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Review

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Therapeutic Vaccines and Nucleic Acid Drugs for Cardiovascular Disease

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ABSTRACT

To combat the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), novel vaccine modalities, such as messenger RNA vaccines, were rapidly developed and have shown high efficacy. This new vaccine technology, underpinned by intensive immunological analysis, is now being applied to the production of other vaccines. For over 10 years, we have been developing therapeutic vaccines for non-infectious diseases. The epitope vaccine approach, which combines a B-cell epitope with exogenous T-cell epitopes presented through major histocompatibility complex molecules, has been proposed to induce antibody production. This vaccine type is designed to efficiently induce a blocking antibody response against the self-antigen without activating cytotoxic T cells. If therapeutic vaccines become established as treatment options for conditions such as hypertension or dyslipidemia, their administration—potentially only a few times per year—could replace the need for daily medication. Nucleic acid drugs, including small interfering RNA and antisense oligonucleotides, have recently received attention as long-term agonists, similar to vaccines. Therefore, therapeutic vaccines or nucleic acid drugs could represent a novel strategy for controlling the progression of cardiovascular diseases. It is hoped that the accumulation of immunological findings and advances in vaccine technology will provide valuable insights into the development of vaccines for treating cardiovascular diseases.

Keywords: Nucleic acid; Epitope; Vaccine; Cardiovascular disease

INTRODUCTION

The global spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has once again highlighted the urgent need to address the threat posed by emerging infectious diseases. When faced with a novel infectious disease, it becomes apparent that controlling the spread is challenging. Initial public health measures such as strengthening quarantine and isolating patients are insufficient, especially in a world where international travel is frequent and rapid. The lack of information about the infectivity, severity, and incubation period of these diseases complicates matters further. Therefore, vaccine development has been seen as a crucial strategy to reduce the morbidity rates among high-risk groups, including healthcare workers and individuals with underlying health conditions. In this context, the expedited approval and global deployment of a messenger RNA (mRNA)

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Data Availability Statement

The datasets analyzed during this study are available from the corresponding author upon reasonable request.

Author Contributions

Conceptualization: Nakagami H, Hayashi H; Project administration: Hayashi H; Supervision: Morishita R; Writing - original draft: Nakagami H; Writing - review & editing: Nakagami H.

vaccine against coronavirus disease 2019 (COVID-19) marked a pivotal moment in vaccine development, transforming the landscape of the pandemic. The use of both traditional inactivated vaccine technology and innovative approaches, including mRNA vaccines, recombinant protein vaccines, nucleic acid vaccines, and virus vector vaccines employing gene therapy technology, has not only been accelerated but has also seen significant technological advancements.[1-4] This progress is expected to accelerate vaccine development in areas beyond infectious diseases.

Vaccines are traditionally used as a preventive treatment against infectious diseases. However, their application in treating chronic inflammatory diseases such as Alzheimer's disease and hypertension is currently under investigation. Unlike preventive vaccines, these are therapeutic vaccines aimed at treating diseases. The objective is to eventually administer these vaccines a few times per year, replacing daily oral medications. One significant clinical advantage of vaccine therapy is the improvement in drug adherence. The rise in polypharmacy, particularly among older adults, has increased the number of patients who struggle with medication management and adherence. Our research has focused on developing novel therapeutic vaccines targeting self-antigens to combat cardiovascular diseases, including diabetes, heart failure, and age-related conditions.[5-9] In this paper, we outline our concept of therapeutic vaccines and provide an overview of the existing knowledge on vaccines for dyslipidemia.

THERAPEUTIC VACCINES AGAINST SELF-ANTIGENS

Immunity is categorized into two types: humoral immunity, where B cells differentiate into plasma cells that produce antibodies specific to antigens, and cellular immunity, where T cells sensitized to specific antigens acquire cytotoxic properties. Vaccine-induced immune responses are initiated through the coactivation of innate immunity and antigen presentation by antigen-presenting cells. In vaccines for infectious diseases, both humoral and cellular immunity are essential to combat and eliminate invading bacteria and viruses. However, therapeutic vaccines, such as those targeting amyloid-β in Alzheimer's disease and angiotensin II in hypertension, involve endogenous proteins. Therefore, it is crucial to primarily activate humoral immunity to promote antibody production while avoiding the activation of cellular immunity. Our proposed vaccine strategies differ from those used for infectious diseases (**Fig. 1**).

In designing a therapeutic peptide vaccine, there are three important elements: the selection of the antigen sequence (B cell epitope), the use of a carrier protein (T cell epitope), and the choice of an efficient adjuvant. When selecting the antigen, it is advisable to exclude major histocompatibility complex molecules sequences from the antigen sequence to prevent the antigen itself from activating T cells. However, for stable antibody production, helper T cells are necessary for the differentiation of B cells into plasma and memory cells.[10] Thus, the antigens (B cell epitope) should be conjugated with foreign T-cell epitopes (carrier proteins). Keyhole limpet hemocyanin (KLH) is widely recognized for its immunogenicity, containing a potent T-cell epitope, and is commonly used as a carrier protein.[11] In immune tolerance, T cells become functionally unresponsive to self-antigen. However, the coadministration of an adjuvant is essential to disrupt this T-cell peripheral immune tolerance, known as "anergy," by activating the CD28-B7 pathway through the innate immune response. Consequently, a therapeutic peptide vaccine typically comprises a combination of the antigen, carrier protein, and adjuvant.

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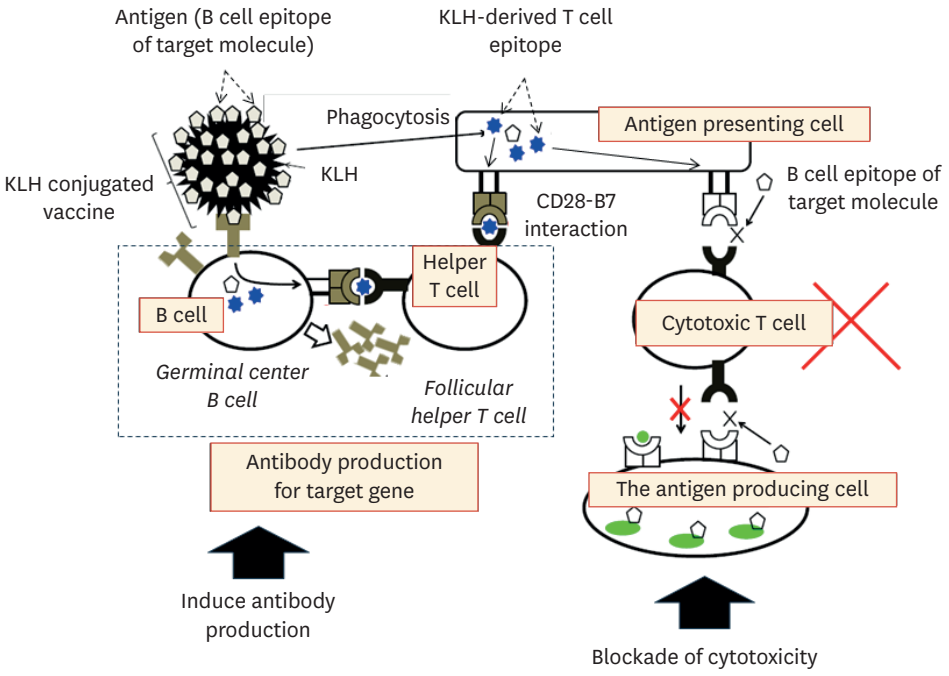

Fig. 1. Scheme for a KLH-conjugated vaccine against self-antigens.
An antigen (B-cell epitope of the target molecule) is conjugated with KLH and administered alongside adjuvants as a cotreatment. Antigen-presenting cells ingest these antigen-carrier protein conjugates and present the T-cell epitope of KLH to T cells via major histocompatibility complex molecules. The cotreatment with adjuvants effectively triggers the CD28-B7 interaction through the activation of innate immunity. Consequently, helper T cells recognize the corresponding epitope via T-cell receptors, which leads to their proliferation and differentiation. With the support of activated T cells, B cells then differentiate into plasmacytes and proliferate. As a result, B cells produce antibodies that target the antigen (i.e., antibody for the target molecule). Tfhs promote the efficient and sustained production of antibodies by B cells within the germinal center. Tfh-activated B cells are capable of producing high-affinity antibodies over an extended period. Since the antigen lacks a T-cell epitope, cytotoxic T cells are not activated by the target antigen and therefore do not attack endogenous proteins.
KLH, keyhole limpet hemocyanin; Tfh, follicular helper T cell.

The development of peptide vaccines has faced challenges, primarily in simplifying the formulation process. Conjugating peptides with a carrier protein often complicates the establishment and control of manufacturing standards, necessitating a simpler peptide vaccine formulation. Previously, we utilized KLH as a carrier protein; however, our recent focus has shifted to the peptide epitope of follicular helper T cells (Tfhs). Studies have demonstrated that Tfhs play a crucial role in promoting efficient and sustained antibody production by B cells within the germinal center.[12] Tfh-activated B cells can produce high-affinity antibodies; this is supported by the observed correlation with the neutralizing antibody levels of COVID-19 patients and the number of peripheral Tfhs in a human clinical study.[13] We have identified the AJ peptide (AJP001) as a novel helper T-cell epitope. Notably, AJP001 also activates innate immunity, allowing this peptide, when linked to an antigen, to be used in vaccine formulations without the need for additional adjuvants. Indeed, conjugating AJP001 with angiotensin II using a spacer and administering it to mice led to a concentration-dependent increase in antibody titers.[14,15] A simple peptide vaccine that includes both T and B cell epitopes can induce antibody production, representing a promising strategy for therapeutic vaccines.

HISTORY OF CLINICAL TRIALS

In the past, vaccines for Alzheimer's disease and hypertension have undergone clinical trials. For Alzheimer's disease, a vaccine targeting β-amyloid was developed but discontinued

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when participants developed aseptic meningoencephalitis, likely due to an autoimmune response.[16] For hypertension, several vaccines targeting the renin-angiotensin system have been conducted. An angiotensin I vaccine (PMD3117) significantly increased the anti-angiotensin I antibody titer without any changes in blood pressure in a phase 1 clinical trial.[17] An angiotensin II vaccine (AngQb-Cyt006) significantly increased anti-angiotensin II antibody titers,[18,19] and blood pressure significantly decreased in the high-dose group. However, subsequent clinical studies failed to replicate this blood pressure reduction with AngQb-Cyt006. Another vaccine targeting angiotensin, using the novel adjuvant CoVaccine HT™, was evaluated in a randomized, double-blind, placebo-controlled phase 2 clinical trial, but this study was terminated due to dose-limiting adverse effects. We also examined the efficacy of an angiotensin II vaccine in an animal model.[20,21] Administration of this vaccine led to a reduction in blood pressure and decreased angiotensin II-induced perivascular fibrosis in the heart [22,23] Building on these preclinical findings, we conducted a placebo-controlled dose escalation study to assess the safety, tolerability, and immunological responses of this angiotensin II vaccine (AGMG0201).[24] AGMG0201 was administered to 12 participants across low-dose and high-dose groups. In the safety evaluation, the most common treatment-related adverse events were pain and erythema at the injection site, which were classified as mild or moderate in severity, indicating that AGMG0201 was well tolerated. Anti-angiotensin II antibodies were notably present in patients receiving AGMG0201, particularly in the high-dose group. Future clinical trials should focus on evaluating the vaccine's effect on blood pressure reduction.

NOVEL THERAPIES FOR CARDIOVASCULAR DISEASES

Similar to hypertension, dyslipidemia is also a candidate for therapeutic vaccines. A vaccination strategy targeting apolipoprotein B100 (ApoB100), the primary component of low-density lipoprotein (LDL), and cholesterol ester transferase protein (CETP), which facilitates the transfer of cholesterol esters and triglycerides from high-density lipoprotein to LDL, has been explored in animal models.[25-28] Following these animal studies, a phase I clinical trial of the CETP vaccine was initiated.[28] However, the vaccine-induced antibody titers proved insufficient. Recently, the significant role of proprotein convertase subtilisin/kexin type 9 (PCSK9) antibodies, which lower LDL by preventing LDL receptor degradation, has been recognized as a therapeutic option for dyslipidemia. The established safety of PCSK9 inhibition has spurred the development of PCSK9 inhibitors,[29] and monoclonal antibodies (mAbs) such as evolocumab and alirocumab have been shown to significantly reduce plasma LDL cholesterol levels.[30] Despite their efficacy, PCSK9 mAbs are not cost-effective.[31] Additionally, a vaccine targeting PCSK9 in animal models has been reported.[32] When administered with adjuvants to male apolipoprotein E (ApoE)-deficient mice, the PCSK9 vaccine significantly elicited an antibody response against PCSK9 and maintained reduced plasma levels of total cholesterol, very low-density lipoprotein, and chylomicron for up to 24 weeks after the initial vaccination. Moreover, a peptide vaccine targeting a novel molecule, ANGPTL3, has been developed.[33] Administering the ANGPTL3 vaccine to obese mice has shown remarkable effects in lowering LDL cholesterol and triglycerides, as well as in preventing the accumulation of triglycerides in the liver, positioning it as a promising new therapeutic target molecule.

Importantly, nucleic acid drugs, such as small interfering RNA (siRNA) and antisense oligonucleotides, have garnered attention as potent therapeutics akin to vaccines.[34] One

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promising approach involves targeting PCSK9 with RNA-based treatments that bind to RNA and alter protein expression.[35] Antisense oligonucleotides are composed of a single-stranded DNA sequence of 15–30 nucleotides and function by inhibiting RNA translation through binding to the target mRNA. In contrast, RNA-interfering (RNAi) drugs consist of a short double-stranded RNA sequence that disrupts mRNA expression. Most RNAi-based therapies utilize noncoding siRNAs that are 20–24 bases long. These siRNAs enter the cell via endocytosis, escape from the endosome, and subsequently bind to the RNA-induced silencing complex (RISC).[35] The antisense strand is recognized as a guide strand and is retained.[36] The RISC cleaves the guide strand's complementary target mRNA, which silences its target gene.[37] Interestingly, this RISC recycling may contribute to prolonged target inhibition, extending the duration of action to several months.[38]

At present, the use of siRNAs for PCSK9 has been approved as a novel therapeutic strategy.[39] Inclisiran, which increases the uptake of LDL cholesterol by inhibiting the translocation of PCSK9, has been found to result in effective and sustained reductions in LDL cholesterol levels by 50% lasting for 6 months after a single injection. Initially used to treat rare genetic disorders, the application of inclisiran has expanded to include familial hypercholesterolemia and more common conditions such as primary dyslipidemia. In terms of advancements in drug delivery systems, N-acetylgalactosamine (GalNAc)-conjugated chemically modified siRNA specifically targets PCSK9 mRNA in the liver. This specificity is due to GalNAc's affinity for the asialoglycoprotein receptor, which is abundantly expressed on hepatocytes and facilitates rapid endocytosis.[40] Indeed, this approach has been employed to increase siRNA delivery to hepatocytes by more than 10-fold in a preclinical model. Similarly, antisense oligonucleotides (ASOs) and siRNAs targeting ANGPTL3 have been developed as targeted ANGPTL3 inhibitors and are undergoing clinical testing. The anti-ANGPTL3 antibody evinacumab has cholesterol-lowering effects, even against treatment-resistant human homozygous familial hyperlipidemia.[41] Moreover, an siRNA targeting angiotensinogen has been developed for hypertension treatment.[42] Angiotensinogen, the sole precursor of angiotensin I or II, is a promising target for gene silencing. Silencing angiotensinogen can suppress the formation of angiotensin I or II without triggering the counterregulatory renin–angiotensin system, potentially leading to more effective and stable blood pressure control. A siRNA targeting angiotensinogen is currently undergoing clinical trials for hypertension treatment. Zilebesiran, an angiotensinogen-targeting siRNA, has shown dose-dependent reductions in serum angiotensinogen levels (reduction >90%) with sustained effects on blood pressure reduction in the high-dose group.[42] The future success of siRNA drugs will depend on their safety and efficacy in reducing blood pressure among large and diverse populations of hypertensive patients, particularly in combination with other antihypertensive medications.

We are also developing an anti-thrombotic vaccine aimed at preventing cerebral and myocardial infarction in patients with cardiovascular diseases. This therapeutic vaccine targets S100A9 and has been designed to significantly extend the time to vascular occlusion following vascular injury, performing comparably to the antiplatelet drug clopidogrel, but without an increase in bleeding time. It shows promise for clinical use as a vaccine for the secondary prevention of cerebral and myocardial infarction.[43] Additionally, we have recently developed a novel vaccine specifically targeting myocardial infarction. This vaccine focuses on epoxyeicosatrienoic acids (EETs), which are part of the arachidonic acid cascade extracted from lipid bilayers. The vaccine is designed to increase the levels of activated EETs, and has been shown to significantly reduce the number of myocardial infarction sites.[8]

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Recently, vaccine technology has been applied to the field of aging research. When cells are exposed to various stresses and accumulate DNA damage, they enter a state of senescence, ceasing to divide and proliferate. It has been reported that senescent cells secrete large amounts of inflammatory cytokines, negatively impacting surrounding cells and accelerating cellular senescence. This phenomenon is referred to as the senescence-associated secretory phenotype. Studies using genetically engineered mice to create a model in which these senescent cells are eliminated from the body have shown an extension of lifespan and a reduction in various age-related symptoms.[44] This has led to the proposal of senolytic therapy, which involves the elimination of senescent cells. A novel approach to treating cardiovascular diseases involves a vaccine targeting senescent cells. To test this hypothesis, studies were conducted using a mouse model. We selected senescent T cells as target cells, as their surface antigens are well characterized. CD153, a relatively specific surface marker for senescent T cells, was chosen for vaccine development. We observed increased antibody titers in mice administered the CD153 vaccine along with an adjuvant. It is known that senescent T cells accumulate throughout the body with aging. However, it has been reported that their numbers increase in the adipocytes of mice on a high-fat diet. Therefore, we utilized a high-fat diet mouse model to assess the removal of senescent T cells in adipose tissue. Consistent with previous findings, the proportion of senescent T cells in visceral fat increased from 6.8% to 16.0% in high-fat diet mice. Importantly, the administration of the CD153 vaccine significantly reduced the number of senescent T cells in these mice to 7.6%. Additionally, macrophage infiltration, a key player in inflammation within adipose tissue, was also suppressed by the CD153 vaccine. Furthermore, the glucose tolerance of the vaccinated mice improved, indicating the prevention of high-fat-induced diabetes by the CD153 vaccine.[6] This is the first report of a senolytic vaccine in the world. Building on this concept, another group identified a new aging antigen, glycoprotein nonmetastatic melanoma protein B (GPNMB), as a molecular target for senolytic therapy. GPNMB expression was upregulated in vascular endothelial cells and/or leukocytes of patients and mice with atherosclerosis. We developed a vaccine targeting GPNMB and confirmed a reduction in GPNMB-positive cells following vaccination. This vaccination also improved normal and pathological aging phenotypes and extended the lifespan of male progeroid mice.[9] Vaccination targeting seno-antigens could be a potential strategy for new senolytic therapies, offering a promising concept for future practical applications.

FUTURE PROSPECTS

Therapeutic vaccines and nucleic acids for hypertension or dyslipidemia are advancing toward clinical application. In this context, we outline the future prospects of our therapeutic vaccine. Initially, for conditions such as hypertension or dyslipidemia, we suggest a novel therapeutic approach using vaccines or nucleic acids as alternatives to daily medication (**Fig. 2**). If a vaccine can effectively substitute for daily medication in managing hypertension or dyslipidemia, this alternative therapy could enhance drug adherence and reduce polypharmacy in our aging population. A significant challenge to the practical use of these therapies is that vaccines and nucleic acid drugs act as long-term agonists and cannot be quickly discontinued in the event of adverse effects. The potential risks associated with the pharmacodynamics of the hypertension vaccine and nucleic acid drugs may include hypotension or elevated potassium levels in patients with chronic kidney disease. If these therapies cause severe hypotension or high potassium due to an overly potent effect lasting several months, reversing the effects would be impossible once antibodies have been generated. To date, no immunological neutralization method for the vaccine has been developed, although the adverse effects can

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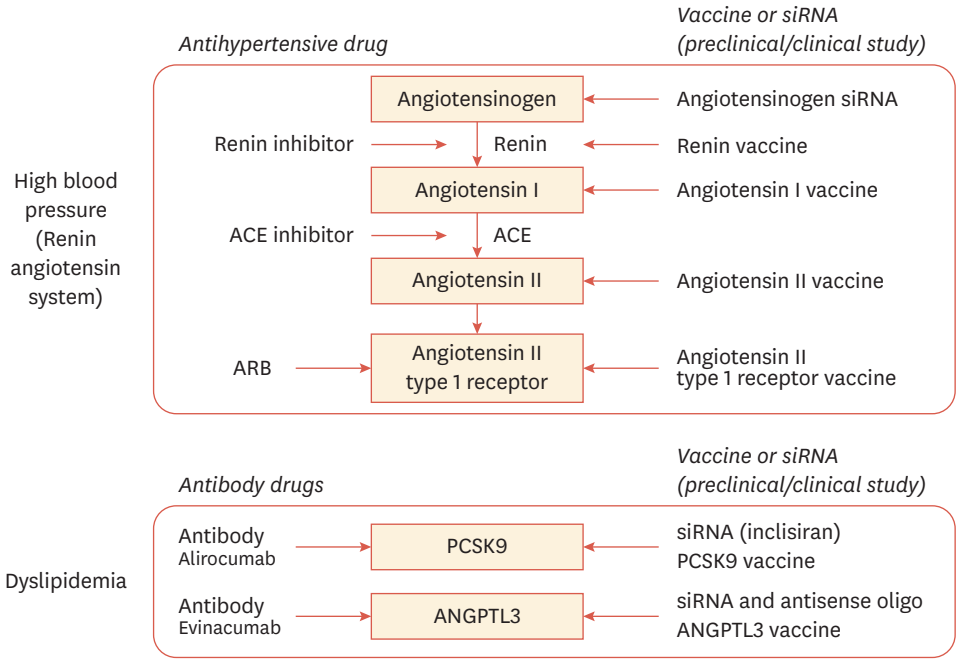

Fig. 2. Vaccine or siRNA development for the treatment of hypertension.
The target molecules for hypertension treatment include components of the renin-angiotensin system, such as angiotensinogen, angiotensin I or II, and the angiotensin II type 1 receptor. For dyslipidemia treatment, the targets are PCSK9 and ANGPTL3. The primary antihypertensive medications are renin inhibitors, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers. A current challenge in this field is the development of vaccines or siRNAs targeting these same molecules. Similarly, novel antibody therapies targeting PCSK9 and ANGPTL3 have been developed for dyslipidemia treatment, with ongoing efforts to create vaccines or siRNAs for these targets as well.
ARB, angiotensin receptor blocker; siRNA, small interfering RNA; PCSK9, proprotein convertase subtilisin/kexin type 9; Antisense oligo, antisense oligonucleotides.

be managed clinically with the judicious use of other medications. Given these potential adverse effects, and considering the variability in immune response and blood pressure regulation, there is a growing need for personalized medicine where these long-term therapies are tailored to individual patients.

Second, in inflammatory diseases such as rheumatoid arthritis and in cancer treatment, targeting disease-causing proteins with specific antibodies can significantly reduce disease activity during the acute phase. However, continuous administration is necessary to maintain low disease activity in the chronic phase, which may diminish a patient's quality of life and increase medical costs. Therefore, we propose transitioning from monthly antibody therapy to vaccination administered a few times per year during the chronic phase. Third, we will explore the challenge of developing personalized and preventive vaccines, potentially for genetic and age-related diseases. Advances in genome diagnostics have improved the accuracy of assessing disease risk, even in healthy individuals. We aim to leverage vaccines as a tool for early prevention and therapeutic intervention. Additionally, while senolytic therapy remains largely theoretical in animal studies, we have recently reported on a therapeutic vaccine designed to deplete senescent cells, demonstrating improvements in age-related diseases.[6,9]

The development of long-term agonists through vaccines and nucleic acid drugs could mitigate hypertension or dyslipidemia resulting from low drug adherence. For these treatments to be practically applied, it is essential to simultaneously promote home blood pressure management and personalized medicine, alongside advancements in wearable devices and the utilization of personal health records. Looking ahead, a diverse array of

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antihypertensive and cholesterol-lowering treatment options will enable the provision of optimal individualized medical care.

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