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Review / Обзорная статья



# PCSK9 Antagonists: Clinical Efficacy and Main Trends in the Development of New Medicines

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## ABSTRACT

**SCIENTIFIC RELEVANCE.** Cardiovascular diseases (CVD) are the leading cause of death worldwide. Dyslipidaemia, as the pathophysiological basis of atherosclerosis, is the most important cause of CVD. Among the factors that modify this pathology, the World Health Organisation lists statins, which effectively reduce cholesterol levels. However, statin treatment compliance is not sufficient to achieve population-based lipid targets. This is a powerful stimulus for the creation of fundamentally new groups of lipid-lowering agents, in particular, antagonists of proprotein convertase subtilisin/kexin type 9 (PCSK9).

**AIM.** The study aimed to review innovative approaches to developing a new generation of lipid-lowering agents, PCSK9 antagonists, and to evaluate the effectiveness, safety, and clinical potential of these medicines.

**DISCUSSION.** PCSK9 antagonists significantly increase the effectiveness of lipid-lowering therapy when combined with statins and are an effective monotherapy in patients with contraindications for statins. PCSK9 monoclonal antibodies, as well as inclisiran, have a favourable risk–benefit ratio. However, the high cost of commercially available PCSK9 antagonists limits their clinical use. A number of promising directions exist for developing new PCSK9 antagonists that have fundamentally different mechanisms of action, such as adnectins; genome editing with CRISPR/Cas9; combining small molecules with low molecular weight PCSK9 inhibitors; PCSK9 vaccines; and antisense oligonucleotides. Medicinal products from these groups are currently at various stages of preclinical and clinical development.

**CONCLUSIONS.** Therefore, new lipid-lowering agents can be developed by synthesising high and low molecular weight PCSK9 ligands and by altering the genetic mechanisms of PCSK9 synthesis. The innovative medicines considered in this review are highly effective, and most have shown no signs of toxicity at the pre-authorisation stage.

**Keywords:** PCSK9 antagonists; proprotein convertase subtilisin/kexin type 9; statins; monoclonal antibodies; alirocumab; evolocumab; small interfering RNA; inclisiran; hypolipidemic agents; cholesterol; low-density lipoproteins

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# Антагонисты PCSK9: эффективность в клинической практике и основные направления создания новых лекарственных средств

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## РЕЗЮМЕ

**АКТУАЛЬНОСТЬ.** Сердечно-сосудистые заболевания являются ведущей причиной смерти в мире. Дислипидемия как патофизиологическая основа атеросклероза — важнейшая причина развития сердечно-сосудистых заболеваний. В число факторов, модифицирующих эту патологию, Всемирная организация здравоохранения включает статины, которые эффективно снижают уровень холестерина. Вместе с тем приверженность лечению статинами недостаточна для достижения популяционных контрольных показателей уровня липидов. Этот факт является мощным стимулом для создания принципиально новых групп гиполипидемических средств, в частности антагонистов пропротеиновой конвертазы субтилизин/кексин типа 9 (PCSK9).

**ЦЕЛЬ.** Обзор инновационных подходов к созданию нового поколения гиполипидемических средств — антагонистов PCSK9, оценка их эффективности, безопасности и перспектив применения в клинической практике.

**ОБСУЖДЕНИЕ.** Применение антагонистов PCSK9 значительно повышает эффективность гиполипидемической терапии при комбинировании со статинами или в случае монотерапии при наличии противопоказаний для назначения с татинов. Препараты моноклональных антител к PCSK9, а также препарат инклисиран характеризуются благоприятным соотношением «польза–риск». Вместе с тем высокая стоимость находящихся в гражданском обороте антагонистов PCSK9 ограничивает их применение в клинической практике. Показано, что перспективными направлениями создания новых антагонистов PCSK9 с принципиально иными механизмами действия являются аднектины, технология редактирования генома CRISPR/Cas9, малые молекулы и низкомолекулярные ингибиторы PCSK9, вакцины против PCSK9, антисмысловые олигонуклеотиды. Препараты из данных групп находятся на различных этапах доклинических и клинических исследований.

**ВЫВОДЫ.** Таким образом, разработка новых гиполипидемических средств может реализовываться как путем синтеза высоко- и низкомолекулярных лигандов PCSK9, так и путем воздействия на генетические механизмы синтеза этого белка. Рассмотренные в обзоре инновационные лекарственные средства отличаются высокой эффективностью, для большинства из них на дорегистрационном этапе не было отмечено проявлений токсичности.

**Ключевые слова:** антагонисты PCSK9; пропротеиновая конвертаза субтилизин/кексин типа 9; статины; моноклональные антитела; алирокумаб; эволокумаб; малая интерферирующая РНК; инклисиран; гиполипидимические средства; холестерин; липопротеины низкой плотности

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## Introduction

According to the Global Burden of Disease study, acute coronary syndrome (ACS) is one of the most important conditions in the world in terms of its prevalence, mortality, and associated healthcare costs [1]. Patients with a history of ACS are at risk of major adverse cardiovascular events [2]. In 2019, 523 million cardiovascular disease (CVD) cases were reported worldwide, with 18.6 million deaths [1]. Based on World Health Organization (WHO) data, atherosclerotic cardiovascular disease (ASCVD) was associated with approximately 32% of all global deaths in 2019. ASCVD accounted for 33–40% of all-age all-cause mortality in the USA and European Union (EU).[1]

In order to reduce low-density lipoprotein cholesterol (LDL-C) in patients with ASCVD to the target levels, the Guideline on the Management of Blood Cholesterol by the American College of Cardiology (ACC) and American Heart Association (AHA) [3] recommends high-intensity statin therapy (rosuvastatin, atorvastatin) or maximally tolerated statin therapy. It is reasonable to supplement maximally tolerated statin therapy with other lipid-lowering agents, in particular, ezetimibe (a cholesterol absorption inhibitor) or antagonists of proprotein convertase subtilisin/kexin type 9 (PCSK9), in ASCVD patients at very high risk (namely, patients with a history of multiple major ASCVD events or 1 major ASCVD event and multiple high-risk conditions, such as an age of over 65 years, heterozygous familial hypercholesterolaemia, a history of prior coronary artery bypass surgery or percutaneous coronary intervention, diabetes mellitus, arterial hypertension, chronic kidney disease, smoking, persistently elevated LDL-C, or a history of congestive heart failure) whose LDL-C levels remain ⩾70 mg/dL (⩾1.8 mmol/L) [3, 4].

PCSK9 inhibitors lower plasma LDL-C levels by up to 60%, even in patients on maximally tolerated statin therapy, significantly reducing the risk of severe CVD, with no risk of serious adverse drug reactions (ADRs). The clinical benefit of PCSK9 inhibitors is an unprecedented reduction in LDL-C levels [3, 4]. Currently, various PCSK9 inhibition approaches are used, which can potentially lead to breakthroughs in dyslipidaemia management.

**This study aimed** to review innovative approaches to developing a new generation of lipid-lowering agents, PCSK9 antagonists, and to evaluate the effectiveness, safety, and clinical potential of these medicines.

## Statins

Statins are competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme for cholesterol biosynthesis.[2] HMG-CoA reductase inhibition reduces cholesterol levels in hepatocytes and myocytes. In its turn, cellular cholesterol reduction upregulates the expression of cell-surface LDL receptors (LDL-R), which leads to an increase in LDL uptake from plasma and, thus, a decrease in circulating blood LDL-C *(Fig. 1, pathways 1–3)*.

When used for hypercholesterolaemia, statins decrease the risk of major vascular events by 22% per 1.0 mmol/L LDL-C reduction [5]. According to clinical trials with a high degree of evidence, statins are effective in lessening cardiovascular morbidity and mortality when used in primary or secondary prevention of ASCVD [6]. A meta-analysis of 14 randomised trials including over 90,000 participants demonstrated a significant downward shift in mortality due to ischaemic coronary heart disease (19%, significance level $p<0.0001$), myocardial infarction or coronary death (23%, $p<0.0001$), and fatal stroke (17%, $p<0.0001$) with statin therapy. Generally, a 1.0 mmol/L decrease in LDL-C levels reduces the incidence of major vascular events by 21% [7]. The reduction in major vascular events is directly proportional to the achieved mean absolute decrease in LDL-C levels. According to an analysis of statin regimens varying in intensity, more intensive regimens produce a further 15% reduction in major vascular events ($p<0.0001$), which suggests additional clinical benefits of such therapy [5, 8].

Adherence to statin therapy, despite its positive effects on cardiovascular outcomes, is often poor, with a non-adherence rate as high as 50% [9]. A meta-analysis of 15 studies on statin adherence [10] showed a 45% increase in all-cause mortality and a 15% increase in CVD in patients with adherence below 80% compared with more adherent patients. In general, high discontinuation rates are attributed to statin-associated ADRs [11], mainly statin-associated muscle symptoms (SAMS). There are various hypotheses that explain SAMS development, ranging from the association of SAMS with low cholesterol levels in muscle cell membranes to the causative role of mevalonate pathway intermediates, such as prenylated proteins, dolichols, and electron transport chain proteins [12]. However, no consensus has been reached on SAMS causes in statin-treated patients.

[1] Cardiovascular diseases (CVDs). WHO; 2021. https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)
[2] https://grls.rosminzdrav.ru/

According to large randomised clinical trials, statin-treated patients also face an increased risk of diabetes mellitus. The estimated attributable (excess) risk of developing diabetes mellitus is approximately 10–20 cases per 10,000 patients continuously treated with statins [13]. Furthermore, a persistent elevation in plasma transaminases in 0.5–3.0% of patients receiving statins has been reported [14].

Statin monotherapy can reduce LDL-C levels to a significant extent (up to 50% at the highest doses of the most effective statins, such as atorvastatin and rosuvastatin). However, this reduction may be insufficient to achieve the target levels that depend on a patient's individual cardiovascular risk. Furthermore, doubling the dose of a statin, on average, generates only a 6% further decrease in LDL-C levels (commonly referred to as the 6% rule) [15]. This necessitates additional approaches involving combining statins with other lipid-lowering agents. For example, ezetimibe might be added to the combination if LDL-C levels remain ⩾70 mg/dL with maximally tolerated statin therapy (class IIb recommendation) [16].

At present, an effective alternative to combination hyperlipidaemia therapy is LDL inhibition by proprotein convertase subtilisin/kexin type 9 proprotein convertase (PCSK9).

## PCSK9/LDL-R binding inhibitors

PCSK9 regulates the degradation of the LDL-C/LDL-R complex during endocytosis *(Fig. 1)*. Hepatocytes internalise LDL-R, which are transported to lysosomes and either degraded or recycled back to the cell surface *(Fig. 1, pathway 4)*. PCSK9 binds to LDL-R at a location other than the LDL-binding site and prevents LDL-R from adopting a closed conformation that protects the receptors from enzymatic degradation [17]. When not bound to PCSK9, the receptors are much more likely to resurface. Ultimately, the LDL-R density on the hepatocyte surface increases, which increases the hepatocyte ability to uptake LDL from plasma [18].

## PCSK9 monoclonal antibodies

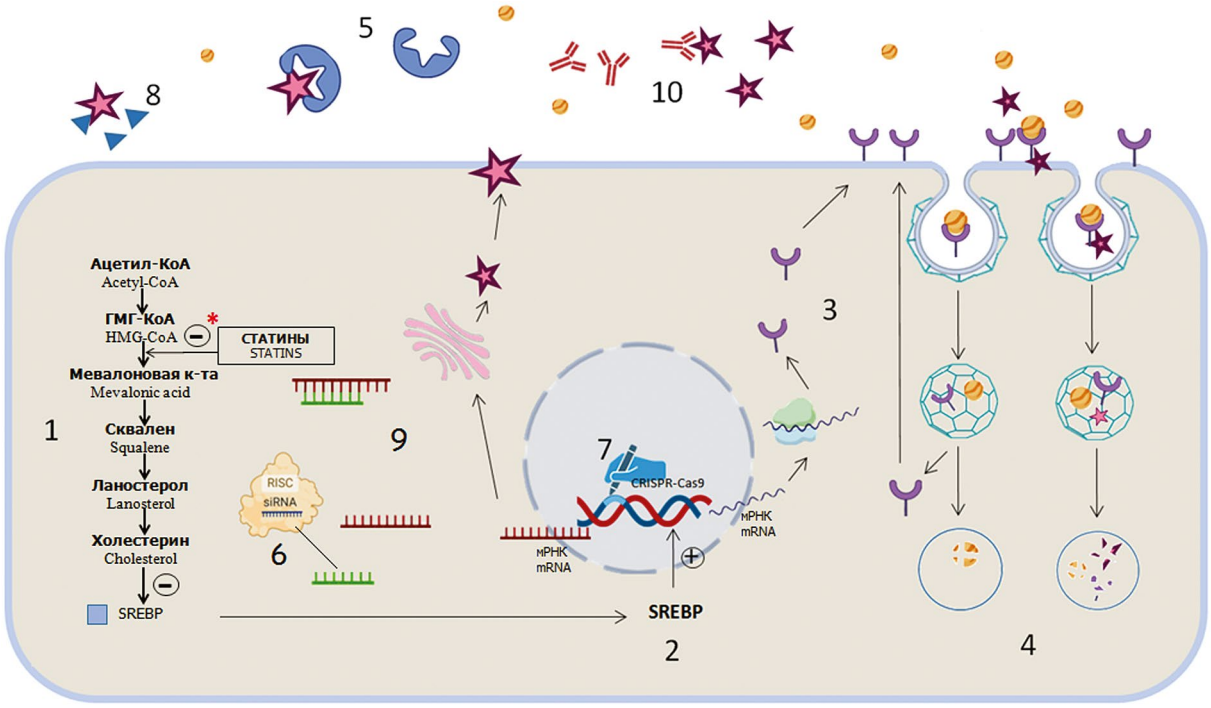
Monoclonal antibodies to PCSK9 became the first clinically effective medicinal products to appear as part of the development of a PCSK9 inhibition strategy. PCSK9 monoclonal antibodies selectively bind to the active centre of the enzyme and prevent its binding to LDL-R *(Fig. 1, pathway 5)*. Currently, the fully human PCSK9 monoclonal antibodies alirocumab and evolocumab[3] are approved for human use in the Russian Federation. Bococizumab, a chimeric antibody retaining 3% of the murine sequence, was withdrawn from development at a late stage because of a relatively high frequency of anti-drug antibodies [19–22].

Evolocumab is a fully humanised monoclonal immunoglobulin G2 (IgG2). After a single subcutaneous injection, the maximum plasma concentration ($C_{max}$) is reached in 3–4 days, the absolute bioavailability is 72%, and the half-life is 11–17 days. Evolocumab is metabolised by proteolysis to small peptides and amino acids. Its metabolism involves immunoglobulin-specific pathways of the reticuloendothelial system. After a single dose, the maximum suppression of circulating PCSK9 occurs in 4 h, providing a gradual decrease (in 14–21 days) in plasma LDL-C levels. According to the package leaflet, the recommended dosing regimen is 140 mg once every 2 weeks or 420 mg once a month. With regular medication, LDL-C levels are reduced by 57–72% from baseline. Evolocumab does not trigger compensatory mechanisms to increase PCSK9 and cholesterol production; it has no rebound effect (increase in PCSK9 and cholesterol) after discontinuation. The most frequent ADRs associated with evolocumab at recommended doses are nasopharyngitis (4.6%), upper respiratory tract infection (4.6%), back pain (4.4%), arthralgia (3.9%), influenza (3.2%), and injection site reactions (2.2%).[4]

Alirocumab, another fully humanised monoclonal antibody of the IgG1 isotype, has similar pharmacokinetics. After a single subcutaneous dose, plasma $C_{max}$ is reached in 3–7 days, and the absolute bioavailability of alirocumab is 85%. The main parameters of its pharmacokinetic profile and efficacy are similar to those of evolocumab [23].

A 50–60% reduction in LDL-C concentration was demonstrated by monoclonal antibodies combined with maximally tolerated statin therapy in large post-registration studies of evolocumab (FOURIER: further cardiovascular outcomes research with PCSK9 inhibition in subjects with elevated risk [24]) and alirocumab (ODYSSEY OUTCOMES: evaluation of cardiovascular outcomes after acute coronary syndrome during treatment with alirocumab [25]). This reduction in LDL-C lowered future cardiovascular risks by ~15% over a median follow-up period of 2.3–2.8 years [24, 25].

[3] https://grls.rosminzdrav.ru/
[4] Ibid.




Гепатоцит / *Hepatocyte*

Пропротеиновая конвертаза субтилизин/кексин типа 9 (PCSK9) / *Proprotein convertase subtilisin/kexin type 9 (PCSK9)*

Липопротеины низкой плотности (ЛПНП) / *Low-density lipoproteins (LDL)*

Рецепторы ЛПНП / *LDL receptors (LDL-R)*

Моноклональные антитела к PCSK9 / *Monoclonal antibodies to PCSK9*

Низкомолекулярные лиганды PCSK9 и аднектины / *Low molecular weight PCSK9 ligands and adnectins*

Вакцина к PCSK9 / *Vaccine against PCSK9*

3-гидрокси-3-метилглутарил-коэнзим А (ГМГ-КоА) редуктаза / *3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase*

Антисмысловой фрагмент инклисирана / *Antisense fragment of inclisiran*

Синтез рецепторов ЛПНП / *Synthesis of LDL receptors*

Аппарат Гольджи / *Golgi apparatus*

Блокада / *blockade*

Активация / *activation*

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**Fig. 1.** Mechanisms of action of lipid-lowering agents
(1) Statins block HMG-CoA reductase, the enzyme catalysing the synthesis of mevalonic acid. The inhibition of HMG-CoA reductase is a rate-limiting step in the metabolic pathway of cholesterol production, decreasing cholesterol levels in hepatocytes. Decreased cholesterol levels activate transcription factors (sterol regulatory element-binding proteins (SREBP)). (2) SREBP transcription factors bind sterol regulatory element DNA sequences and stimulate the expression of LDL-R. (3) An increase in the density of LDL-R leads to an increase in LDL uptake from plasma. (4) Pro-protein convertase subtilisin/kexin type 9 (PCSK9) regulates the LDL-R density on the hepatocyte surface. PCSK9

binds LDL-R on the cell surface without affecting the affinity for LDL. After LDL uptake, the receptors undergo endocytosis. In the presence of PCSK9, the receptors are destroyed in lysosomes, whereas in its absence, the receptors return to the surface of the hepatocyte. (5) Alirocumab and evolocumab, monoclonal antibodies to PCSK9, bind this protein and thus prevent the subsequent destruction of LDL-R. (6) Inclisiran, a small interfering RNA (siRNA), interacts with the RNA-induced silencing complex (RISC); the antisense strand of inclisiran binds to the messenger RNA (mRNA) encoding PCSK9, thus disrupting the synthesis of this protein. (7) CRISPR/Cas9 genome editing prevents the synthesis of PCSK9 by stopping the transcription of the gene encoding this protein, which increases LDL levels on the hepatocyte surface. (8) Low molecular weight ligands for PCSK9 prevent its binding to LDL-R. (9) Antisense nucleotides bind the mRNA encoding PCSK9. (10) Vaccines against PCSK9 cause the production of antibodies that neutralise the activity of PCSK9.

**Рис. 1.** Различные механизмы действия гиполипидемических средств
(1) Статины блокируют ГМГ-КоА редуктазу, фермент, катализирующий синтез мевалоновой кислоты, что лимитирует стадии метаболического пути синтеза холестерина. Снижение уровня холестерина в гепатоците приводит к активации фактора транскрипции SREBP, белка, связывающего регуляторный элемент стерола. (2) SREBP связывается со стерол-регуляторным участком ДНК, стимулируя синтез рецепторов ЛПНП. (3) Повышение плотности ЛПНП-рецепторов приводит к увеличению захвата ЛПНП из плазмы крови. (4) Плотность рецепторов ЛПНП на поверхности гепатоцита регулируется PCSK9. На поверхности клетки белок PCSK9 связывается с рецепторами ЛПНП, не влияя на сродство рецептора к ЛПНП. После захвата ЛПНП рецептор подвергается эндоцитозу. В присутствии PCSK9 рецептор ЛПНП разрушается в лизосомах, а при отсутствии PCSK9 рецептор возвращается на поверхность гепатоцита. (5) Моноклональные антитела к PCSK9 алирокумаб и эволокумаб связывают белок PCSK9, предотвращая разрушение рецепторов ЛПНП. (6) Малая интерфирирующая РНК инклисиран связывается с РНК-индуцированным комплексом сайленсинга, антисмысловой фрагмент инклисирана блокирует м-РНК, кодирующую PCSK9, нарушая синтез этого белка. (7) Редактирование генома с помощью системы CRISPR/Cas9 вызывает угнетение синтеза PCSK9 за счет остановки считывания кодирующего его гена, что способствует повышению содержания ЛПНП на поверхности гепатоцитов. (8) Низкомолекулярные лиганды PCSK9 блокируют его связывание с рецептором ЛПНП. (9) Антисмысловые олигонуклеотиды связывают матричную РНК, кодирующую PCSK9. (10) Вакцина к эпитопам PCSK9 вызывает выработку антител, нейтрализующих активность PCSK9.

The available study data were further confirmed by a meta-analysis of 39 randomised controlled studies in 66,478 patients. The authors of this meta-analysis, Guedeney et al., estimated that alirocumab and evolocumab reduced the risk of cardiovascular disease by 15–20% [26]. At the same time, there was no increased risk of neurocognitive impairment, transaminase activity elevation, rhabdomyolysis, or new-onset diabetes mellitus [27]. Evolocumab and alirocumab combined with statins altered the coronary plaque characteristics in ACS patients, leading to significant thickening of the fibrous cap, thereby stabilising it. According to the HUYGENS and PACMAN-AMI studies, this contributed to atheroma regression [28, 29].

High adherence is required to achieve optimal outcomes with alirocumab and evolocumab. Despite the proven efficacy of PCSK9 inhibitors for hyperlipidaemia management, potential problems may arise because of the need for parenteral administration and, consequently, reduced patient adherence. PCSK9 monoclonal antibodies require parenteral (subcutaneous) administration once or twice a month. Data from 6 clinical trials showed a high level of adherence to treatment with alirocumab for at least 1 year. Treatment-emergent adverse events, including injection site reactions, were rare [30].

However, in contrast to clinical trials, the use of injectable PCSK9 monoclonal antibodies may be difficult in asymptomatic patients in primary care settings because initiation of PCSK9 monoclonal antibodies requires training in the administration of the medicinal product, adherence to the recommended injection schedule, and adequate storage of the medicinal product. In the long term, these limitations may lead to lower adherence to treatment and, consequently, to a decrease in the efficacy of lipid-lowering therapy [30].

Since their first authorisation in 2015, the widespread use of evolocumab and alirocumab has been limited by their low cost-effectiveness. In 2017, the data from the FOURIER trial were analysed to build a cost-effectiveness model and compare statin monotherapy and statin combinations with evolocumab and alirocumab [31]. With the cost of PCSK9 inhibitors at $14,300 per year, the additional cost of using a combination of a PCSK9 inhibitor with a statin was $337,729. This sum was more than three times higher than the cost of conventional lipid-lowering therapy ($100,000). Therefore, the spending limits required that the cost of monoclonal antibodies should be reduced by at least $5,459 per year. The ODYSSEY OUTCOMES study [25] concluded that alirocumab would be considered cost-effective if the cost of

therapy reduced from $14,560 to $1,974 per year. The cost-effectiveness of PCSK9 monoclonal antibodies is expected to improve with the targeted selection of patients at high CVD risk [31, 32]. Thus, economics is a powerful motivation to search for PCSK9 inhibitors that have other mechanisms of action [33].

## PCSK9 synthesis inhibitors

Inclisiran is a medicinal product that targets PCSK9 but has a fundamentally different mechanism of action than anti-PCSK9 monoclonal antibodies. Inclisiran was approved for hypercholesterolaemia or mixed dyslipidaemia by the European Medicines Agency (EMA) in 2020 [34] and by the Russian national regulatory authorities in 2022.[5]

Inclisiran is a long-acting synthetic small interfering RNA (siRNA) that blocks PCSK9 synthesis. The double-stranded siRNA, which consists of a guide (antisense) and a passenger (sense) strand, is conjugated to a vector with N-acetylgalactosamine. Using the N-acetylgalactosamine ligand, inclisiran binds to the asialoglycoprotein receptors (ASGPR) expressed on hepatocytes. The ASGPR–inclisiran complex is internalised into hepatocytes through endocytosis—it is trapped in endocytic vesicles that fuse with endosomes/lysosomes. After its separation from N-acetylgalactosamine and ASGPR, the double-stranded siRNA is slowly released from endosomes/lysosomes into the cytoplasm, while the asialoglycoprotein receptors are recycled back to the cell surface. This is followed by siRNA incorporation into an RNA-induced silencing complex (RISC), and the sense strand is degraded. Afterwards, in the cytoplasm, the RISC-bound antisense strand interacts with the complementary sequence of its target, PCSK9 mRNA *(Fig. 1, pathway 6)*. The RISC uses catalytic slicer activity to cleave PCSK9 mRNA, thereby reducing the amount of mRNA that is available for translation. This results in fewer PCSK9 molecules available for binding with LDL-R, thus minimising the effect of PCSK9 on LDL-R degradation. Reduced LDL-R degradation provides for a higher LDL-R density on the cell surface available for LDL binding and capture, which results in lower plasma LDL-C levels [35].

Inclisiran is injected into the deltoid muscle, first at 3-month and then at 6-month intervals. Inclisiran is used as an adjunct to statin therapy; it is combined with other lipid-lowering agents in statin-intolerant patients.[6]

The safety, tolerability, and efficacy of inclisiran were thoroughly studied in the ORION clinical trial [36]. According to data obtained at Phases I and II, the optimum dosing regimen for inclisiran involves subcutaneous injections at a dose of 284 mg (equivalent to 300 mg of inclisiran sodium) at days 0, 90, and 180 and then every 6 months. Sustained PCSK9 synthesis inhibition by approximately 80% and LDL-C reduction by approximately 50% from baseline were shown by a meta-analysis of patient data from ORION Phase III, which enrolled 3,660 patients with familial hypercholesterolaemia, ASCVD, or at high risk of ASCVD. Moreover, the safety profile of inclisiran was found to be comparable with that of PCSK9 monoclonal antibodies, with no serious ADRs. The most frequent ADRs were mild to moderate transient injection site reactions, which led to therapy discontinuation only in a very small number of patients [36]. Notably, the cost of inclisiran is higher than that of PCSK9 monoclonal antibodies, so the pharmacoeconomics of its use remains a relevant issue.

## Adnectins, polymer PCSK9 ligands

A promising method to influence PCSK9 levels may be the use of adnectin, a small protein (~10 kDA) with variable loops that can be engineered to generate binding surfaces with high affinity and specificity for therapeutically relevant targets [37]. Adnectin BMS-962476 is a polypeptide conjugated to polyethylene glycol to improve pharmacokinetics. BMS-962476 binds to PCSK9 with subnanomolar affinity. Such a substrate affinity inhibits the interaction between PCSK9 and the LDL-R domain [38]. Highly stable PCSK9-binding adnectin has the functional properties of inhibitory PCSK9 monoclonal antibodies and an intermediate size between peptides and immunoglobulins. The abovementioned and the use of bacterial expression systems make adnectin production easier and cheaper than antibody production [38]. In a preclinical study in cynomolgus monkeys, BMS-962476 rapidly lowered free PCSK9 levels by more than 99% and LDL-C levels by ~55%, while total plasma PCSK9 levels increased 6-fold from baseline [38]. A first-in-human study of BMS-962476 combined with statins showed that a single subcutaneous injection of adnectin at the maximum dose reduced free PCSK9 levels by more than 90% and LDL-C levels by ~48% compared with baseline [39]. Hence, adnectin is an effective PCSK9 inhibitor without serious adverse

[5] https://grls.rosminzdrav.ru/
[6] Ibid.

events; therefore, it has the potential to become an alternative to monoclonal antibodies.

A novel adnectin candidate is the PCSK9 antagonist LIB003, a recombinant fusion protein consisting of a PCSK9-binding domain (adnectin) and human serum albumin. In a Phase I clinical trial in volunteers with elevated LDL-C levels randomised to diet or statin therapy, a single injection of LIB003 at doses of 150–600 mg (in volumes of ~0.6–2 mL) was well tolerated and resulted in a sustained reduction in LDL-C levels [40].

Compared with PCSK9 monoclonal antibodies, LIB003 is effective at a smaller injection volume, which may be attributed not so much to the size of the adnectin molecule (11 kDa), as to its high PCSK9 binding efficiency. Moreover, LIB003 binds to human serum albumin, which increases the half-life of the medicinal product to up to 12–15 days [41]. LIB003 can be administered as a small volume injection (about 1 mL) once a month and achieve maximal and stable LDL-C reduction. The medicinal product remains stable at room temperature for more than 6 months. Currently, LIB003 is being evaluated in Phase III clinical trials [42].

## CRISPR/Cas9 genome-editing technology

In experiments *in vivo*, the CRISPR/Cas9 system effectively immobilised the PCSK9 gene in the liver of mice, which led to a decrease in PCSK9 protein levels in plasma and, consequently, to an increase in LDL content in hepatocytes. This resulted in a significant 35–40% decrease in total cholesterol levels with no ADRs [43]. In cynomolgus monkeys, K. Musunuru et al. [44] demonstrated that CRISPR base editors, which were delivered to the liver using lipid nanoparticles, effectively and accurately modified the PCSK9 gene. A single infusion of lipid nanoparticles resulted in a nearly complete knockdown of the PCSK9 gene, with approximately 90% and 60% decreases in blood PCSK9 protein and LDL-C levels, respectively. All these changes remained stable for at least 8 months after the single infusion. T. Rothgangl et al. conducted another study on PCSK9 editing in non-human primates [45]. Similarly to the study by K. Musunuru et al., T. Rothgangl et al. used an adenine base editor, which was delivered in lipid nanoparticles containing editor mRNA and guide gRNA [45]. The use of gRNA, which was selected from a number of analogues, resulted in a high base-editing rate and a significant reduction in the levels of PCSK9-encoding mRNA and the PCSK9 protein *(Fig. 1, pathway 7)*. After successful studies in mice, mRNA- and gRNA-containing lipid nanoparticles were injected into cynomolgus monkeys. The treatment induced a mean of 26% base editing, a 32% decrease in PCSK9 protein levels, and a 14% decrease in LDL-C levels. Since repeated administration did not increase the rate of base editing, T. Rothgangl et al. investigated the potential immune response and found that the animals produced IgG against the base editor when re-injected. Off-target editing, which can lead to unintentional inhibition of other genes, was not observed in studies [46].

## Small-molecules and low-molecular-weight PCSK9 inhibitors

Low-molecular-weight PCSK9 inhibitors, which disrupt the interaction between PCSK9 and LDL-C, can be taken orally and do not cause ADRs typical of injectable medicinal products. The use of low-molecular-weight PCSK9 inhibitors *(Fig. 1, pathway 8)* in combination with statins may be an additional treatment strategy for ASCVD. Moreover, the production of low-molecular-weight PCSK9 inhibitors is simpler and cheaper than the production of PCSK9 monoclonal antibodies [47].

Using GOLD (Genetic Optimisation for Ligand Docking) software, D. Min et al. [48] conducted *in silico* screening of the ChemBridge database and selected 100 potential PCSK9 ligands. D. Min et al. determined the activity of these compounds by assays of PCSK9 binding to LDL-C and LDL-C uptake *in vitro* and by measuring serum LDL-C levels *in vivo*. Several small molecules dose-dependently decreased PCSK9 binding to LDL and significantly increased LDL levels in hepatocytes, as evidenced by an increase in the uptake of fluorescence-labelled LDL by the HepG2 cell line. In addition, one compound significantly reduced total cholesterol and LDL-C levels in the serum of wild-type mice [48].

Mimetic peptides, intended as a therapeutic alternative to monoclonal antibodies, occupy a niche between monoclonal antibodies and low-molecular-weight compounds. As inhibitors of protein–protein interactions, mimetic peptides have the potential for highly specific substrate binding and low immunogenicity [49]. The peptides that mimic epidermal growth factor-like repeat A (EGF-A) and EGF-like repeat B (EGF-B), the binding sites of LDL-R, have been developed as competitive PCSK9 inhibitors. These mimetic peptides bind to the catalytic domain of PCSK9, thus inhibiting the interaction between PCSK9 and LDL-R [50]. The oral macrocyclic peptide MK-0616 by Merck is the first experimental PCSK9 inhibitor that binds to PCSK9 and

inhibits the interaction between PCSK9 and LDL-R. A study in healthy volunteers found that MK-0616 reduced free plasma PCSK9 levels by more than 90% without serious ADRs [51]. Recent Phase II clinical trials of MK-0616 demonstrated a statistically significant, reliable, dose-dependent, placebo-adjusted reduction in LDL-C levels of up to 60.9% from baseline at week 8. MK-0616 was well tolerated during the 8-week treatment and an additional 8-week follow-up period [52].

## PCSK9 vaccines

Medicinal products for active immunisation against PCSK9 were developed as a new alternative to PCSK9 monoclonal antibodies for sustained reduction of LDL-C levels [53]. The PCSK9 vaccine AT04A consists of short peptides that mimic fragments of the mature human PCSK9 protein and are conjugated to a foreign carrier protein that provides a source of T-helper cell epitopes [54]. The AT04A vaccine stimulates the immune system to produce persistent high-affinity PCSK9-specific antibodies that block the ability of PCSK9 to bind to LDL-R *(Fig. 1, pathway 10)*. Induced by the vaccine *in vivo*, PCSK9 antibodies reduce total cholesterol levels by 30% and LDL-C levels by 50%. AT04A has a long half-life period of approximately 4 months and induces a reduction in cholesterol levels that lasts for at least 1 year. The results obtained in mice indicate that a booster dose 1 year after the first immunisation successfully reactivates the immune response to PCSK9, suggesting the possibility of annual booster immunisation [55]. Additionally, vaccines based on virus-like particles self-assembling from nucleic acid-free viral proteins induce pronounced antibody responses against self-antigens. In a study in rhesus macaques, anti-PCSK9 vaccination reduced LDL-C levels by 10–15% and LDL levels by 28% [56].

## Antisense oligonucleotides

SPC5001 is a 14-mer oligonucleotide with locked nucleic acid (LNA) modifications. This oligonucleotide can act as an antisense inhibitor by reducing intracellular and extracellular PCSK9 protein levels *(Fig. 1, pathway 9)*. Preclinical studies in mice and non-human primates have shown no evidence of SPC5001 toxic effects on kidney and liver function. In a randomised double-blind placebo-controlled study [57], SPC5001 was administered to healthy volunteers with elevated cholesterol and LDL levels. The volunteers demonstrated a decrease in plasma PCSK9 and LDL levels, while having signs of renal tubular toxicity, and one patient developed acute tubular necrosis. Further studies are needed to clarify the molecular mechanism of renal toxicity of antisense oligonucleotides and to explore ways to minimise adverse effects.

Another study[7] evaluated the lipid-lowering efficacy of a PCSK9 synthesis antagonist, the antisense oligonucleotide AZD8233, in high-risk hypercholesterolaemia patients taking high doses of statins. Patients received 3 subcutaneous injections of AZD8233 at doses of 15, 50, and 90 mg during a 12-week period. By week 12, the reduction in LDL-C levels from baseline was 39, 73, and 79%, and the reduction in PCSK9 levels from baseline was 58, 89, and 94%, respectively. Clinically significant reduction in LDL-C levels was observed one week after the first injection.[8] In general, patients tolerated AZD8233 well. All patients receiving AZD8233 maintained a clinically significant reduction in the investigated parameters until week 14 (6 weeks after the last dose).

Summarised information on the groups of PCSK9 antagonists described in this article is presented in *Table 1*: Main trends in the development of PCSK9 antagonists (published on the journal's website[9]).

## Conclusion

To achieve the milestones set for modern lipid-lowering therapy, statins should be combined with effective agents that can influence cholesterol levels through other mechanisms of action. PCSK9 inhibitors have already been successfully used for this purpose. However, the high cost of medicinal products comprising this group significantly limits their widespread use in medical practice. Intensive research in this area has led to the development of novel experimental medicines with mechanisms of hypolipidaemic action fundamentally different from those of statins, such as PCSK9 synthesis inhibitors, adnectins (polymeric PCSK9 ligands), CRISPR/Cas9 genome-editing systems, low-molecular-weight PCSK9 inhibitors, and PCSK9 vaccines. The high efficacy and absence of severe ADRs hold promise for the appearance of medicinal products of the described groups in clinical practice.

[7] https://classic.clinicaltrials.gov/ct2/show/NCT04641299
[8] https://s3.amazonaws.com/ctr-med-7111/D7990C00003/e0de9a3b-3e99-46dd-a46f-c217b8279d7e/ffb71037-b725-4e35-a923-b2993a1fb05c/d7990c00003_CSR_synopsis_Redacted-v1.pdf
[9] https://doi.org/10.30895/2312-7821-2023-11-3-279-291-table1

## Литература / References


1. Iannuzzo G, Gentile M, Bresciani A, Mallardo V, Di Lorenzo A, Merone P, et al. Inhibitors of protein convertase subtilisin/kexin 9 (PCSK9) and acute coronary syndrome (ACS): the state-of-the-art. *J Clin Med*. 2021;10(7):1510. https://doi.org/10.3390/jcm10071510
2. Schwartz GG, Chaitman BR. Initiating PCSK9 inhibition in hospital for ACS: we can, but does that mean we should? *J Am Coll Cardiol*. 2019;74(20):2463–5. https://doi.org/10.1016/j.jacc.2019.09.039
3. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK., Blumenthal RS et al. AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *J Am Coll Cardiol*. 2018;73(24);E285–E350. https://doi.org/10.1016/j.jacc.2018.11.003
4. Ferri N, Grego MF, Corsini A, Ruscica M. Proprotein convertase subtilisin/kexin type 9: an update on the cardiovascular outcome studies. *Eur Heart J Suppl*. 2020;22(Suppl E):E64–E67. https://doi.org/10.1093/eurheartj/suaa063
5. Cholesterol Treatment Trialists' (CTT) Collaboration; Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, et al. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. *Lancet*. 2010;376(9753):1670–81. https://doi.org/10.1016/S0140-6736(10)61350-5
6. Taylor F, Huffman MD, Macedo AF, Moore TH, Burke M, Davey Smith G, et al. Statins for the primary prevention of cardiovascular disease. *Cochrane Database Syst Rev*. 2013;2013(1):CD004816. https://doi.org/10.1002/14651858.CD004816.pub5
7. Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, et al. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet*. 2005;366(9493):1267–78. https://doi.org/10.1016/S0140-6736(05)67394-1
8. Dimmitt SB, Stampfer HG, Warren JB. The pharmacodynamic and clinical trial evidence for statin dose. *Br J Clin Pharmacol*. 2018;84(6):1128–35. https://doi.org/10.1111/bcp.13539
9. Blackburn DF, Dobson RT, Blackburn JL, Wilson TW, Stang MR, Semchuk WM. Adherence to statins, beta-blockers and angiotensin-converting enzyme inhibitors following a first cardiovascular event: a retrospective cohort study. *Can J Cardiol*. 2005;21(6):485–8. PMID: 15917876
10. Chowdhury R, Khan H, Heydon E, Shroufi A, Fahimi S, Moore C, et al. Adherence to cardiovascular therapy: a meta-analysis of prevalence and clinical consequences. *Eur Heart J*. 2013;34(38):2940–8. https://doi.org/10.1093/eurheartj/eht295
11. Corrao G, Conti V, Merlino L, Catapano AL, Mancia G. Results of a retrospective database analysis of adherence to statin therapy and risk of nonfatal ischemic heart disease in daily clinical practice in Italy. *Clin Ther*. 2010;32(2):300–10. https://doi.org/10.1016/j.clinthera.2010.02.004
12. Oesterle A, Laufs U, Liao JK. Pleiotropic effects of statins on the cardiovascular system. *Circ Res*. 2017;120(1):229–43. https://doi.org/10.1161/CIRCRESAHA.116.308537
13. Pinal-Fernandez I, Casal-Dominguez M, Mammen AL. Statins: pros and cons. *Med Clin (Barc)*. 2018;150(10):398–402. https://doi.org/10.1016/j.medcli.2017.11.030
14. Egom EE, Hafeez H. Biochemistry of statins. *Adv Clin Chem*. 2016;73:127–68. https://doi.org/10.1016/bs.acc.2015.10.005
15. Буланова ЕЮ. Статинотерапия: доказательства, мнения экспертов, перспективы. *Лечебное дело*. 2013;(3):59–77.
Bulanova EU. Statin therapy: evidence, expert opinions, perspective. *Lečebnoe delo*. 2013;(3):59–77 (In Russ.).
16. Reiter-Brennan C, Osei AD, Iftekhar Uddin SM, Orimoloye OA, Obisesan OH, Mirbolouk M, et al. ACC/AHA lipid guidelines: personalized care to prevent cardiovascular disease. *Cleve Clin J Med*. 2020;87(4):231–9. https://doi.org/10.3949/ccjm.87a.19078
17. Leren TP. Sorting an LDL receptor with bound PCSK9 to intracellular degradation. *Atherosclerosis*. 2014;237(1):76–81. https://doi.org/10.1016/j.atherosclerosis.2014.08.038
18. Page MM, Watts GF. PCSK9 inhibitors—mechanisms of action. *Aust Prescr*. 2016;39(5):164–7. https://doi.org/10.18773/austprescr.2016.060
19. Baruch A, Mosesova S, Davis JD, Budha N, Vilimovskij A, Kahn R, et al. Effects of RG7652, a monoclonal antibody against PCSK9, on LDL-C, LDL-C subfractions, and inflammatory biomarkers in patients at high risk of or with established coronary heart disease (from the Phase 2 EQUATOR Study). *Am J Cardiol*. 2017;119(10):1576–83. https://doi.org/10.1016/j.amjcard.2017.02.020
20. Zhang L, McCabe T, Condra JH, Ni YG, Peterson LB, Wang W, et al. An anti-PCSK9 antibody reduces LDL-cholesterol on top of a statin and suppresses hepatocyte SREBP-regulated genes. *Int J Biol Sci*. 2012;8(3):310–27. https://doi.org/10.7150/ijbs.3524
21. Yokote K, Suzuki A, Li Y, Matsuoka N, Teramoto T. Pharmacokinetics and exploratory efficacy biomarkers of bococizumab, an anti-PCSK9 monoclonal antibody, in hypercholesterolemic Japanese subjects. *Int J Clin Pharmacol Ther*. 2019;57(12):575–89. https://doi.org/10.5414/CP203418
22. Ridker PM, Revkin J, Amarenco P, Brunell R, Curto M, Civeira F, et al. Cardiovascular efficacy and safety of bococizumab in high-risk patients. *N Engl J Med*. 2017;376(16):1527–39.

https://doi.org/10.1056/NEJMoa1701488
23. Li H, Wei Y, Yang Z, Zhang S, Xu X, Shuai M, et al. Safety, tolerability, pharmacokinetics, and pharmacodynamics of alirocumab in healthy Chinese subjects: a randomized, double-blind, placebo-controlled, ascending single-dose study. *Am J Cardiovasc Drugs*. 2020;20(5):489–503. https://doi.org/10.1007/s40256-020-00394-1
24. Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, et al. Evolocumab and clinical outcomes in patients with cardiovascular disease. *N Engl J Med*. 2017;376(18):1713–22. https://doi.org/10.1056/NEJMoa1615664
25. Schwartz GG, Steg PG, Szarek M, Bhatt DL, Bittner VA, Diaz R, et al. Alirocumab and cardiovascular outcomes after acute coronary syndrome. *N Engl J Med*. 2018;379(22):2097–107. https://doi.org/10.1056/NEJMoa1801174
26. Guedeney P, Giustino G, Sorrentino S, Claessen BE, Camaj A, Kalkman DN, et al. Efficacy and safety of alirocumab and evolocumab: a systematic review and meta-analysis of randomized controlled trials. *Eur Heart J*. 2022;43(7):e17–e25. https://doi.org/10.1093/eurheartj/ehz430
27. Evolocumab. In: *LiverTox: Clinical and Research Information on Drug-Induced Liver Injury*. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012. https://www.ncbi.nlm.nih.gov/books/NBK548469/
28. Nicholls SJ, Kataoka Y, Nissen SE, Prati F, Windecker S, Puri R, et al. Effect of evolocumab on coronary plaque phenotype and burden in statin-treated patients following myocardial infarction. *JACC Cardiovasc Imaging*. 2022;15(7):1308–21. https://doi.org/10.1016/j.jcmg.2022.03.002
29. Räber L, Ueki Y, Otsuka T, Losdat S, Häner JD, Lonborg J, et al. Effect of alirocumab added to high-intensity statin therapy on coronary atherosclerosis in patients with acute myocardial infarction: the PACMAN-AMI randomized clinical trial. *JAMA*. 2022;327(18):1771–81. https://doi.org/10.1001/jama.2022.5218
30. Farnier M. Alirocumab for the treatment of hyperlipidemia in high-risk patients: an updated review. *Expert Rev Cardiovasc Ther*. 2017;15(12):923–32. https://doi.org/10.1080/14779072.2017.1409115
31. Korman MJ, Retterstøl K, Kristiansen IS, Wisløff T. Are PCSK9 inhibitors cost effective? *Pharmacoeconomics*. 2018;36(9):1031–41. https://doi.org/10.1007/s40273-018-0671-0
32. Arrieta A, Page TF, Veledar E, Nasir K. Economic evaluation of PCSK9 inhibitors in reducing cardiovascular risk from health system and private payer perspectives. *PLoS One*. 2017;12(1):e0169761. https://doi.org/10.1371/journal.pone.0169761
33. Rosenson RS, Hegele RA, Fazio S, Cannon CP. The evolving future of PCSK9 inhibitors. *J Am Coll Cardiol*. 2018;72(3):314–29. https://doi.org/10.1016/j.jacc.2018.04.054
34. Fitzgerald K, White S, Borodovsky A, Bettencourt BR, Strahs A, Clausen V, et al. A highly durable RNAi therapeutic inhibitor of PCSK9. *N Engl J Med*. 2017;376(1):41–51. https://doi.org/10.1056/NEJMoa1609243
35. Lagace TA. PCSK9 and LDLR degradation: regulatory mechanisms in circulation and in cells. *Current Opinion in Lipidology*, 2014; 25(5), 387–93. https://doi.org/10.1097/MOL.0000000000000114
36. Wright RS, Ray KK, Raal FJ, Kallend DG, Jaros M, Koenig W, et al. Pooled patient-level analysis of inclisiran trials in patients with familial hypercholesterolemia or atherosclerosis. *J Am Coll Cardiol*. 2021;77(9):1182–93. https://doi.org/10.1016/j.jacc.2020.12.058
37. Lipovsek D. Adnectins: engineered target-binding protein therapeutics. *Protein Eng Des Sel*. 2011;24(1–2):3–9. https://doi.org/10.1093/protein/gzq097
38. Mitchell T, Chao G, Sitkoff D, Lo F, Monshizadegan H, Meyers D, et al. Pharmacologic profile of the adnectin BMS-962476, a small protein biologic alternative to PCSK9 antibodies for low-density lipoprotein lowering. *J Pharmacol Exp Ther*. 2014;350(2):412–24. https://doi.org/10.1124/jpet.114.214221
39. Stein EA, Kasichayanula S, Turner T, Kranz T, Arumugam U, Biernat L, et al. LDL cholesterol reduction with BMS-962476, an adnectin inhibitor of PCSK9: results of a single ascending dose study. *J Am Coll Cardiol*. 2014;63(12):A1372. https://doi.org/10.1016/S0735-1097(14)61372-3
40. Арабидзе ГГ. Обзор материалов 88 Конгресса Европейского общества по атеросклерозу (EAS), проходившего 04–07 октября 2020 г. в online-формате *Атеросклероз и дислипидемии*. 2020;4(41):44–6. Arabidze GG. Review based on the materials of the 88th Congress of the European Atherosclerosis Society (EAS) held on October 04–07, 2020 in on-line format. *Atherosclerosis and Dyslipidemia*. 2020;4(41):44–6 (In Russ.). https://doi.org/10.34687/2219-8202.JAD.2020.04.0006
41. Catapano AL, Pirillo A, Norata GD. New pharmacological approaches to target PCSK9. *Curr Atheroscler Rep*. 2020;22(7):24. https://doi.org/10.1007/s11883-020-00847-7
42. Brandts J, Ray KK. Familial hypercholesterolemia: JACC focus seminar 4/4. *J Am Coll Cardiol*. 2021;78(18):1831–43. https://doi.org/10.1016/j.jacc.2021.09.004
43. Ding Q, Strong A, Patel KM, Ng SL, Gosis BS, Regan SN, et al. Permanent alteration of PCSK9 with *in vivo* CRISPR-Cas9 genome editing. *Circ Res*. 2014;115(5):488–92. https://doi.org/10.1161/CIRCRESAHA.115.304351
44. Musunuru K, Chadwick AC, Mizoguchi T, Garcia SP, DeNizio JE, Reiss CW, et al. *In vivo* CRISPR base editing of PCSK9 durably lowers cholesterol in primates. *Nature*. 2021;593(7859):429–34. https://doi.org/10.1038/s41586-021-03534-y
45. Rothgangl T, Dennis MK, Lin PJC, Oka R, Witzigmann D, Villiger L, et al. *In vivo* adenine base editing

of PCSK9 in macaques reduces LDL cholesterol levels. *Nat Biotechnol*. 2021;39(8):949–57. https://doi.org/10.1038/s41587-021-00933-4
46. Katzmann JL, Cupido AJ, Laufs U. Gene therapy targeting PCSK9. *Metabolites*. 2022;12(1):70. https://doi.org/10.3390/metabo12010070
47. Hernandez I, Bott SW, Patel AS, Wolf CG, Hospodar AR, Sampathkumar S, Shrank WH. Pricing of monoclonal antibody therapies: higher if used for cancer? *Am J Manag Care*. 2018;24(2):109–12. PMID: 29461857
48. Min DK, Lee HS, Lee N, Lee CJ, Song HJ, Yang GE, et al. *In silico* screening of chemical libraries to develop inhibitors that hamper the interaction of PCSK9 with the LDL receptor. *Yonsei Med J*. 2015;56(5):1251–7. https://doi.org/10.3349/ymj.2015.56.5.1251
49. Tombling BJ, Zhang Y, Huang YH, Craik DJ, Wang CK. The emerging landscape of peptide-based inhibitors of PCSK9. *Atherosclerosis*. 2021;330:52–60. https://doi.org/10.1016/j.atherosclerosis.2021.06.903
50. Lavecchia A, Cerchia C. Recent advances in developing PCSK9 inhibitors for lipid-lowering therapy. *Future Med Chem*. 2019;11(5):423–41. https://doi.org/10.4155/fmc-2018-0294
51. Johns DG, Almonte A, Bautmans A, Campeau L, Cancilla MT, Chapman J, et al. The clinical safety, pharmacokinetics, and LDL-cholesterol lowering efficacy of MK-0616, an oral PCSK9 inhibitor. *Circulation*. 2021;144:e573.
52. Ballantyne CM, Banka P, Mendez G, Garcia R, Rosenstock J, Rodgers A et al. Phase 2b randomized trial of the oral PCSK9 inhibitor MK-0616. *J Am Coll Cardiol*. 2023;81(16), 1553–64. https://doi.org/10.1016/j.jacc.2023.02.018
53 Chackerian B, Remaley A. Vaccine strategies for lowering LDL by immunization against proprotein convertase subtilisin/kexin type 9. *Curr Opin Lipidol*. 2016;27(4):345–50. https://doi.org/10.1097/MOL.0000000000000312
54. Landlinger C, Pouwer MG, Juno C, van der Hoorn JWA, Pieterman EJ, Jukema JW, et al. The AT04A vaccine against proprotein convertase subtilisin/kexin type 9 reduces total cholesterol, vascular inflammation, and atherosclerosis in APOE*3Leiden.CETP mice. *Eur Heart J*. 2017;38(32):2499–507. https://doi.org/10.1093/eurheartj/ehx260
55. Zeitlinger M, Bauer M, Reindl-Schwaighofer R, Stoekenbroek R, Lambert G, Berger-Sieczkowski E, et al. A phase I study assessing the safety, tolerability, immunogenicity, and low-density lipoprotein cholesterol-lowering activity of immunotherapeutics targeting PCSK9 . *Eur J Clin Pharmacol*. 2021;77:1473–84. https://doi.org/10.1007/s00228-021-03149-2
56. Crossey E, Amar MJA, Sampson M, Peabody J, Schiller JT, Chackerian B, Remaley AT. A cholesterol-lowering VLP vaccine that targets PCSK9. *Vaccine*. 2015;33(43):5747–55. https://doi.org/10.1016/j.vaccine.2015.09.044
57. Чаулин АМ, Дупляков ДВ. О роли PCSK9 в развитии атеросклероза: молекулярные аспекты. *Молекулярная медицина*. 2021;19(2):8–15. Chaulin AM, Duplyakov DV. On the role of PCSK9 in the development of atherosclerosis: molecular aspects. *Molecular Medicine*. 2021;19(2):8–15 (In Russ.). https://doi.org/10.29296/24999490-2021-02-02

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