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# Immunotherapy and vaccine-based approaches for atherosclerosis prevention: a systematic review study

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## Abstract

**Introduction** Cardiovascular disease is a major global health issue, and atherosclerosis is a leading cause of cardiovascular conditions. Traditional approaches for managing atherosclerosis have limitations, creating a need for alternative preventive strategies such as vaccines.

**Methods** The authors conducted a systematic review following Cochrane Handbook and PRISMA guidelines. They searched multiple databases for studies on preventive vaccines against atherosclerosis, including clinical trials and experimental models. The search period was from 1950 to August 2024.

**Results** After screening and evaluation, 47 studies were included in the systematic review. The studies investigated various vaccine candidates and immunization strategies. Vaccination goals involve targeting proteins that are found in higher quantities in individuals with atherosclerosis, such as oxidized low-density lipoprotein (LDL), apolipoprotein B-100, proprotein convertase subtilisin/kexin type-9 serine protease (PCSK9), cholesteryl ester transfer protein (CETP), and heat shock proteins HSP60 and HSP65. The review highlights the potential of vaccines in preventing atherosclerosis by targeting specific antigens, modulating lipoprotein metabolism, and enhancing immune responses. Promising approaches included PCSK9 inhibitors, virus-like particle (VLP)-based vaccines, and gene-editing techniques. Monoclonal antibodies like alirocumab, designed to inhibit PCSK9, were also effective in reducing LDL cholesterol levels.

**Conclusion** This systematic review provides insights into the progress, challenges, and future directions of preventive vaccine research against atherosclerosis. The findings support the development of effective vaccines to complement existing preventive strategies and reduce the global burden of cardiovascular diseases.

**Clinical trial number** It is not applicable.

**Keywords** Cardiovascular disease, Atherosclerosis, Vaccine, PCSK9, CETP

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## Introduction

Cardiovascular disease has emerged as one of the primary contributors to both early death and disability on a worldwide scale [1, 2]. Based on the 2015 Global Burden of Diseases, Injuries, and Risk Factor Study, approximately 422.7 million individuals were affected by cardiovascular disease and it was responsible for approximately 17.9 million deaths across the globe that year, constituting 31% of total worldwide mortality [1, 3]. By the year 2030, it is projected that around 23.6 million individuals will experience annual fatalities as a result of cardiovascular diseases [4]. The significant and continuously increasing impact of cardiovascular diseases on individuals, families, and healthcare systems highlights the pressing requirement for research on atherosclerotic diseases and the adoption of preventive measures [4].

Atherosclerosis, the primary underlying mechanism of numerous cardiovascular conditions, can initiate at a young age and remain dormant and symptom-free for extended periods before advancing to more advanced stages [5]. The prevention and management of atherosclerosis, a chronic inflammatory disease of the arteries, remains a global health challenge [6]. Atherosclerosis is a leading cause of cardiovascular diseases like heart attacks and strokes, which contribute to significant morbidity and mortality worldwide [7, 8]. The traditional strategies for managing atherosclerosis have primarily focused on lifestyle modifications and pharmacotherapy [9]. Among these, immunotherapy-based approaches have gained attention as potential adjuncts to traditional therapies. These approaches include traditional vaccine candidates, which stimulate the immune system to recognize and neutralize specific atherosclerosis-related antigens, and other immunomodulatory therapies, such as monoclonal antibodies (e.g., PCSK9 inhibitors like alirocumab and evolocumab) and RNA-based therapeutics, which regulate lipid metabolism and inflammation but do not induce active immunity in the classical sense [10, 11].

The development of preventive vaccines against atherosclerosis is grounded in the understanding of its underlying pathophysiology [12]. Atherosclerosis is characterized by the accumulation of lipids, immune cells, and fibrous tissue in the arterial walls, leading to the formation of plaques [13, 14]. These plaques can rupture, triggering the formation of blood clots that block blood flow and cause severe cardiovascular events. Immune cells, including monocytes and T lymphocytes, play key roles in plaque formation and progression, providing potential targets for vaccine-mediated interventions [15].

In recent years, significant progress has been made in elucidating the immunological mechanisms involved in atherosclerosis and identifying potential vaccine candidates. Various approaches have been explored, such as targeting specific antigens associated with atherosclerotic plaques, modifying lipoprotein metabolism, modulating immune responses, and utilizing novel delivery systems to enhance vaccine efficacy. These advances hold great promise for the development of effective preventive vaccines tailored to tackle atherosclerosis, potentially complementing existing preventive strategies [16, 17].

In this systematic review, we comprehensively evaluated the existing literature on preventive vaccine development against atherosclerosis. We systematically analyzed published studies, clinical trials, and experimental models to summarize the current understanding of vaccine candidates, immunization strategies, and their potential impact on atherosclerotic lesion development, plaque stabilization, and clinical outcomes. By consolidating the available evidence, this review aims to provide valuable insights into the progress, challenges, and future directions in the field of atherosclerosis vaccine research, with the ultimate goal of preventing and reducing the burden of cardiovascular diseases worldwide.

## Methods

To carry out this systematic review, we followed the guidelines outlined in the Cochrane Handbook for Systematic Reviews [18]. In addition, we used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines [19] in writing this review article.

### Information sources and search strategy

For this systematic review study, we systematically searched valid databases including PubMed, Scopus, Web of Science, Embase, and Cochrane using the following keywords: Atherosclerosis OR Arteriosclerosis OR Cardiovascular Disease OR CVD OR Coronary Artery Disease OR Heart Disease AND Vaccine OR Vaccines OR Immunization OR Prophylactic Vaccines OR Preventive Immunization OR Vaccine Therapy. The strategy included an extensive list of keywords showed in Table 1. The time period for searching the databases was from 1950 to August 2024. No restrictions to language or publication status were applied. Proper Boolean operators and database filters were applied to optimize the search. The strategies were peer reviewed by another author

**Table 1** Keywords used in search strategy for literature review on vaccine

| Concept 1 | Combine by | Concept2 |
|---|---|---|
| Atherosclerosis | AND | vaccin* |
| Atherosclero* | | Immunis* |
| Atherogenesis | | Immuniz* |
| Anti-atherosclerosis | | Vaccines |
| Cardiovascular Disease | | Immunization |
| CVD | | Prophylactic Vaccines |
| Coronary Artery Disease | | |

prior to execution using the Peer Review of Electronic Search Strategies Checklist [20]. The reference lists of relevant reviews and included studies were screened for additional references.

### Eligibility criteria

After the initial search, the records obtained from each database are transferred to the EndNote software to start the screening process. After eliminating duplicate studies, the initial screening process was done independently by two researchers. We included studies that investigated a vaccine or a vaccine adjuvant in the prevention of atherosclerosis. Studies that were conducted on human or animal samples and even cell culture media were included. We excluded studies that investigated a biological agent in the treatment of atherosclerosis. Also, other type of the studies included review, abstract, seminar presentation and posters were excluded. Any disagreement between two authors in the selection of articles was resolved through consultation with a third and experienced person.

### Selection process

Data related to the included studies were extracted by two researchers independently. Two authors independently reviewed the identified citations in a three-step process to determine their suitability for inclusion. Titles were initially screened based on their relevance to the main keywords. Abstracts of potentially eligible articles were then examined. Finally, full-text articles meeting the inclusion criteria were independently reviewed by both authors. Disagreements between the two authors were resolved collaboratively or by seeking the opinion of another author.

### Data extraction

Pairs of authors independently extracted data from the eligible studies using a pre-designed Microsoft Excel spreadsheet. A third author conducted a quality check of the extracted data.

This data included the name of the first author, the location of the study, the year of publication, the factor used as a vaccine, type of sample, intervention duration and a summary of the results.

### Risk of bias assessment

Two authors independently appraised the risk of bias in each included study. In the event of disagreement, a third author was consulted to provide a final assessment. SYRCLE's risk of bias tool for animal studies was used to assess the risk of bias [21]. The selected studies were assessed using the following types of bias: selection bias (domains: sequence generation, baseline characteristics, allocation concealment), performance bias (domains: randomization of animal housing conditions, blinding), detection bias (domains; random outcome assessment, blinding), attrition bias (domain: incomplete outcome data), reporting bias (domain: selective outcome reporting), other (domain: other sources of bias). The risk level for each domain was determined by whether the judgment was yes, no, or unclear. Accordingly, domains were classified as low risk, high risk, or unclear risk. SYRCLE's risk of bias tool was recalibrated to AHRQ standards, categorizing studies as good, fair, or poor quality based on the risk levels of the individual domains.

### Data synthesis

Due to the high heterogeneity between the results, meta-analysis was not possible in this study.

## Results

### Identified literature

A total of 7890 records from various languages were found in the databases through the use of combined search techniques (Fig. 1). Following the elimination of duplicate entries, a total of 5700 records were selected for initial screening based on their titles and abstracts, and out of these, 525 records were considered for thorough evaluation of their full texts. In the second phase of screening, we evaluated the full text of the included studies and after evaluation, 47 studies were included in this systematic study [11, 22–67]. The included studies were conducted between 1989 and 2024. The results of quality assessment among the animal studies were summarized in Table 2. Most of the studies except nine studies obtained fair and poor quality.

### Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors

Recently, there has been significant interest in Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) inhibitors as a potential therapeutic strategy for reducing LDL-C levels in patients with high cholesterol [68]. These agents have shown promising results in lowering lipid levels and reducing cardiovascular risk. However, it is important to clarify that while some PCSK9-targeting strategies, such as virus-like particle (VLP)-based approaches, share similarities with vaccine technologies, they do not meet the classical definition of a vaccine. Instead, monoclonal antibodies (e.g., alirocumab, evolocumab) and gene-based therapies (e.g., small interfering RNA like inclisiran) function as immunotherapies or targeted interventions rather than traditional vaccines that induce active immunity [69].

PCSK9, originating from the liver, is a plasma protease. It is first produced as a 75 kDa precursor protein and later transformed into a mature form weighing 62 kDa through an autocatalytic cleavage process in the Golgi

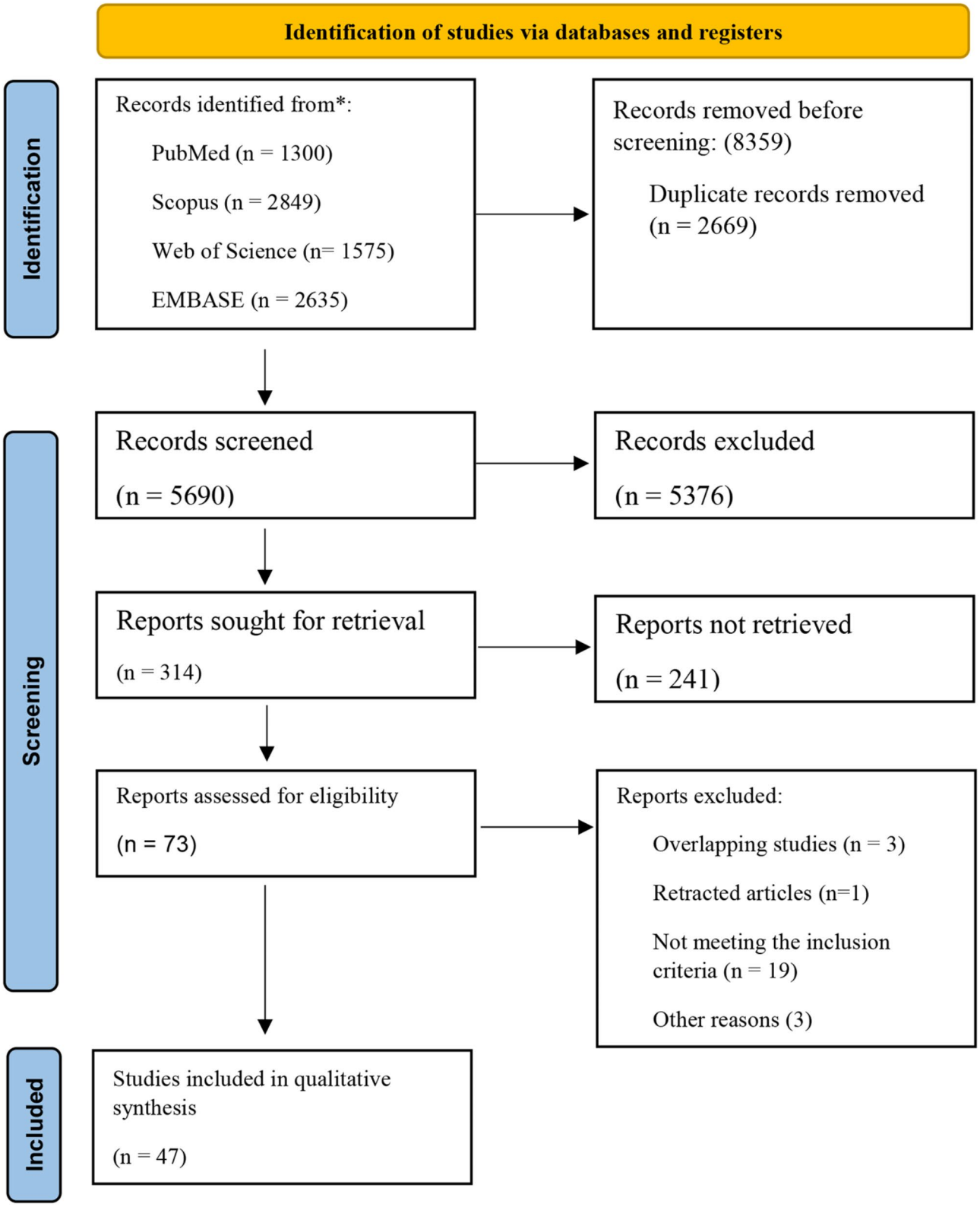


**Fig. 1** Preferred Reporting Items For Systematic Reviews Diagram for search process

**Table 2** SYRCLE's risk of Bias tool for included Studies *

| Studies | Risk of Bias Tool | | | | | | AHRQ Rating |
|---|---|---|---|---|---|---|---|
| | SB | PB | DB | AB | RB | OB | |
| Kimura et al./2023 | Low | Low | High | Low | Low | Low | Fair |
| Graham et al/2007 | Low | Low | Unclear | Unclear | Low | Low | Fair |
| Momtazi-Borojeni et al./2021 | Low | Low | High | High | High | Low | Poor |
| Crossey et al./2015 | High | Low | Unclear | Low | Unclear | Low | Poor |
| Ding et al.2018 | Low | Low | Low | Unclear | Unclear | Unclear | Poor |
| Wu et al.2021 | Low | Low | Low | Low | Low | Low | Good |
| Galabova et al./2014 | Low | Low | Low | Low | Low | Low | Good |
| Landlinger et al./2017 | Low | Unclear | Low | Unclear | Low | Low | Fair |
| Kawakami et al./2018 | Unclear | Unclear | Low | Unclear | Low | Low | Poor |
| Asgary et al./2007 | Low | Low | Low | Low | Low | Low | Good |
| Zhong et al./2012 | Low | Low | High | Low | Low | Low | Fair |
| Dunér et al.2021 | Low | Low | Low | High | Low | Low | Fair |
| Fredrikson et al.2003 | Low | High | High | Low | Low | Low | Poor |
| Chyu et al. 2005 | Low | Low | Unclear | Unclear | Low | Low | Fair |
| Wigren et al.2016 | Low | Low | Low | Low | Low | Low | Good |
| Hermansson et al.2011 | Low | Low | Low | Low | Low | Low | Good |
| Herbin et al. 2012 | Low | Unclear | Low | Low | High | Low | Poor |
| Tse et al.2013 | Low | Low | Low | Unclear | Low | Low | Fair |
| Aghebati et al.2020 | Low | Low | Low | Low | Low | Low | Good |
| Badiee et al.2016 | Unclear | Low | Low | Low | Low | Unclear | Fair |
| Sugano et al.1998 | Unclear | Unclear | Low | High | Low | Unclear | Poor |
| Whitlock et al. 1998 | Low | Low | Unclear | High | Low | Low | Poor |
| Mao et al.2006 | Low | Unclear | Low | Low | High | High | Poor |
| Yuan et al.2008 | Low | Low | Low | Low | Low | Low | Good |
| Gutiérrez-Vidal et al.2018 | Low | Low | Unclear | Low | Low | Low | Fair |
| Aghebati et al.2016 | Low | Low | Low | Low | Low | Low | Good |
| Thomas et al.2009 | Low | Low | Low | Unclear | Unclear | Low | Fair |
| Joo et al.2020 | Low | Low | Low | Low | Low | Low | Good |
| Grundtman et al. 2015 | Low | Low | Low | Low | Unclear | Low | Fair |
| Harats et al.2002 | Low | Low | High | Low | Low | High | Poor |

AHRQ indicates Agency for Healthcare Research and Quality; AB, attrition bias; DB, detection bias; OB, other bias; PB, performance bias; RB, reporting bias, SB, selection bias

apparatus [70]. When PCSK9 is present in the bloodstream, it attaches to the extracellular domain of the LDL receptor (LDLR) known as epidermal growth factor-like repeat A (EGF-A), leading to the internalization of the receptor [71]. Afterwards, the PCSK9/LDLR complex moves to the endosome-lysosomal compartment, where the LDLR undergoes degradation. When the expression of LDLR on the cell surface is decreased, the levels of circulating LDL-C (low-density lipoprotein cholesterol) increase. Therefore, it would be advantageous to lower the level or activity of PCSK9 in the bloodstream in order to increase the expression of LDLR in the liver and decrease the levels of circulating LDL-C. This approach would help mitigate the risk of atherosclerosis in human beings [72, 73].

Various studies, especially animal models, have investigated different approaches for PCSK9 inhibition, including monoclonal antibodies, antisense oligonucleotides, and CRISPR-based gene editing [23, 74]. A summary of the studies that investigated the effect of PCSK9 inhibitors on the prevention of atherosclerosis is shown in Table 3. For instance, Graham et al. demonstrated that a PCSK9 antisense oligonucleotide (ASO) significantly lowered total cholesterol levels and increased hepatic LDL receptor expression in animal models [23]. In another study, Momtazi-Borojeni et al. evaluated a nano-liposomal anti-PCSK9 formulation, which effectively induced PCSK9-specific antibodies in preclinical models [25]. While this approach mimics aspects of a vaccine, it primarily acts as an immunotherapy aimed at neutralizing PCSK9 activity rather than conferring long-term acquired immunity.

The use of disease-causing genes editing techniques has also been evaluated in the case of PCSK9. It has been reported that after administering a single infusion of lipid nanoparticles, researchers observed a significant reduction in PCSK9 levels in the liver, nearly eliminating it completely. This also resulted in approximately 90%

**Table 3** The effect of PCSK9 inhibitors on the prevention of atherosclerosis

| Reference | PCSK9 inhibitors type | Model type | Main Results |
|---|---|---|---|
| Liu et al.2023 | small nucleolar RNA host gene 16 (SNHG16) | apoE−/− C57BL/6 mice | The protective control of the PCSK9 inhibitor was observed in mice fed HFD and in VSMCs treated with ox-LDL. |
| Kong et al.2022 | PCSK9 inhibitor, inclisiran | ApoE(-/-) mice | Significant reduction in TG, LDL and total cholesterol concentration and increase in HDL level. It also notably suppressed the formation of plaques and oil droplets in a manner that depended on the dosage. |
| Räber et al.2021 | PCSK9 antibody alirocumab | Patients with acute myocardial infarction | Significant greater reduction in the mean change in percent atheroma volume in non–infarct-related arteries after 52 weeks (−2.13% vs. −0.92%). |
| Wu et al.2021 | PCSK9Qβ-003 Vaccine | Male ApoE$^{-/-}$ mice | Significant reduction in total cholesterol and LDL cholesterol, lesion area and significant improvement in the stability of atherosclerotic plaque |
| Momtazi-Borojen,2021 | nano liposomal antiPCSK9 vaccine | Five male rhesus macaque monkeys | Increase the number of antiPCSK9 antibodies in immunized monkeys. |
| Kawakami et al.,2018 | Peptide-based anti-PCSK9 vaccines | male ApoE deficient mice | Mice that received immunization showed higher LDL receptor expression on their cell surface. |
| Pan,2017 | VLP -PCSK9 peptide vaccines with high titer IgG antibodies (PCSK9Qβ-003) | Specific Pathogen-Free (SPF grade) male Balb/c mice | The PCSK9Qβ-003 vaccine reduced the amount of PCSK9 in the bloodstream and increased the expression of LDLR in the liver |
| Landlinger et al.2017 | AT04A anti-PCSK9 vaccine | Mice | The AT04A vaccine triggered the production of elevated and enduring levels of antibodies against PCSK9, leading to a notable decrease in both plasma total cholesterol (by 53%, $P < 0.001$), atherosclerotic lesion area and LDLc when compared to the control group. |
| Ray et al.2017 | Inclisiran | human | 41.9% reduction in LDL cholesterol |
| Crossey et al.,2015 | virus-like particle (VLP)-based vaccines | Mice and macaques | Significant reduction in TG, total cholesterol and phospholipids |
| Ding et al.2014 | Genome editing of PCSK9 gene with CRISPR/Cas9 | Mice | Significant reduction in LDLR and cholesterol levels |
| Galabova et al., 2014 | Peptide-based anti-PCSK9 vaccines | Ldlr+/- mice | Total cholesterol (TC) levels dropped by as much as 30%, and LDLc levels decreased by up to 50% in the animals receiving treatment. Additionally, the mice developed a lasting immune response to the PCSK9 vaccine for up to one year, resulting in significant and ongoing reductions in cholesterol levels throughout the entire study. |
| Graham, 2007 | Antisense oligonucleotide (ASO) inhibitors | High fat-fed mice for 6 weeks | Giving high fat-fed mice a PCSK9 ASO for a duration of 6 weeks resulted in a 53% decrease in total cholesterol and a 38% decrease in LDL levels. Additionally, suppressing the expression of PCSK9 led to a twofold increase in the levels of hepatic LDLR protein. |

ApoE, apolipoprotein E; oxLDL, oxidized low-density lipoprotein; HFD, high-fat diet; LDL, low density lipoprotein; PCSK9, proprotein convertase subtilisin/kexin type 9; SNHG16, Small Nucleolar RNA Host Gene 16; VSMCs, vascular smooth muscle cells; TG, Triglyceride

reduction in blood levels of PCSK9 and about 60% reduction in low-density lipoprotein cholesterol. These effects remained stable for at least 8 months following the treatment with a single dose [24].

Similarly, viral vector-based strategies, such as virus-like particle (VLP) vaccines, have been investigated for their ability to elicit an immune response against PCSK9 [26]. For example, some types of virus-based vaccines such as Qβ-PCSK9 vaccines were created by combining specific human PCSK9 peptides (PCSK968–76, PCSK9153–163, or PCSK9207–223) with bacteriophage Qβ particles in a laboratory setting. On the other hand, MS2-PCSK9 vaccines were produced by genetically fusing PCSK9 peptide sequences (PCSK9153–163, PCSK9188–200, PCSK9208–222, and PCSK9368–381) with the MS2 coat protein [75]. It has been reported that (VLP)-based vaccines can suppresses the PCSK9 combination with LDL-R and lead to a significant reduction in total cholesterol and LDL-C levels [76].

Alirocumab and evolocumab, which are monoclonal antibodies called PCSK9 inhibitors, are designed to target the PCSK9 protein and disrupt its interaction with the LDL receptor. These antibodies are of fully human origin. Evolocumab and alirocumab were granted approval by the European Medicines Agency in July 2015 and September 2015, respectively [77, 78]. These medications are specifically prescribed for adult patients with primary hypercholesterolemia (both heterozygous familial and non-familial) or mixed dyslipidemia, under certain specified conditions: When used alongside a statin (with or without other lipid-lowering treatments), these drugs are recommended for patients who are unable to achieve the

desired LDL-C levels even with the highest tolerated dose of a statin. Additionally, they can be used either alone or in combination with other lipid-lowering therapies for patients who experience intolerance to statins or have medical contraindications against statin usage. In a RCT study with 300 patients, results showed that the injecting alirocumab subcutaneously every two weeks alongside a high-intensity statin treatment showed a much larger decrease in the average percentage of atheroma volume in non-infarct-related arteries after 52 weeks [79].

When compared to a placebo, Evolocumab has shown significant efficacy. It is approved for both adults and adolescents aged 12 and older, who have homozygous familial hypercholesterolemia and are receiving other lipid-lowering treatments, making it a versatile treatment option across different age groups [80, 81].

Building on previous methods, a different strategy may be required in cases where the current approach is ineffective. This alternative strategy could include utilizing a synthetic small interfering RNA (siRNA) like inclisiran to inhibit the production of PCSK9 in the liver [82]. The results of the human trial showed that inclisiran administration to patients with cardiovascular risk and elevated LDL cholesterol led to a 41.9% reduction in LDL-C in first dose and 52.6% reduction after second dose [27]. Also, they found in another study that inclisiran administration led to a significant reduction in LDLR degradation and LDL-C [28].

Besides controlling cholesterol metabolism, PCSK9 also plays a role in regulating various other physiological processes, such as adipogenesis, immune function, and interactions with several cell surface receptors, including LOX-1, VLDLR, ApoER2, CD36, and LRP-1. CD36 and LRP-1, which are important receptors in signaling pathways, are commonly found on hematopoietic and vascular-related cells [30, 31]. Therefore, it is highly likely that PCSK9 can influence important hemostatic processes like inflammation, hemostasis, tissue regeneration, and repair [83]. Ding et al. (2018) discovered that when TNF-α-primed murine macrophages were treated with recombinant PCSK9, it resulted in an upregulation of CD36 expression [32]. This finding suggests that PCSK9 plays a role in promoting the advancement of atherosclerosis. A different research study has indicated that when CD36 is suppressed in endothelial cells, it leads to the transport of fatty acids across cells and reduced lipid levels in the heart. This outcome may be attributed to the notion that the uptake of oxidized low-density lipoprotein (ox-LDL) depends on fatty acids [84, 85]. Finally, some studies were showed that CSK9Qβ-003 vaccine led to a significant reduction in the area of plaques and improved the stability of atherosclerotic plaque [33].

### Targeting oxidized LDL (oxLDL)

Multiple antigens have been identified as potential triggers for immune responses in the development of atherosclerosis. The extensively researched internal antigen is oxidized low-density lipoprotein (oxLDL) [86]. The process of lipoprotein oxidation within the arterial intima, subsequent ingestion by macrophages, and the resulting formation of foam cells, plays a crucial role in the progression of atherosclerosis. Moreover, the oxidation of LDL brings about numerous changes to apoB-100's structure, resulting in the creation of various neo-epitopes [87]. This modification makes the oxidized LDL capable of triggering an immune response, involving both cellular and humoral components. Macrophages and dendritic cells, acting as antigen-presenting cells (APCs), identify the pro-atherogenic protein oxLDL [88]. They subsequently process this protein and display its epitopes to T cells [37]. Macrophages utilize a defensive mechanism of engulfing oxLDL, leading to the presentation of epitopes on the cell surface through MHC-I and MHC-II molecules. Afterward, CD8 + cytotoxic T cells and CD4 + helper T cells identify and connect with these MHC molecules, triggering the generation of T cells that are specific to oxLDL This series of reactions stimulates the production of pro-inflammatory cytokines and the formation of cholesterol-rich foam cells [89].

In recent years, several studies have shown that increasing the level of oxLDL-specific autoantibodies, which are mainly are the Th1-specific IgG2a isotype, through stimulating macrophages and increasing the production of antibodies can increase the level of immunity against cardiovascular diseases [17]. Habets et al. in an animal study showed that vaccination using oxidized low-density lipoprotein-pulsed dendritic cells led to a significant reduction (about 87%) in carotid artery lesion size and improvement in plaque stability [38]. Table 4 summarizes the results of studies conducted on oxLDL. In a human study, it was shown that there was a positive correlation between OxLDL/LDL-C ratio with the incidence of atherosclerosis in diabetic patients [39]. Also, it has been reported in an animal study that nasal oxLDL administration could reduce the risk and progression of atherosclerosis through some important mechanisms such as increasing the level of CD4 + latency-associated peptide (LAP) + regulatory T cells (Tregs) and $CD4^+CD25^+Foxp3^+$ Tregs in spleens and cervical lymph nodes, together with increased transforming growth factor (TGF)-β production and suppressed T-helper cells type 1, 2, and 17 immune responses [41].

### Targeting apolipoprotein B-100

ApoB-100, a significant constituent of the LDL protein, interacts with oxidized LDL products like MDA through its histidine and lysine residues [44]. After MDA attaches

**Table 4** Results of preclinical studies related to oxidized low-density lipoprotein atherosclerosis vaccines

| Reference | Intervention type | Model type | Main Results |
|---|---|---|---|
| Zhong et al.2012 | Nasal oxLDL | ApoE-/- mice | Nasal oxLDL significantly improved the onset and advancement of atherosclerosis |
| Geng et al.2010 | oxLDL | ApoE-/- mice | They proposed that the adverse impact of ox-LDL on atherosclerosis may be partially mediated through the TLR4 pathway. Additionally, suppressing TLR4 expression could reduce NF-kappa B activity and the release of MCP-1 and IL-8 in monocytes triggered by oxidized LDL, leading to the improvement of atherosclerosis progression |
| Habets et al.2010 | oxidized low-density lipoprotein-pulsed dendritic cells | LDLr−/− mice | significant reduction (about 87%) in carotid artery lesion size and improvement in plaque stability |
| Asgary et al.2007 | immunized with MDA-LDL or Cu-LDL | hypercholesterolemic rabbits | Immunization with Cu2+-LDL and MDA-LDL resulted in the production of antibodies against ox-LDL that showed statistical significance |
| Piarulli et al. 2005 | autoantibodies against oxLDL | Patients with type 2 diabetes | Antibodies against IgG OxLDL could potentially serve as indicators of the later stage of the atherosclerotic process, as well as the immune system's reaction to the presence of OxLDL within atherosclerotic plaques. |

oxLDL, oxidized low-density lipoprotein; MCP-1, IL-8, interleukin-8; Monocyte Chemoattractant Protein-1; TLR4, toll-like receptor family; MDA-LDL, Malondialdehyde-modified low density lipoprotein

to ApoB-100, the immune system specifically recognizes and targets both the ApoB-100 protein and its peptide sequences. In the past years, this protein has been the target of many studies related to the atherosclerosis vaccine [75].

In an animal study conducted by Dunér et al., it was demonstrated that administering antibodies against apoB100 to apoE(-/-) mice resulted in a notable decrease in atherosclerotic plaque development in the aorta, accompanied by a reduction in MDA-LDL levels within the lesions [43]. The results of some other studies have shown that major histocompatibility complex (MHC) class II-restricted ApoB peptides strengthen the immune system against atherosclerosis by stimulating the production of interleukin-10 (IL-10) and also by inducing regulatory T cells (Tregs) [11]. The results of other studies are summarized in Table 5.

The ApoB-100 protein, consisting of 302 amino acids, possesses multiple peptides that are highly capable of triggering an immune response [12, 45]. It has been reported that administration of some type of MDA-modified ApoB-100 peptides specially p143 and p210 resulted in a significant 60% decrease in the area of atherosclerotic lesions [44]. Also, Fredrikson et al. found that MDA-modified apoB-100 peptide p45 administration in animal models led to a considerable reduction in the atherosclerotic lesion area [46].

### Cholesteryl ester transfer protein

Over a decade ago, the notion that cholesteryl ester transfer protein (CETP) could have proatherogenic effects and that inhibiting its activity could be beneficial in preventing atherosclerosis was initially proposed [90]. Additionally, there have been suggestions that CETP may have the capability to suppress the development of atherosclerosis by promoting the efficient removal of cholesterol from peripheral tissues to the liver through the Reverse Cholesterol Transport (RCT) pathway, which facilitates its elimination through bile [91]. Additional research on vaccinations targeting atherosclerosis explores the use of CETP vaccination to raise levels of HDL (high-density lipoprotein) cholesterol [92]. CETP attaches to HDL and facilitates the elimination of cholesterol from tissues, transporting it to the liver for reverse cholesterol transfer, ultimately resulting in the excretion of cholesterol [93].

Some animal studies have investigated the effect of CETP-containing vaccines on the prevention of atherosclerosis (Table 5). Diet-induced atherosclerosis is known to occur easily in rabbits, primarily due to their high susceptibility. Moreover, rabbits possess an inherent, elevated level of CETP [91]. It has been reported in the Sugano et al. study that administration of antisense oligonucleotides against CETP on rabbit models led to a significant reduction in the CETP mRNA and total cholestrol [53]. Also, it has been reported that anti-CETP antibody administration on rabbits inhibited the CETP activity, without any significant preventive effects against atherosclerosis development [54]. In subsequent studies, researchers have used newer in this method by administering a DNA vaccine containing the plasmid pCR-X8-HBc-CETP, which carries the genetic information for the B-cell epitope of the CETP C-terminal fragment (CETPC) displayed by hepatitis B virus core (HBc) particles, specific antibodies targeting CETP have been successfully generated [55]. Similar results were found in another study on female rabbits [56].

In another study, researchers used an ATV-8 vaccine that included micellar nanoparticles consisting of the C-terminal residues H486–S496 of CETP in pig samples

**Table 5** Results of preclinical studies related to anti-atherosclerosis vaccines

| Reference | Intervention type | Model type | Main Results |
|---|---|---|---|
| Soto et al.2024 | Monoclonal Antibody chP3R99 | CR: LAcp (cp/cp) male rats | In a 5-week vaccination study in insulin resistant rats with (200 µg subcutaneously, once a week), chP3R99 reduced arterial lipoprotein retention, and was associated with the production of antichondroitin sulfate antibodies (Ab3) able to accumulate in the arteries (dot-blot). |
| Tang et al. 2024 | COL6A6 peptide (named the Pep_A6 vaccine) | Apoe−/− mice | Pep_A6 vaccine significantly reduced the atherosclerotic plaque area in Apoe−/− mice fed with a high-fat diet for 20 weeks. Pep_A6 vaccine intervention inhibited Th1 cell differentiation, which led to a decrease in inflammatory markers, including reduced production of IFN-γ and TNF-α, suggesting a shift towards a more regulatory immune response. |
| Dunér et al.2021 | Antibodies against apoB100 peptide 210 | ApoE(-/-) mice | A notable decrease in atherosclerotic plaque development in the aorta |
| Kimura et al.2017 | MHC-II-restricted ApoB peptides | $ApoE^{-/-}$Mice | Their findings suggest that immunizing with autologous ApoB peptides restricted to MHC class II prompts the activation of Tregs and IL-10, providing a possible explanation for the protective effects against atherosclerosis. |
| Tse et al.2013 | MHC-II Restricted Peptides from ApoB-100 | ApoE−/− Mice | 40% reduction in overall plaque burden, more than 60% reduction in aortic sinus plaque development |
| Herbin et al.2012 | ApoB100-derived peptides | $Apoe^{-/-}$ mice | The administration of ApoB100 peptides resulted in a notable decrease in the progression of lesions in young Apoe-/- mice. |
| Wigren et al.2011 | aBp210, a prototype atherosclerosis vaccine | ApoE−/− Mice | After 12 weeks, mice that received immunization showed higher levels of the Treg marker CD25 on CD4 cells present in the bloodstream. Additionally, the release of interferon-γ, IL-4, and IL-10 from splenocytes induced by concanavalin A (Con A) was significantly reduced. |
| Fredrikson et al.2005 | MDA-modified apo B-100 peptide | Apo E deficient mice | In 25-week-old mice, immunization using MDA-modified apo B-100 peptide resulted in a 48% and 31% reduction in atherosclerosis in the aorta, respectively. Additionally, there was a 33% and 39% decrease in macrophage content within atherosclerotic plaques after immunization with P45 and P74, respectively. |
| Chyu et L. 2005 | Apo B-100 related epitope | ApoE−/− Mice | Immunization with Peptide-2 resulted in a 40% decrease in aortic atherosclerosis and an 80% reduction in plaque inflammation compared to the control group, without affecting circulating cholesterol levels. |
| Fredrikson et al.2003 | ApoB-100 Peptide 210 | C57BL/6 mice | Reduce atherosclerosis by about 60% and increase the collagen content of subvalvular lesions. |
| Aghebati et al.2019 | CETP | rabbit model | Rabbits vaccinated with Lip-CETP exhibited a significantly lower grade of atherosclerosis thickness in the aorta compared to the buffer group |
| Aghebati et al.2016 | Tetanus toxoid-CETP (TT-CETP) peptide | rabbit model | TT-CETP was observed to inhibit CETP activity and increase HDL-C levels. However, when compared to the control group, the vaccine did not effectively prevent the development of aortic lesions in immunized rabbits |
| Gutiérrez-Vidal et al.2018 | Micellar nanoparticle composed of lipids and a peptide segment derived from the CETP | Pigs | The HB-ATV-8 vaccine administration resulted in the production of anti-CETP IgG antibodies and decreased atherosclerotic and hepatic lesions caused by the high-fat diet |
| Thomas et al.2009 | Comparison of PADRE-CET vaccine with TT-CETP vaccine | Mice and rabbit | Both mice and rabbits demonstrated a more robust production of anti-CETP antibodies in response to the vaccine peptide that incorporated the PADRE T cell epitope, compared to the TT-CETP vaccine |
| Yuan et al.2008 | plasmid pCR-X8-HBc-CETP (pCETP) encoding B-cell epitope | rabbit model | Rabbits that received intranasal vaccination with nanoparticles exhibited a 59.2% reduction in the average percentage of aortic lesions compared to those treated with saline |
| Mao et al. 2005 | plasmid pCR-X8-HBc-CETP encoding a B-cell epitope of CETP C-terminal fragment (CETPC) | rabbit model | 80.60% reduction in the aortic lesions in the entire aorta area.<br>There was a notable decrease in the average thickness of the hypertrophic coronary artery. |
| Joo et al.2020 | PgHSP60-derived peptide 14 | mice | Significant reduction in atherosclerotic plaques.<br>Significant induction of Tregs |
| Jing et al.2020 | mycobacterial HSP65 (mbHSP65) | Low-density lipoprotein receptor-deficient (LDL-RD) mice | Inducible HSP65-specific tolerance had a positive impact on the development of atherosclerotic plaques and protection against endothelial damage. |
| Grundtman et al.2015 | mycobacterial HSP65 (mbHSP65) | ApoE(-/-) mice | Decreased lesion size |

**Table 5** (continued)

| Reference | Intervention type | Model type | Main Results |
|---|---|---|---|
| Puijvelde et al.2007 | HSP60 | LDLr(-/-) mice | Administering HSP60 led to a noteworthy 80% decrease in plaque size in the carotid arteries and a 27% decrease in plaque size at the aortic root. |
| Harats et al. 2002 | HSP65 | mice | Early atherosclerosis was attenuated in HSP65-fed mice |

ApoE, apolipoprotein E; apoB100, Apolipoprotein B100; IL-10, interleukin-10; IL-4, interleukin-4; Con A, concanavalin A; CETP, Cholesteryl ester transfer protein; Tregs, regulatory T cells

that were fed a high-fat diet, and the results showed that this type of vaccine had a significant effect in reducing atherosclerotic lesions [57]. Scientists have also created vaccines that merge B-cell epitopes of CETP with residues of tetanus toxoid (TT), which function as helper T cell epitopes. It has been reported in an animal study on rabbit models that the administration of tetanus toxoid-CETP (TT-CETP) peptide caused a significant reduction in atherosclerotic lesions [51]. Other studies that investigated the effects of TT-CETP in animal samples reported positive results [58, 59].

#### Heat shock proteins (HSP)

The peptides derived from HSP have been discovered to stimulate the synthesis of cytokines that have anti-inflammatory effects, suggesting their ability to regulate the immune response [94]. Different epitope peptides from bacterial HSPs have the potential to act as either regulatory or effector molecules in the autoimmune response associated with infection-induced atherosclerosis. it was suggested that two peptides from PgHSP60, namely Pep14 and Pep19, could have contrasting effects on atherosclerosis in an ApoE knockout (ApoE KO) mouse model, with one peptide potentially having anti-atherogenic properties and the other peptide possibly promoting atherogenesis [95, 96]. Humans have developed both cellular and humoral defenses targeting bacterial HSP65, which is analogous to the 60-kDa human heat shock protein 60 (HSP60). Nevertheless, in the presence of stressors like smoking, hypertension, as well as the accumulation of oxLDL on arterial walls, the expression of HSP60 increases within cells. This elevation in HSP60 levels can potentially act as a catalyst for an autoimmune response, playing a crucial role in the onset and advancement of atherosclerosis. An increase in the levels of extracellular HSP60 causes it to bind more to Toll-like receptor 4 (TLR4) and subsequently increases the level of pro-inflammatory cytokines.

Scientists have investigated the potential of vaccines for restoring tolerance to HSP60/HSP65. Nevertheless, the subcutaneous application of HSP60/HSP65 had an adverse effect, leading to the development of larger atherosclerotic plaques. On the flip side, oral and nasal vaccines provoked the intended tolerance. In Joo et al.'s study, nasal immunization of mice with PgHSP60-derived peptide 14 (Pep14) caused a significant reduction in atherosclerotic plaques [60]. Similar results were observed in other studies [61–64].

#### Clinical trials

In human studies, 2 active and passive approaches have been used to reduce the risk of cardiovascular diseases. In the passive approach, the goal of vaccination is to produce antibodies with the aim of reducing the level of factors involved in the pathogenesis of atherosclerosis [97]. While in the active vaccination approach, the goal is to increase the body's immunity to reduce the inflammatory responses in the artery wall [98]. The influenza vaccine is widely known as the most extensively proven case of an active immunization that reduces the risk of cardiovascular issues. There is compelling epidemiological data indicating a heightened likelihood of heart attacks and strokes during the immediate weeks following an influenza infection [99]. The exact reasons behind this connection are not fully understood, but it is plausible that the buildup of virus particles in susceptible atherosclerotic plaques could worsen inflammation, potentially heightening the chances of plaque rupture [100]. Several studies have shown that influenza vaccination significantly reduces the risk of certain cardiovascular events [99, 101, 102]. Similar positive results following pneumococcal vaccination were reported in some studies [103, 104].

The use of PCSK9 antibodies (Evolocumab and inclisiran) with the aim of a vaccine to prevent atherosclerosis has been investigated in recent years and some studies have shown positive results, however, the big obstacle in using these drugs is their high cost [98]. Donoghue and colleagues conducted a lengthy clinical trial to examine the impact of evolocumab with a statin, in comparison to a control group, on 6635 patients with atherosclerotic cardiovascular disease who had LDL-C levels of 70 mg/dL or higher. The outcome revealed that 63.2% of patients on evolocumab achieved LDL-C levels below 40 mg/dL. Additionally, the evolocumab group exhibited a 15% reduced risk of cardiovascular death, myocardial infarction, stroke, or hospitalization for unstable angina or coronary revascularization [65].

### Difficulties linked to the clinical advancement of vaccines for atherosclerosis

Despite the encouraging experimental outcomes of various vaccines that help decrease atherosclerosis progression, it's regrettable that the translation of these discoveries into practical clinical use has been sluggish [17]. The experimental studies on atherosclerosis vaccines have a common limitation in that they primarily investigate the impact of prevention on early stages of atherosclerosis. During this phase, lesion development follows a mostly linear pattern, allowing for a quantitative assessment of the effectiveness of treatment protocols in reducing the extent of the disease. However, in real and clinical conditions, the conditions of many patients are complicated. Additionally, there are significant differences between the immune systems of experimental animals raised in a sterile environment since birth and humans, who have a more evolved immune system due to exposure to various foreign pathogens [17, 105].

A major challenge of peptide-based vaccines is ensuring proper HLA class II matching. In order for a peptide to activate a T-cell response by being presented on MHC (HLA in humans) class II, it must be capable of binding to an HLA class II allele expressed by the individual. In an ideal scenario, multiple peptides that bind to different HLA molecules would have to be identified, tested for clinical effectiveness, and the patient's HLA types would need to be screened to select the appropriate peptide for vaccination. Fortunately, there are ways to address this issue, such as attaching the peptide to proteins that strongly bind to all HLA molecules [17, 106].

## Conclusions and suggestions for future studies

The results of this study indicate that in recent years, the vaccines aimed at reducing the risk of atherosclerosis have primarily taken the form of active vaccines, which decrease the inflammation levels in the artery walls, and passive vaccines, which generate antibodies that neutralize a factor contributing to the development of atherosclerosis. Although the results of pre-clinical studies have been mostly positive, clinical studies are still very limited and to prove the results of pre-clinical studies, clinical studies with strong design and high sample size are needed. On the other hand, in the design of clinical studies, special attention should be paid to the limitations mentioned in preclinical studies.

#### Author contributions

MSE, MS and RSS designed the research. ARA, MK, MA, and MSh conducted the research. ARA and MS wrote the article. MSh and MA revised the article. MS had primary responsibility for the final content. All authors read and approved the final manuscript.

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#### Data availability

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author/s.

## Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

The authors provided final approval for the publication.

#### Competing interests

The authors declare no competing interests.

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#### References

1. Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, et al. Global, regional, and National burden of cardiovascular diseases for 10 causes, 1990 to 2015. J Am Coll Cardiol. 2017;70(1):1–25.
2. Beaglehole R, Yach D. Globalisation and the prevention and control of non-communicable disease: the neglected chronic diseases of adults. Lancet. 2003;362(9387):903–8.
3. Mozaffarian D. Global scourge of cardiovascular disease: time for health care systems reform and precision population health. J Am Coll Cardiol. 2017;70(1):26–8.
4. Song P, Fang Z, Wang H, Cai Y, Rahimi K, Zhu Y, et al. Global and regional prevalence, burden, and risk factors for carotid atherosclerosis: a systematic review, meta-analysis, and modelling study. Lancet Global Health. 2020;8(5):e721–9.
5. Hong YM. Atherosclerotic cardiovascular disease beginning in childhood. Korean Circ J. 2010;40(1):1–9.
6. McGill HC Jr., McMahan CA, Herderick EE, Malcom GT, Tracy RE, Strong JP. Origin of atherosclerosis in childhood and adolescence. Am J Clin Nutr. 2000;72(5 Suppl):s1307–15.
7. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. JAMA. 2002;287(19):2570–81.
8. Herrington W, Lacey B, Sherliker P, Armitage J, Lewington S. Epidemiology of atherosclerosis and the potential to reduce the global burden of atherothrombotic disease. Circul Res. 2016;118(4):535–46.
9. Pederiva C, Capra ME, Viggiano C, Rovelli V, Banderali G, Biasucci G. Early prevention of atherosclerosis: detection and management of hypercholesterolaemia in children and adolescents. Life (Basel). 2021;11(4).
10. Oude Nijhuis MM, van Keulen JK, Pasterkamp G, Quax PH, de Kleijn DP. Activation of the innate immune system in atherosclerotic disease. Curr Pharm Des. 2007;13(10):983–94.
11. Kimura T, Tse K, McArdle S, Gerhardt T, Miller J, Mikulski Z, et al. Atheroprotective vaccination with MHC-II-restricted ApoB peptides induces peritoneal IL-10-producing CD4 T cells. Am J Physiol Heart Circ Physiol. 2017;312(4):H781–90.
12. Amirfakhryan H. Vaccination against atherosclerosis: an overview. Hellenic J Cardiol. 2020;61(2):78–91.
13. Poznyak AV, Bezsonov EE, Popkova TV, Starodubova AV, Orekhov AN. Vaccination against atherosclerosis: is it real?? Int J Mol Sci. 2022;23(5).
14. Jebari-Benslaiman S, Galicia-García U, Larrea-Sebal A, Olaetxea JR, Alloza I, Vandenbroeck K, et al. Pathophysiology of atherosclerosis. Int J Mol Sci. 2022;23(6):3346.
15. Frąk W, Wojtasińska A, Lisińska W, Młynarska E, Franczyk B, Rysz J. Pathophysiology of cardiovascular diseases: new insights into molecular mechanisms of atherosclerosis, arterial hypertension, and coronary artery disease. Biomedicines. 2022;10(8):1938.
16. Roy P, Ali AJ, Kobiyama K, Ghosheh Y, Ley K. Opportunities for an atherosclerosis vaccine: from mice to humans. Vaccine. 2020;38(28):4495–506.
17. Nilsson J, Hansson GK. Vaccination strategies and immune modulation of atherosclerosis. Circ Res. 2020;126(9):1281–96.
18. Chandler J, Cumpston M, Li T, Page MJ, Welch V. Cochrane handbook for systematic reviews of interventions. Hoboken: Wiley; 2019.

19. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372.
20. McGowan J, Sampson M, Salzwedel DM, Cogo E, Foerster V, Lefebvre C. PRESS peer review of electronic search strategies: 2015 guideline statement. J Clin Epidemiol. 2016;75:40–6.
21. Hooijmans CR, Rovers MM, De Vries RB, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. BMC Med Res Methodol. 2014;14:1–9.
22. Liu Y, Zhao Y, Feng P, Jiang H. PCSK9 inhibitor attenuates atherosclerosis by regulating SNHG16/EZH2/TRAF5-mediated VSMC proliferation, migration, and foam cell formation. Cell Biol Int. 2023;47(7):1267–80.
23. Graham MJ, Lemonidis KM, Whipple CP, Subramaniam A, Monia BP, Crooke ST, Crooke RM. Antisense Inhibition of proprotein convertase subtilisin/kexin type 9 reduces serum LDL in hyperlipidemic mice. J Lipid Res. 2007;48(4):763–7.
24. 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.
25. Momtazi-Borojeni AA, Jaafari MR, Banach M, Gorabi AM, Sahraei H, Sahebkar A. Pre-clinical evaluation of the nanoliposomal antipcsk9 vaccine in healthy non-human primates. Vaccines. 2021;9(7).
26. 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.
27. Ray KK, Landmesser U, Leiter LA, Kallend D, Dufour R, Karakas M, et al. Inclisiran in patients at high cardiovascular risk with elevated LDL cholesterol. N Engl J Med. 2017;376(15):1430–40.
28. Ray KK, Wright RS, Kallend D, Koenig W, Leiter LA, Raal FJ, et al. Two phase 3 trials of inclisiran in patients with elevated LDL cholesterol. N Engl J Med. 2020;382(16):1507–19.
29. Ding Q, Strong A, Patel KM, Ng S-L, Gosis BS, Regan SN, et al. Permanent alteration of PCSK9 with in vivo CRISPR-Cas9 genome editing. Circul Res. 2014;115(5):488–92.
30. Ragusa R, Basta G, Neglia D, De Caterina R, Del Turco S, Caselli C. PCSK9 and atherosclerosis: looking beyond LDL regulation. Eur J Clin Invest. 2021;51(4):e13459.
31. Ricci C, Ruscica M, Camera M, Rossetti L, Macchi C, Colciago A, et al. PCSK9 induces a pro-inflammatory response in macrophages. Sci Rep. 2018;8(1):2267.
32. Ding Z, Liu S, Wang X, Theus S, Deng X, Fan Y, et al. PCSK9 regulates expression of scavenger receptors and ox-LDL uptake in macrophages. Cardiovascular Res. 2018;114(8):1145–53.
33. Wu D, Pan Y, Yang S, Li C, Zhou Y, Wang Y, et al. PCSK9Qβ-003 vaccine attenuates atherosclerosis in Apolipoprotein E-Deficient mice. Cardiovasc Drugs Ther. 2021;35(1):141–51.
34. Galabova G, Brunner S, Winsauer G, Juno C, Wanko B, Mairhofer A, et al. Peptide-based anti-PCSK9 vaccines - an approach for long-term LDLc management. PLoS ONE. 2014;9(12):e114469.
35. 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.
36. Kawakami R, Nozato Y, Nakagami H, Ikeda Y, Shimamura M, Yoshida S, et al. Development of vaccine for dyslipidemia targeted to a proprotein convertase subtilisin/kexin type 9 (PCSK9) epitope in mice. PLoS ONE. 2018;13(2):e0191895.
37. Asgary S, Saberi SA, Azampanah S. Effect of immunization against ox-LDL with two different antigens on formation and development of atherosclerosis. Lipids Health Dis. 2007;6:32.
38. Habets KL, van Puijvelde GH, van Duivenvoorde LM, van Wanrooij EJ, de Vos P, Tervaert JW, et al. Vaccination using oxidized low-density lipoprotein-pulsed dendritic cells reduces atherosclerosis in LDL receptor-deficient mice. Cardiovasc Res. 2010;85(3):622–30.
39. Xu L, Yan X, Tang Z, Feng B. Association between Circulating oxidized OxLDL/LDL-C ratio and the severity of coronary atherosclerosis, along with other emerging biomarkers of cardiovascular disease in patients with type 2 diabetes. Diabetes Res Clin Pract. 2022;191:110040.
40. Kong N, Xu Q, Cui W, Feng X, Gao H. PCSK9 inhibitor inclisiran for treating atherosclerosis via regulation of endothelial cell pyroptosis. Ann Transl Med. 2022;10(22):1205.
41. Zhong Y, Wang X, Ji Q, Mao X, Tang H, Yi G, et al. CD4 + LAP + and CD4 + CD25 + Foxp3 + regulatory T cells induced by nasal oxidized low-density lipoprotein suppress effector T cells response and attenuate atherosclerosis in ApoE−/− mice. J Clin Immunol. 2012;32:1104–17.
42. Geng H, Wang A, Rong G, Zhu B, Deng Y, Chen J, Zhong R. The effects of ox-LDL in human atherosclerosis May be mediated in part via the toll-like receptor 4 pathway. Mol Cell Biochem. 2010;342(1–2):201–6.
43. Dunér P, Mattisson IY, Fogelstrand P, Glise L, Ruiz S, Farina C, et al. Antibodies against apoB100 peptide 210 inhibit atherosclerosis in apoE(-/-) mice. Sci Rep. 2021;11(1):9022.
44. Fredrikson GN, Söderberg I, Lindholm M, Dimayuga P, Chyu KY, Shah PK, Nilsson J. Inhibition of atherosclerosis in apoE-null mice by immunization with apoB-100 peptide sequences. Arterioscler Thromb Vasc Biol. 2003;23(5):879–84.
45. Chyu KY, Zhao X, Reyes OS, Babbidge SM, Dimayuga PC, Yano J, et al. Immunization using an Apo B-100 related epitope reduces atherosclerosis and plaque inflammation in hypercholesterolemic Apo E (-/-) mice. Biochem Biophys Res Commun. 2005;338(4):1982–9.
46. Frederikson GN, Andersson L, Söderberg I, Dimayuga P, Chyu KY, Shah PK, Nilsson J. Atheroprotective immunization with MDA-modified Apo B-100 peptide sequences is associated with activation of Th2 specific antibody expression. Autoimmunity. 2005;38(2):171–9.
47. Wigren M, Kolbus D, Dunér P, Ljungcrantz I, Söderberg I, Björkbacka H, et al. Evidence for a role of regulatory T cells in mediating the atheroprotective effect of Apolipoprotein B peptide vaccine. J Intern Med. 2011;269(5):546–56.
48. Hermansson A, Johansson DK, Ketelhuth DF, Andersson J, Zhou X, Hansson GK. Immunotherapy with tolerogenic Apolipoprotein B-100-loaded dendritic cells attenuates atherosclerosis in hypercholesterolemic mice. Circulation. 2011;123(10):1083–91.
49. Herbin O, Ait-Oufella H, Yu W, Fredrikson GN, Aubier B, Perez N, et al. Regulatory T-cell response to Apolipoprotein B100-derived peptides reduces the development and progression of atherosclerosis in mice. Arterioscler Thromb Vasc Biol. 2012;32(3):605–12.
50. Tse K, Gonen A, Sidney J, Ouyang H, Witztum JL, Sette A, et al. Atheroprotective vaccination with MHC-II restricted peptides from ApoB-100. Front Immunol. 2013;4:493.
51. Aghebati T, Arabsalmani M, Mohammadpour AH, Afshar M, Jaafari MR, Abnous K, et al. Development of an effective liposomal cholesterol ester transfer protein (CETP) vaccine for protecting against atherosclerosis in rabbit model. Pharm Dev Technol. 2020;25(4):432–9.
52. Aghebati T, Badiee A, Mohammadpour AH, Afshar M, Jaafari MR, Abnous K, et al. Anti-atherosclerosis effect of different doses of CETP vaccine in rabbit model of atherosclerosis. Biomed Pharmacother. 2016;81:468–73.
53. Sugano M, Makino N, Sawada S, Otsuka S, Watanabe M, Okamoto H, et al. Effect of antisense oligonucleotides against cholesteryl ester transfer protein on the development of atherosclerosis in cholesterol-fed rabbits. J Biol Chem. 1998;273(9):5033–6.
54. Whitlock ME, Swenson TL, Ramakrishnan R, Leonard MT, Marcel YL, Milne RW, Tall AR. Monoclonal antibody Inhibition of cholesteryl ester transfer protein activity in the rabbit. Effects on lipoprotein composition and high density lipoprotein cholesteryl ester metabolism. J Clin Invest. 1989;84(1):129–37.
55. Mao D, Kai G, Gaofu Q, Zheng Z, Li Z, Jie W, et al. Intramuscular immunization with a DNA vaccine encoding a 26-amino acid CETP epitope displayed by HBc protein and containing CpG DNA inhibits atherosclerosis in a rabbit model of atherosclerosis. Vaccine. 2006;24(23):4942–50.
56. Yuan X, Yang X, Cai D, Mao D, Wu J, Zong L, Liu J. Intranasal immunization with Chitosan/pCETP nanoparticles inhibits atherosclerosis in a rabbit model of atherosclerosis. Vaccine. 2008;26(29–30):3727–34.
57. Gutiérrez-Vidal R, Delgado-Coello B, Méndez-Acevedo KM, Calixto-Tlacomulco S, Damián-Zamacona S, Mas-Oliva J. Therapeutic intranasal vaccine HB-ATV-8 prevents atherogenesis and Non-alcoholic fatty liver disease in a pig model of atherosclerosis. Arch Med Res. 2018;49(7):456–70.
58. Aghebati T, Mohammadpour AH, Afshar M, Jaafari MR, Abnous K, Nazemi S, et al. A novel atheroprotective role of MF59-like adjuvant when co-administered with CETP vaccine in rabbit model of atherosclerosis. Iran J Basic Med Sci. 2016;19(12):1345.
59. Thomas LJ, Hammond RA, Forsberg EM, Geoghegan-Barek KM, Karalius BH, Marsh HC Jr., Rittershaus CW. Co-administration of a CpG adjuvant (VaxImmune, CPG 7909) with CETP vaccines increased immunogenicity in rabbits and mice. Hum Vaccin. 2009;5(2):79–84.

60. Joo JY, Cha GS, Kim HJ, Lee JY, Choi J. Atheroprotective nasal immunization with a heat shock protein 60 peptide from Porphyromonas gingivalis. J Periodontal Implant Sci. 2020;50(3):159–70.
61. Grundtman C, Jakic B, Buszko M, Onestingel E, Almanzar G, Demetz E, et al. Mycobacterial heat shock protein 65 (mbHSP65)-induced atherosclerosis: preventive oral tolerization and definition of atheroprotective and atherogenic mbHSP65 peptides. Atherosclerosis. 2015;242(1):303–10.
62. Harats D, Yacov N, Gilburd B, Shoenfeld Y, George J. Oral tolerance with heat shock protein 65 attenuates Mycobacterium tuberculosis-induced and high-fat-diet-driven atherosclerotic lesions. J Am Coll Cardiol. 2002;40(7):1333–8.
63. Jing H, Yong L, Haiyan L, Yanjun M, Yun X, Yu Z, et al. Oral administration of Lactococcus lactis delivered heat shock protein 65 attenuates atherosclerosis in low-density lipoprotein receptor-deficient mice. Vaccine. 2011;29(24):4102–9.
64. van Puijvelde GH, van Es T, van Wanrooij EJ, Habets KL, de Vos P, van der Zee R, et al. Induction of oral tolerance to HSP60 or an HSP60-peptide activates T cell regulation and reduces atherosclerosis. Arterioscler Thromb Vasc Biol. 2007;27(12):2677–83.
65. O'Donoghue ML, Giugliano RP, Wiviott SD, Atar D, Keech A, Kuder JF, et al. Long-Term Evolocumab in patients with established atherosclerotic cardiovascular disease. Circulation. 2022;146(15):1109–19.
66. Tang D, Liu Y, Duan R, Lin R, Li Z, Liu X, et al. COL6A6 peptide vaccine alleviates atherosclerosis through inducing immune response and regulating lipid metabolism in Apoe(-/-) mice. Cells. 2024;13:18.
67. Soto Y, Hernández A, Sarduy R, Brito V, Marleau S, Vine DF, et al. Monoclonal antibody chP3R99 reduces subendothelial retention of atherogenic lipoproteins in Insulin-Resistant rats: acute treatment versus Long-Term protection as an idiotypic vaccine for atherosclerosis. J Am Heart Assoc. 2024;13(13):e032419.
68. Zhang D-W, Lagace TA, Garuti R, Zhao Z, McDonald M, Horton JD, et al. Binding of proprotein convertase subtilisin/kexin type 9 to epidermal growth factor-like repeat A of low density lipoprotein receptor decreases receptor recycling and increases degradation. J Biol Chem. 2007;282(25):18602–12.
69. Tibolla G, Norata GD, Artali R, Meneghetti F, Catapano A. Proprotein convertase subtilisin/kexin type 9 (PCSK9): from structure–function relation to therapeutic Inhibition. Nutr Metabolism Cardiovasc Dis. 2011;21(11):835–43.
70. Benjannet S, Rhainds D, Essalmani R, Mayne J, Wickham L, Jin W, et al. NARC-1/PCSK9 and its natural Mutants: Zymogen cleavage and effects on the low density lipoprotein (LDL) receptor and LDL cholesterol. J Biol Chem. 2004;279(47):48865–75.
71. Nassoury N, Blasiole DA, Tebon Oler A, Benjannet S, Hamelin J, Poupon V, et al. The cellular trafficking of the secretory proprotein convertase PCSK9 and its dependence on the LDLR. Traffic. 2007;8(6):718–32.
72. Martin WR, Lightstone FC, Cheng F. In Silico insights into protein–protein interaction disruptive mutations in the PCSK9-LDLR complex. Int J Mol Sci. 2020;21(5):1550.
73. Benn M, Nordestgaard BG, Grande P, Schnohr P, Tybjaerg-Hansen A. PCSK9 R46L, low-density lipoprotein cholesterol levels, and risk of ischemic heart disease: 3 independent studies and meta-analyses. J Am Coll Cardiol. 2010;55(25):2833–42.
74. Rinaldi C, Wood MJ. Antisense oligonucleotides: the next frontier for treatment of neurological disorders. Nat Reviews Neurol. 2018;14(1):9–21.
75. Moreno-Gonzalez MA, Ortega-Rivera OA, Steinmetz NF. Two decades of vaccine development against atherosclerosis. Nano Today. 2023;50.
76. Pan Y, Zhou Y, Wu H, Chen X, Hu X, Zhang H, et al. A therapeutic peptide vaccine against PCSK9. Sci Rep. 2017;7(1):12534.
77. van den Heuvel TW, Cohen AF, Rissmann R. European drug market entries 2015 with new mechanisms of action. Clin Med. 2016;16(5):475.
78. Tomlinson B, Hu M, Zhang Y, Chan P, Liu Z-M. Alirocumab for the treatment of hypercholesterolemia. Expert Opin Biol Ther. 2017;17(5):633–43.
79. 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.
80. Toth PP, Worthy G, Gandra SR, Sattar N, Bray S, Cheng LI, et al. Systematic review and network meta-analysis on the efficacy of Evolocumab and other therapies for the management of lipid levels in hyperlipidemia. J Am Heart Association. 2017;6(10):e005367.
81. Steffens D, Bramlage P, Scheeff C, Kasner M, Hassanein A, Friebel J. Rauch-Kröhnert U. PCSK9 inhibitors and cardiovascular outcomes. Expert Opin Biol Ther. 2020;20(1):35–47.
82. Ray KK, Landmesser U, Leiter LA, Kallend D, Dufour R, Karakas M et al. Inclisiran in patients with high CV risk and elevated LDL-cholesterol. 2017.
83. Silverstein RL. PCSK9 (proprotein convertase subtilisin/kexin 9) goes DAMP. Am Heart Assoc; 2021. pp. 62–4.
84. Son N-H, Basu D, Samovski D, Pietka TA, Peche VS, Willecke F, et al. Endothelial cell CD36 optimizes tissue fatty acid uptake. J Clin Investig. 2018;128(10):4329–42.
85. Xu S, Jay A, Brunaldi K, Huang N, Hamilton JA. CD36 enhances fatty acid uptake by increasing the rate of intracellular esterification but not transport across the plasma membrane. Biochemistry. 2013;52(41):7254–61.
86. van Puijvelde GH, Hauer AD, de Vos P, van den Heuvel R, van Herwijnen MJ, van der Zee R, et al. Induction of oral tolerance to oxidized low-density lipoprotein ameliorates atherosclerosis. Circulation. 2006;114(18):1968–76.
87. Jiang H, Zhou Y, Nabavi SM, Sahebkar A, Little PJ, Xu S, et al. Mechanisms of oxidized LDL-Mediated endothelial dysfunction and its consequences for the development of atherosclerosis. Front Cardiovasc Med. 2022;9:925923.
88. Khatana C, Saini NK, Chakrabarti S, Saini V, Sharma A, Saini RV, Saini AK. Mechanistic insights into the oxidized Low-Density Lipoprotein-Induced atherosclerosis. Oxid Med Cell Longev. 2020;2020:5245308.
89. Zheng H, Pei Y, Zhou C, Hong P, Qian ZJ. Amelioration of atherosclerosis in ox-LDL induced HUVEC by sulfated polysaccharides from Gelidium crinale with antihypertensive activity. Int J Biol Macromol. 2023;228:671–80.
90. Brown ML, Inazu A, Hesler CB, Agellon LB, Mann C, Whitlock ME, et al. Molecular basis of lipid transfer protein deficiency in a family with increased high-density lipoproteins. Nature. 1989;342(6248):448–51.
91. Barter PJ, Brewer HB Jr., Chapman MJ, Hennekens CH, Rader DJ, Tall AR. Cholesteryl ester transfer protein: a novel target for Raising HDL and inhibiting atherosclerosis. Arterioscler Thromb Vasc Biol. 2003;23(2):160–7.
92. Zhao TX, Mallat Z. Targeting the immune system in atherosclerosis: JACC State-of-the-Art review. J Am Coll Cardiol. 2019;73(13):1691–706.
93. Colombo GI, Bianconi V, Bonomi A, Simonelli S, Amato M, Frigerio B et al. The association between HDL-C and subclinical atherosclerosis depends on CETP plasma concentration: insights from the IMPROVE study. Biomedicines. 2021;9(3).
94. van Eden W, van der Zee R, Prakken B. Heat-shock proteins induce T-cell regulation of chronic inflammation. Nat Rev Immunol. 2005;5(4):318–30.
95. Choi J, Lee SY, Kim K, Choi BK. Identification of immunoreactive epitopes of the Porphyromonas gingivalis heat shock protein in periodontitis and atherosclerosis. J Periodontal Res. 2011;46(2):240–5.
96. Jeong E, Kim K, Kim JH, Cha GS, Kim SJ, Kang HS, Choi J. Porphyromonas gingivalis HSP60 peptides have distinct roles in the development of atherosclerosis. Mol Immunol. 2015;63(2):489–96.
97. Nilsson J, Hansson GK. Vaccination strategies and immune modulation of atherosclerosis. Circul Res. 2020;126(9):1281–96.
98. 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.
99. Barnes M, Heywood AE, Mahimbo A, Rahman B, Newall AT, Macintyre CR. Acute myocardial infarction and influenza: a meta-analysis of case-control studies. Heart. 2015;101(21):1738–47.
100. Libby P, Loscalzo J, Ridker PM, Farkouh ME, Hsue PY, Fuster V, et al. Inflammation, immunity, and infection in atherothrombosis: JACC review topic of the week. J Am Coll Cardiol. 2018;72(17):2071–81.
101. Ciszewski A, Bilinska ZT, Brydak LB, Kepka C, Kruk M, Romanowska M, et al. Influenza vaccination in secondary prevention from coronary ischaemic events in coronary artery disease: FLUCAD study. Eur Heart J. 2008;29(11):1350–8.
102. Fröbert O, Götberg M, Angerås O, Jonasson L, Erlinge D, Engstrøm T, et al. Design and rationale for the influenza vaccination after myocardial infarction (IAMI) trial. A registry-based randomized clinical trial. Am Heart J. 2017;189:94–102.
103. Ren S, Newby D, Li SC, Walkom E, Miller P, Hure A, Attia J. Effect of the adult Pneumococcal polysaccharide vaccine on cardiovascular disease: a systematic review and meta-analysis. Open Heart. 2015;2(1):e000247.
104. Ren S, Hure A, Peel R, D'Este C, Abhayaratna W, Tonkin A, et al. Rationale and design of a randomized controlled trial of Pneumococcal polysaccharide vaccine for prevention of cardiovascular events: the Australian study for the prevention through immunization of cardiovascular events (AUSPICE). Am Heart J. 2016;177:58–65.
105. Libby P. Murine model monotheism: an iconoclast at the altar of mouse. Circul Res. 2015;117(11):921–5.

106. Kallinteris NL, Lu X, Blackwell CE, von Hofe E, Humphreys RE, Xu M. Ii-Key/MHC class II epitope hybrids: a strategy that enhances MHC class II epitope loading to create more potent peptide vaccines. Expert Opin Biol Ther. 2006;6(12):1311–21.

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