the EGF-A domain on the LDL receptor described before.

Administration of these drugs leads to a substantial reduction in plasma LDL-C in healthy volunteers and patients with lipid disorders [29], with the maximal reductions of LDL-C in RCTs after 12-52 weeks reaching 83% when compared with placebo [30]. However, due to specific interaction with only circulating, thus extracellular, serum fraction of PCSK9, a monoclonal antibody cannot influence the intracellular portion of the protein inside the hepatic cell. Nevertheless, the clinical value of its intracellular activity remains unknown and further studies are required to establish, whether it conveys a clinical significance.

In the Further Cardiovascular Outcomes With PCSK9 Inhibition in Subjects with Elevated Risk (FOURIER) trial, 27,564 patients with stable CHD, but high CV risk were randomized to receive either placebo or subcutaneous injections of evolocumab [31]. The patients were required to have baseline LDL-C level higher or equal to 70 mg/dL or non-HDL-C level higher or equal to 100 mg/dL despite the treatment with moderate to high intensity statin, to participate in the trial. The primary composite endpoint of the study consisted of CV death, myocardial infarction, stroke, hospitalization for unstable angina, or coronary revascularization, while the key secondary endpoint constituted CV death, myocardial infarction and stroke. Median LDL-C after 48-week therapy was reduced from 92 mg/dL to 30 mg/dL and the reduction in LDL-C levels was followed by a significant, 1.5% absolute reduction in the incidence of the primary endpoint in comparison with intensive statin therapy at a median of 22 months and a 2.0% absolute reduction of this endpoint in 36-month follow-up [31]. Moreover, treatment with evolocumab significantly reduced the concentration of triglycerides (in average by 16.2% at week 48) and lipoprotein (a) (by 26.9% at week 48) [31] with a similar safety profile to placebo. There were no significant differences in the overall occurrence of any adverse effects apart from the injection-site reactions which occurred in 2.1% versus 1.6% in the placebo group [31]. Worth noting is that the occurrence of the adverse effects most frequently responsible for the discontinuation of statin therapy, such as muscle-related events or elevation of hepatic biomarkers was not increased by treatment with evolocumab.

Before the beginning of the trial, there had been concerns about neurocognitive function in patients with extremely low LDL-C levels [32]. Hence, in order to assess the cognition of patients recruited in the FOURIER trial, the Evaluating PCSK9 Binding Antibody Influence on Cognitive Health in High Cardiovascular Risk Subjects (EBBINGHAUS) study was performed. In contrast to the initial doubts regarding the safety of evolocumab, no evidence of serious evolocumab-associated adverse neurocognitive events was found during the study in the median follow-up of 19.4 months [33]. The results were consistent even in patients with LDL-C levels lower than 10 mg/dL [34].

The ODYSSEY OUTCOMES trial was conducted on 18,924 patients who had had an ACS one to twelve months before randomization [35]. The initial lipid profile inclusion criteria were similar as in FOURIER, with an additional possible criterion of apolipoprotein B levels higher or equal to 80 mg/dL. To be enrolled in the study, the patients must have had a high-intensity or maximally tolerated dose statin therapy. According to the study protocol, the dose of alirocumab was adjusted to target the LDL-C concentration between 25 mg/dL and 50 mg/dL. After 48-weeks, treatment with alirocumab was associated with a 54.7% reduction in LDL-C concentration in comparison with statin treatment (53.3 mg/dL vs 101.4 mg/dL) [35]. The reduction in LDL-C resulted in a significant reduction of MACE risk (death from CHD, nonfatal myocardial infarction, fatal or nonfatal ischemic stroke, or unstable angina requiring hospitalization) which was reduced by 15% with an absolute risk reduction of 1.6% in the intention-to-treat analysis during a median follow-up of 2.8 years. It is worth noting that the highest treatment efficacy was observed in patients with baseline LDL-C concentration exceeding 100 mg/dL and according to subanalyses of the trial, no significant differences were observed in the overall efficacy and safety profile with regard to any analysed subgroup [35].

A third monoclonal antibody against PCSK9, bococizumab, has been evaluated in clinical trials. As it was non-fully human antibody, antidrug antibodies developed in 48% of the patients who received bococizumab and neutralizing antibodies developed in 29%, which in some affected

patients substantially attenuated LDL-cholesterol lowering over time. As a result - although treatment with bococizumab was associated with a 21% reduction of the risk of MACE at 12-month follow-up - due to immunogenicity and variability of LDL response, bococizumab was withdrawn from further development [36].

The results of the large clinical trials with PCSK9 inhibitors led to significant changes in the recent European and American guidelines for the management of lipid disorders. The incremental value of anti-PSCK9 monoclonal antibodies is expressed in the recent American Guidelines on the Management of Blood Cholesterol [5]. The recommendations state that although their long-term safety is still uncertain, an addition of PSCK9 inhibitors is reasonable in the very-high risk patients or patients with primary hypercholesterolaemia already treated with maximal tolerated combined therapy with statins and ezetimibe, who cannot reach their therapeutic goal [5]. In the recent, 2019 ESC/EAS Guidelines for the management of lipid disorders, PCSK9 inhibitors are mentioned as a part of combined therapy for patients in secondary prevention of CVD along with patients with FH and very high CV risk, who don't achieve their therapeutic goals on a maximal tolerated dose of statin and ezetimibe [3].

Vaccination against PCSK9 represents an interesting direction for future investigation. According to currently available data from studies on animals, vaccination against PCSK9 protein reduces LDL-C concentration by nearly half, and the effect persists for over 40 weeks [37]. Such long-term efficacy could result in a dosing regimen that was attractive to patients. The results of a clinical study currently being conducted (NCT02508896) will shed new light on this novel approach to the treatment of hypercholesterolaemia.

In this context, it is notable that a meta-analysis encompassing fifteen randomised clinical trials has demonstrated that statin treatment increases the concentration of serum PCSK9 regardless of statin used [38]. It is believed that this mechanism might contribute to the incomplete efficacy of statin pharmacotherapy in some patients. Therefore, from the molecular point of view, simultaneous

treatment with PCSK9 inhibitors and statins is a rational combination which results in additional lipid-lowering, such as demonstrated in FOURIER and ODYSSEY OUTCOMES trials [31,35].

Inclisiran-molecular structure and properties

Inclisiran (ALN-PCSSC; ALN-60212) is a short-chain, synthetic, siRNA, which inhibits the expression of the PCSK9 gene [39]. Inclisiran specifically binds to the mRNA precursor for this protein, which then undergoes degradation. Due to this unique mechanism of action, a molecule of the drug can concomitantly reduce both intra- and extracellular PCSK9 protein levels, which results in a substantial, long-lasting reduction of LDL-C concentrations. The inclisiran molecule consists of two complementary strands of ribonucleic acid strands; out of which one plays the role of a guide strand, while the latter is called the passenger strand.

siRNAs exert their action by the activation of the natural pathway of selective gene expression silencing. Once the inclisiran molecule has been incorporated into the hepatocyte, the guide strand binds to a multiprotein complex named RISC (RNA-induced silencing complex). Afterwards, the guide strand undergoes hybridization with complementary mRNA for PCSK9 and induces its degradation. Concerning the long-term efficacy, it is notable that the silencing complex remains active after mRNA degradation has occurred. Thus a single molecule of siRNA delivered to the hepatocyte can specifically interfere with the expression of multiple mRNA molecules. Degradation of mRNA for PCSK9 limits its translation and therefore reduces synthesis and secretion of this protein into the vascular system leading to a substantial reduction of serum LDL-C [39]. The discovery of the mechanism of RNA interference led to the award of the Nobel Prize in Physiology or Medicine in 2006 to Andrew Fire and Craig Mello.

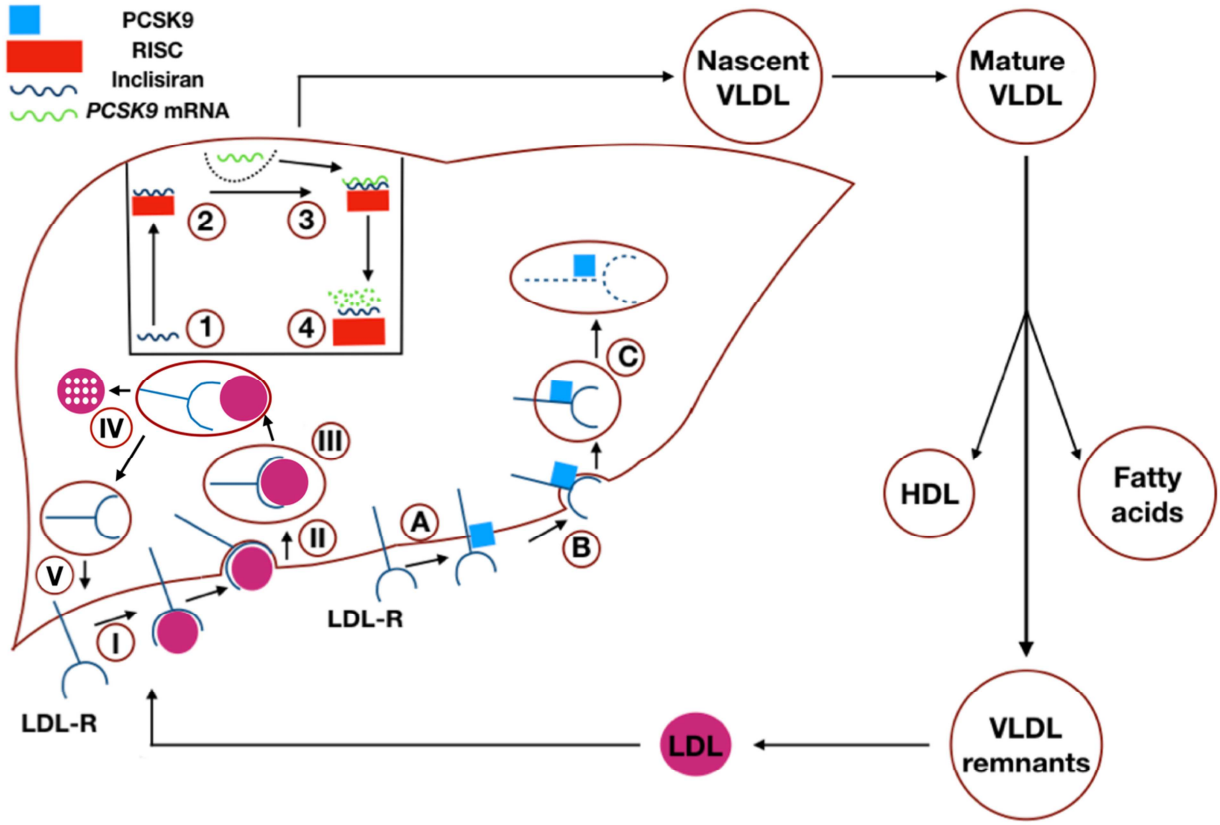

Figure 1: Low-density lipoprotein cholesterol (LDL-C) metabolism and the role of the LDL receptor and proprotein convertase subtilisin/kexin type 9 (PCSK9) in the hepatic influx of LDL. 1-4: Intracellular actions of inclisiran 1) Inclisiran small interfering RNA molecule, 2) RNA-induced silencing complex (RISC) formation 3) RISC complex binds to the complementary mRNA strand of PCSK9 4) mRNA strand is cleaved by the RISC complex. A-D: hepatic degradation of LDL receptor mediated by PCSK9 A: PCSK9 binds to the LDL receptor attached to the cellular membrane, B: The complex is internalised into the endosome, C: LDL receptor is degraded in the lysosome by the proteolytic enzymes. I-V: Metabolism of LDL internalisation from the surface of hepatocyte: I: LDL binds to the receptor, II: The complex is internalised into the endosome and then, III: The LDL particle is transferred to the lysosome IV: where it is digested, while V: LDL receptor is recycled to the hepatocyte plasma membrane.

Pharmacodynamics

An early predecessor of Inclisiran- the ALN-PCS molecule - was encompassed in a lipid nanoparticle. The spherical structure resulted in a rapid 70% reduction of both PCSK9 mRNA and protein concentrations, along with a 60% reduction in LDL-C concentrations lasting for three weeks in an animal model [40]. In healthy volunteers, the largest effect after a 60-minute infusion of the drug was a 70% reduction of PCSK9 protein along with 40% reduction of LDL-C levels on the third day of follow-up [41].

Studies on ALN-PCS were followed by the introduction of ALN-PCSSC, which possesses an additional synthetic triantennary N-acetylgalactosamine (GalNAc) bound to its structure. Addition of GalNAc significantly increased its clinical efficacy and duration of action through increased adhesion to the cellular membrane of hepatocyte and specificity to liver cells.

GalNAC is complementary to asialoglycoprotein receptors on the hepatocyte. It has been demonstrated that 24 hours after intravenous administration, there were no drug molecules in serum, which confirms specific drug influx into the liver. The lack of activity on peripheral tissues reduced the risk of potential adverse effects on organs other than the liver. A linear relationship between dose and efficacy was observed and substitution of molecules in the polynucleotide strands with 2'-O-methylnucleotides or 2'-O-fluoronucleotides led to increased stability of the drug. In preclinical studies conducted on animal models, the therapeutic dose associated with 50% PCSK9 inhibition was 1 mg/kg of body mass, and administration of the highest dose exceeding 3 mg/kg of body mass leads to inhibition of PCSK9 by 85% and a 60% reduction in LDL-C concentrations [40]. An additional clinical benefit of the modified molecule is derived from the way the drug is administered. While ALN-PCS requires intravenous infusion, ALN-PCSSC can be injected subcutaneously.

siRNAs are one of two most frequently used strategies for gene expression silencing, the second being antisense oligonucletides (ASO). Although they both act in the mechanism of natural gene expression pathway silencing, they possess a few differences with clinical implications that are worth mentioning. One of the most important drawbacks of siRNA in comparison with ASO molecules is its significantly lower affinity to the targeted molecule [42]. In combination with different cellular action location (ASOs act predominantly in the nucleus, while the activity of siRNA is restricted to cytoplasm [43]), it presumably increases the risk of off-target hybridization and adverse effects. On the other hand, ASOs require more frequent administration, and in the past concerns were raised on the development of thrombocytopaenia in patients treated with ASOs [44]. Among currently available ASOs, worth mentioning are mipomersen and volanesorsen, the former

used in the treatment of HoFH and the latter to treat familial chylomicronemia [45-47]. Despite their significant efficacy in reducing target lipoprotein levels (mipomersen reduced LDL-C in patients with HoFH by 36% while volanesorsen triglyceride concentration by 77%), they exert multiple adverse effects. In patients treated with mipomersen, the most frequent adverse reactions were injection site-reactions and influenza-like symptoms (which occurred in respectively almost 90% and almost 50% of patient treated with mipomersen), while patients treated with volanesorsen complained mostly of injection-site reactions and thrombocytopaenia, which occurred in respectively 61% and 45% patients included in the Phase III trial with the drug [45-47]. Nonetheless, despite the results of the large analysis by Crooke et al, who found no cumulative evidence of the thrombocytopaenic activity of ASOs based on 16 clinical trials with various molecules, the U.S. Food and Drug Administration rejected the application specifically for volanesorsen, mostly due to thrombocytopaenia suggesting a risk for bleeding in patients treated with the drug [48]. However, as both mipomersen or volanesorsen are lacking GalNAc ligand described above, the adverse effects they exert could be caused by their low tissue specificity and an addition of the GalNAc group could potentially improve their safety profile.

In the past, SPC5001, a specific ASO against PCSK9 underwent phase I study, but due to significant number of injection site reactions and signs of nephrotoxicity, the development of the drug was terminated [49]. Therefore, despite their minor disadvantages, the near future of gene silencing in the inhibition of PCSK9 seems open for siRNA molecules.

Pharmacokinetics

The highest concentration of ALN-PCS is reached almost immediately after completion of the 60-minute intravenous infusion. The maximal concentration and area under the curve of drug concentration rise in a relatively linear relationship with its dose [41]. Modification of the molecular structure of ALN-PCSSC led to substantial increases in stability and prolonged biological activity [50]. In the studies conducted on mice, the largest reduction of PCSK9 protein and LDL concentration were achieved approximately twenty days after subcutaneous drug injection,

independently of the dose administered. The higher the dose of ALN-PCSSC, the longer lipid-lowering effect persisted. A small but progressive increase in LDL concentration was observed 90-120 days after injection [39]. Phase 1 studies indicated, that 180 days after drug administration no lipid-lowering effect was observed in doses lower than 300 mg [39].

Phase I and Phase II studies

Convincing results of preclinical studies on inclisiran triggered the initiation of clinical trials to obtain regulatory approval for its use in pharmacotherapy of lipid disorders. In the phase I trial (NCT01437059) 32 healthy volunteers with LDL-C levels exceeding 116 mg/dL were administered a single intravenous, 60-minute infusion of different doses of ALN-PCS. The primary endpoint of the study was the assessment of safety and tolerance of the drug. The secondary endpoint was the determination of pharmacokinetics of the drug and its pharmacodynamic effects on the concentrations of PCSK9 protein and LDL-C. Twenty-four participants were randomly assigned to receive inclisiran (in doses ranging from 0.015 mg/kg to 0.4 mg/kg), and eight were given a placebo. No difference in the occurrence of adverse effects associated with drug administration was observed between the control and drug group, thus confirming that inclisiran is well-tolerated. It was observed that administration of the highest (0.4 mg/kg) dose was associated with a reduction of serum PCSK9 by nearly 70% and LDL-C by 40%. The lipid-lowering effect of ALN-PCS was observed across all doses of inclisiran and was greatest when the baseline concentration of LDL-C was highest [41].

The first phase I study on the safety of subcutaneously administered ALN-PCSSC was published at the end of 2016 [39]. This randomised, placebo-controlled trial was conducted on 24 healthy volunteers with LDL-C higher than 100 mg/dL. The patients underwent randomization to either a single dose of ALN-PCSSC in doses ranging from 25 mg to 800 mg or multiple injections of this drug in doses from 125 to 500 mg with intervals of at least one week between administrations. Significant reduction of PCSK9 concentration was observed after administration of

a single dose of at least 300 mg of the drug. In order to significantly reduce LDL-C concentration, a single dose of 100 mg or higher was required. At day 84, the maximal reduction of the PCSK9 concentration was 74.5% after the 300 mg dose. LDL-C concentration was reduced maximally by 50.6% after 500 mg dose. PCSK9 and LDL-C concentrations were maintained at reduced levels for over 180 days after administration of 300 mg or a higher dose of a single dose of inclisiran.

In the multiple-dose subgroup, maximal PCSK9 concentration reduction was observed in the group being administered 500 mg for two doses a month throughout two months. The highest LDL-C reduction occurred in the group administered a 300 mg dose with the same frequency. At day 84, these reductions were 83.8% and 59.7% respectively. In all patients assigned to multiple doses, the reduction of LDL-C level was durable over 196 days after the administration of the first dose.

The ORION I (NCT02597127) trial was the first Phase II study on the lipid-lowering effect of inclisiran. It was a multi-centre, randomised, placebo-controlled trial, conducted on 501 patients at high risk of CVD, with elevated baseline serum LDL-C concentration uncontrolled with maximal tolerated conventional pharmacotherapy [51]. LDL-C concentration thresholds were 70 mg/dL in patients with concomitant CVD (345 patients, 69% of the studied group) and 100 mg/dL in patients without CVD (156 patients, 31% of the studied group). Before enrollment in the study, patients were required to have been on a maximal tolerated dose of statin and/or ezetimibe for 30 days. The patients were randomised to a single dose of placebo or 200, 300, or 500 mg of inclisiran or two doses (at the first and the ninetieth day) of placebo or 100, 200, or 300 mg of inclisiran. The primary endpoint was the change in LDL-C concentration 180 days after injection. The study confirmed findings derived from preclinical studies indicating a linear correlation between the dose of the drug and its efficacy. Thirty days after the first injection, the average PCSK9 concentration reduction varied between 66.2 and 74.0% and LDL-C concentration reduction varied between 44.5% and 50.5% depending on the dose. At day 180, the least square mean LDL-C reduction was between 27.9% and 41.9% after administration of a single dose and varied between 35.5% and 52.6% after multiple doses. The highest LDL-C reduction was observed after a double 300 mg

injection of the drug. In this group, the mean reduction of PCSK9 protein concentration was 69.1%, and LDL-C concentration was reduced by 52.6% at the day 180. The percentage of PCSK9 and LDL-C concentration reductions were consistent with the lipid-lowering effects of monoclonal antibodies against PCSK9 seen in clinical trials, however, the effects of inclisiran persist significantly longer. Moreover, the mean reduction of LDL-C concentrations after a 240-day follow-up period varied between 26.7% and 47.2% and was dose-dependent. Significantly reduced levels of LDL-C and PCSK9 concentrations remained consistent among all dose subgroups at day 240, although were elevated in comparison to day 180. These data suggest that in order to achieve the most efficient and durable form of lipid lowering treatment with inclisiran, six monthly dosing may be appropriate.

The analysis of the other lipid parameters demonstrates further important effects of inclisiran [52]. After follow up for 180-days, each dose of the drug significantly reduced not only the concentration of LDL-C but also of total-cholesterol (by 18-33%) and non-HDL-C (by 25-46%) along with apolipoprotein B (by 23-41%) and VLDL-C (by 12-21%). Despite the significant reduction of triglyceride levels after administration of a single 300 mg and 500 mg dose or double 300 mg dose, their level did not decrease significantly when lower doses were administered. The authors stated that the overall lipid-lowering effect did not vary significantly between patients as far as LDL-C, apoB and non-HDL-C concentrations were concerned, but the variability in reduction of VLDL and triglycerides was more pronounced. Although these differences were not explained by the authors, one can speculate that as those lipoproteins contain significantly more triglycerides than cholesterol, their levels might be significantly more affected by multiple factors including dietary habits, concomitant diseases and concomitant pharmacotherapy.

Consistently with the results of the clinical trials on monoclonal anti-PCSK9 antibodies, no significant modification of C-reactive protein (CRP) concentration was observed [52]. Therefore, it could be considered as an additional argument suggesting that PCSK9 is not involved in the

systemic inflammatory processes in the human body, which remains an important target for CV preventive medicine.

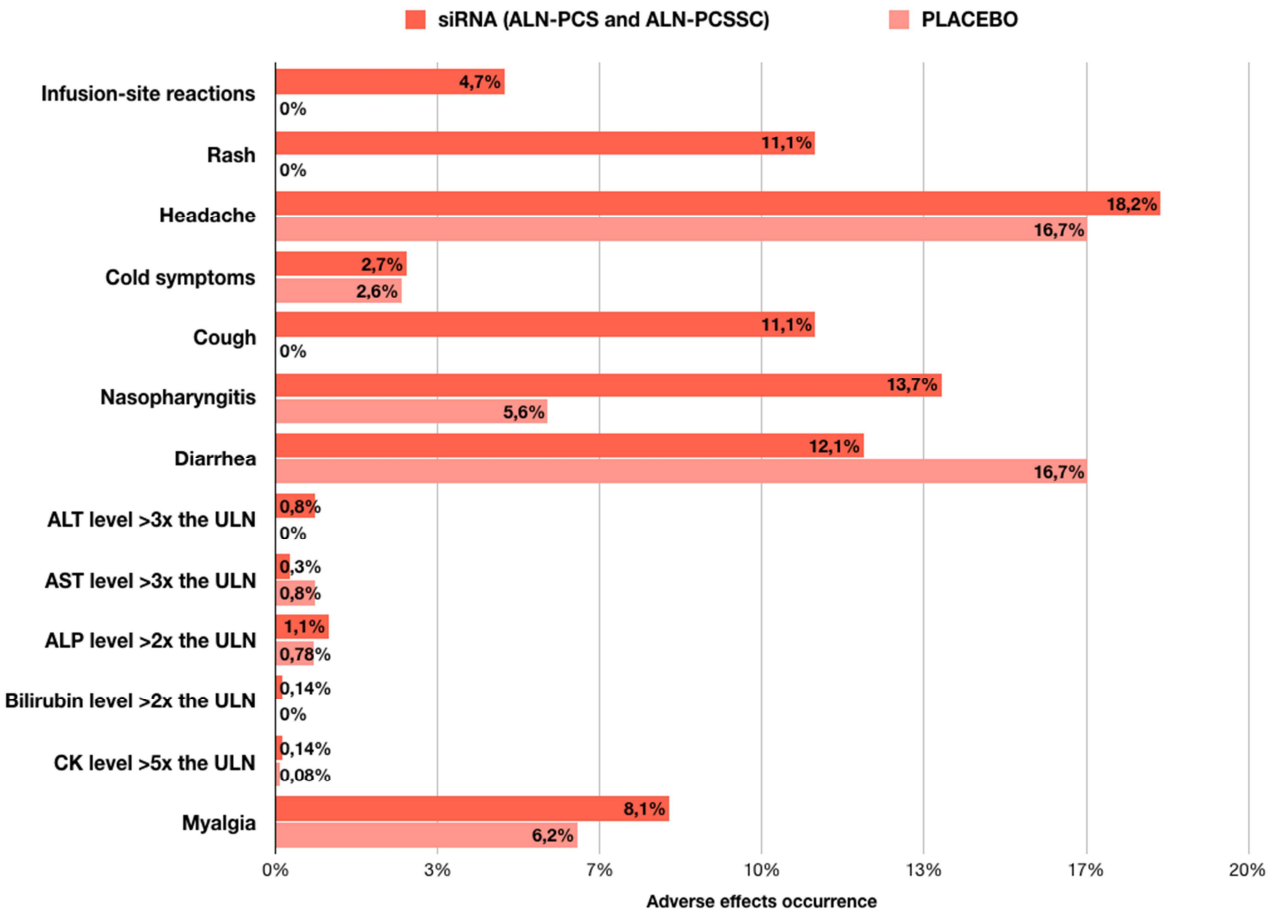

Figure 2: Adverse effects occurrence in Phase I and Phase II clinical studies based on the available data [39, 41, 51].

Drug safety

Drug injections are more commonly associated with the increased risk of adverse effects in comparison with conventional oral pharmacotherapy [53]. However, in currently available studies on inclisiran, the adverse effects occurred in both placebo and drug-treatment arms with a relatively similar frequency. Among the most frequently recorded adverse effects there were local, mild self-limiting rash and hyperpigmentation, cough, musculoskeletal pain, headache and back pain along with acute nasopharyngitis or hiccups [39, 41, 51]. The incidence of adverse effects reported in the

aready conducted clinical trials is summarised in Figure 2. Although none of the patients within the trials discontinued therapy due to side effects caused by the medication, it should be noted that one patient experienced an asymptomatic elevation of γ-glutamyltransferase (GGTP) and alanine aminotransferase (ALT) levels. However, the authors of the study associate these results rather with parallel treatment with atorvastatin, than with inclisiran itself [39]. Apart from this single patient, no significant differences in the profile of adverse effects were observed with and without the use of statins. Although the overall adverse effects occurred in 76% of the patients in both placebo and inclisiran arms of the ORION-1 trial, severe adverse effects were observed in only 8% in the placebo and 11% in the inclisiran group [51]. Even though 3% of patients from the drug-treated subgroup experienced local reactions at the injection site, the overall incidence of adverse effects did not differ significantly between the groups. During the follow-up period, two patients who enrolled in the program died, but neither of these events was attributable to treatment with inclisiran.

One of the adverse reactions observed after siRNA administration is the development of siRNA-induced peripheral neuropathies [54]. Although no mechanism explaining this phenomenon has been demonstrated, it has been proposed that conjugation of siRNA with synthetic strands bound with GalNAc can reduce the risk of its development via a reduction in the peripheral tissue activity. Peripheral neuropathy was not detected in any of the three trials mentioned above.

Though the modified structure of inclisiran with GalNAc molecules increases its liver selectivity, it is essential to remember that along with the other phosphorothioate modifications of ribonucleic acids it may trigger activation of platelet-activating factors and formation of a thrombus even in the therapeutic range of concentrations [55]. Fortunately, to date, no such effect has been observed in clinical trials. However, it must be considered that in the very-high-risk population of patients with CVDs, additionally burdened with hypercholesterolaemia, who are already at elevated risk of thrombosis, the potential pro-thrombotic activity of inclisiran can become clinically pronounced after a longer period of treatment.

Furthermore, in the aforementioned trials, there were no symptoms of immune system activation (such as influenza-like symptoms or elevation of pro-inflammatory markers) which often occur after treatment with ribonucleic acid-based therapeutics. Importantly, the incidence of adverse effects does not differ significantly to that associated with therapy with anti-PCSK9 monoclonal antibodies, such as with evolocumab [31, 35]. However, even though it might be considered an issue similar as with anti-PCSK9 monoclonal antibodies which have still not been utilized for a long period of time, a relatively short follow-up period in the studies conducted to date does not allow confirmation of the long-term safety of inclisiran.

Many studies conducted to date confirm a direct association between statin therapy and new-onset diabetes raising concerns as to whether PCSK9 inhibition will have diabetogenic effects. The Mendelian randomization study performed on more than 550,000 participants reported that patients with PCSK9 genetic variants associated with LDL-C reduction have also an elevated risk of development of type 2 diabetes [56]. To date, according to the data from the RCTs, treatment with neither monoclonal antibodies nor inclisiran has been associated with an increased risk of new onset diabetes [57, 58]. Moreover, in the ORION-1 study, there was a slight tendency for higher reductions of the LDL-C concentration after 180 days in patients with diabetes in almost all treatment regimens, suggesting that these patients might encounter significant benefits from this therapy [59]. We would like, however, to point out that the first studies observing an association between statin use and development of diabetes were published more than 15 years after first clinical trials with those drugs, hence the long-term data are necessary to fully evaluate the relationship between the anti-PCSK9 drugs and new-onset diabetes [60, 61].

Another aspect worth discussion is associated with the method of administration of inclisiran. Even though in short-term follow-up of randomized clinical trials with either monoclonal antibodies or siRNA, the patients did not find the parenteral way of their administration as particularly cumbersome, the analysis of the satisfaction level of patients having the orally- or parenterally-administered drugs leads to the conclusion that they do prefer orally administered drugs [62].

Hence, long-term adherence and satisfaction with inclisiran may decrease over time, although it requires significantly less frequent injections than monoclonal antibodies.

The future

The confirmation of the efficacy of inclisiran in the long-term reduction of LDL-C concentration along with its good tolerability has given the green light for further clinical studies of this drug. In Table 1, the most important phase I-III studies are summarised. The interim results of the primary outcomes from the ORION-3 study, an open-label extension study of the ORION-1 study, have already been published. Regardless of the dose and frequency of dosing of the drug in the ORION-1 study, treatment with 300 mg twice-a-year dose of inclisiran was associated with a 51% reduction of LDL-C concentration in day 210, with an absolute reduction of LDL-C by 59.4 mg/dL [63, 64].

The ORION-4 study will assess the clinical efficacy of a 300 mg dose of inclisiran in a very large population of patients with stable CVD and LDL-C concentrations ≥100 mg/dL [65]. After an introductory 8-week hypolipidaemic optimizing therapy, according to the study protocol, the patients will be randomised to receive the drug or placebo in the first month, the third month and then in consecutive six-month periods. The occurrence of a combined endpoint consisting of CHD, non-fatal myocardial infarction (MI), stroke and urgent coronary revascularisation will be the primary endpoint of the study. The study is designed for a median 5-year follow-up duration [40].

Another important trial, which will shed additional light on inclisiran treatment, will be the ORION-9 trial, which, according to the press release, reached the required number of the patients enrolled significantly ahead of expectations [66]. In this double-blind trial, four hundred patients with HeFH and baseline LDL-C ≥2.6 mmol/l (≥100 mg/dL) will be randomised to either 300 mg inclisiran or placebo injections at days 1, 90, 270 and 450. The mean follow-up is planned for 510 days. The inclusion criterion is that the patients prior to randomization should be treated with a maximal tolerated dose of statin or have documented statin intolerance. The primary endpoint of the study will be the percentage change in LDL-C concentration at day 510 and its time-adjusted

percentage change between day 90 and day 540. Therefore, this trial will measure the real potential of inclisiran in the very-high-risk population who often do not reach their therapeutic goals despite maximal tolerated hypolipidaemic therapy (**Table 1**).

At the ESC 2019 Congress, the 18-month results of the ORION-11 were presented [67, 68]. The objective of the study was to assess the efficacy of inclisiran in patients with atherosclerotic CVD and LDL-C ≥70 mg/dL or with ASCVD equivalent and LDL-C ≥100 mg/dL after treatment with maximal tolerated statin doses [68]. 1,617 patients were randomized in a 1:1 fashion to receive either 300 mg inclisiran at days 1, 90, 270 and 450, or placebo. At day 510, the LDL-C reduction observed among participants treated with inclisiran was 54% in the intention-to-treat analysis. There were no differences between the groups in the occurrence of adverse effects. A numerical 2.5% absolute risk reduction in the pre-specified non-adjudicated CV endpoint, consisting of cardiac death, cardiac arrest, non-fatal MI or stroke, was found in patients treated with the drug.

Study	**Phase**	**Number of patients enrolled**	**Primary endpoint**	**Selected secondary endpoints**
ORION-2 [69]	II	Four patients with HoFH	Percentage change in LDL-C at day 90 Percentage change in LDL-C at day 180	Absolute changes in LDL-C between Day 1 and Days 60, 90, 180
ORION-3 [63, 64]	II	Planned on 490 patients who completed the ORION-1 study	Percentage Change in LDL-C from Day 1 to Day 210 in the Inclisiran Group.	Proportion of Participants in the Inclisiran and Evolocumab Groups with ≥50% LDL-C Reduction.

ORION-4 [65]	III	Planned on 15,000 members of high CV risk population	The occurrence of a composite clinical endpoint of CHD death, MI, fatal or non-fatal ischemic stroke or urgent coronary revascularization.	The occurrence composite of CHD death or MI, or CV death.
ORION-5 [71]	III	Planned on 50-60 patients with HoFH	Analysis of LDL-C change in the course of treatment.	N/A
ORION-7 [70]	I	18 patients with different stages of chronic kidney disease against six patients with normal renal function	Analysis of safety, tolerability, pharmacokinetics and pharmacodynamics in patients with CKD.	Changes in LDL-C and PCSK9 concentrations in at day 60
ORION-9 [66]	III	400 patients with HeFH	Percentage change in LDL-C concentration at day 510 Time-adjusted percentage change between day 90 and day 540.	Absolute change in LDL-C at day 510 Absolute change in LDL-C from baseline between day 90 and day 540
ORION-10 [72]	III	1500 patients with ASCVD from the US	Percentage change in LDL-C concentration at day 510 Time-adjusted percentage change between day 90 and day 540.	Absolute change in LDL-C at day 510 Absolute change in LDL-C from baseline between day 90 and day 540
ORION-11 [67, 68]	III	1500 patients with ASCVD or ASCVD-risk equivalents not from the US	Percentage change in LDL-C concentration at day 510 Time-adjusted percentage change between day 90 and day 540.	Absolute change in LDL-C at day 510 Absolute change in LDL-C from baseline between day 90 and day 540

Table 1. Summary of the most important data from the main trials with inclisiran.

*Abbreviations: ASCVD- atherosclerotic cardiovascular disease. CHD- Coronary Heart Disease, HeFH - Heterozygous Familial Hypercholesterolaemia, HoFH - Homozygous Familial Hypercholesterolaemia US-United States

Conclusions

The widespread introduction of lipid-lowering therapy in CV preventive medicine has contributed substantially to an increase in longevity by reducing CV risk. Despite the demonstrated efficacy of statins and ezetimibe on LDL-C concentration and long-term CVD risk, a large number of patients do not achieve their therapeutic goals, even on maximal tolerated therapy. The introduction of monoclonal antibodies against the PCSK9 protein was a milestone in the treatment of lipid disorders, as their administration leads to unprecedentedly low LDL-C concentrations and a further reduction in mortality in patients with CVD. Inclisiran represents an entirely new mechanism of PSCK9 protein inhibition in hepatocytes, as it targets the mRNA for PCSK9; it has favorable pharmacokinetics, which means that administration is necessary only every 3-6 months. This is an advantage over daily statin therapy and monoclonal antibodies, which are administered every 2-4 weeks, and hence can pose the risk of reduction in the adherence during the long-term treatment. The infrequent administration regimen has the potential to increase the number of patients who maintain their therapeutic goals for the long periods, especially in patients who struggle to comply with daily pharmacotherapy.

Preclinical studies and Phase I and Phase II clinical trials of inclisiran have demonstrated its tolerability and efficacy in promoting the long-term reduction of both PCSK9 protein and LDL-C levels. The efficacy and safety of inclisiran will continue to be assessed in ongoing and forthcoming trials on larger patient groups. If the results of these trials reflect previously published data, they will add further weight to the body of evidence suggesting that inclisiran is a revolutionary new tool in the pharmacological management of plasma lipids.

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Krzysztof Dyrbuś: conceived and designed the review, contributed in the data analysis tool selection, performed the analysis of the subject and wrote the manuscript

Mariusz Gąsior: conceived and designed the review, performed the analysis of the subject and reviewed, discussed and accepted the final version of the manuscript

Peter Penson: conceived and designed the review, performed the analysis of the subject and prepared the figures and tables

Kausik K Ray: conceived and designed the review, performed the analysis of the subject and reviewed, discussed and accepted the final version of the manuscript

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