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REVIEW

Concerns and challenges in clinics-guided nanovaccines design and applications

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Abstract Vaccines play a crucial role in the prevention and treatment of multiple diseases. Given the constraints of conventional vaccines, the development of nanovaccines, characterized by their superior design flexibility and controllability, has emerged as a compelling alternative. By utilizing nanotechnology, nanovaccines optimize the targeted delivery of antigens and adjuvants, augment antigen presentation, and facilitate precise modulation of immune cell responses, thereby exhibiting substantial potential for both preventive and therapeutic applications across a range of diseases. However, research on nanovaccines is currently stalled at the preclinical stage, with numerous challenges and shortcomings hindering their clinical translation. Herein, we discuss various design concepts and strategies for nanovaccines, along with their biomedical applications, with an emphasis on the challenges, future directions, and strategies of their clinical translation. We specifically highlight the core principles that need to be achieved in the preclinical development of nanovaccines, aiming to explore strategies to overcome existing challenges and promote their clinical application.

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1. Introduction

Since Edward Jenner inoculated cowpox pus to prevent smallpox in 1796, making it medically possible to prevent infection in healthy people[1], vaccines have helped people fight infectious diseases, significantly reducing the incidence of infectious diseases and mortality rates and achieving historic success[2]. However, current conventional vaccines have limitations such as weak immune response, instability *in vivo*, toxicity, etc.[3]. Compared with conventional vaccines, nanovaccines use nanocarriers to deliver antigens at the nanoscale. By leveraging the tailored selection and design of nanocarriers with distinct physicochemical properties, nanovaccines exhibit superior attributes, including enhanced vaccine targeting and potentiated immune responses[4], which provide promising strategies for overcoming the inherent limitations of traditional vaccines.

This review provides a comprehensive overview of the design principles of nanovaccines with an in-depth discussion of their impact on the immune system, and highlights their current applications, including their synergistic potential in combination with other therapeutic modalities. Importantly, we explore the future directions for nanovaccine development, emphasizing the existing limitations and challenges associated with their design, delivery, and efficacy, with the ultimate goal of accelerating their clinical translation.

2. Types of nanovaccine carriers

Currently, numerous nanomaterials have been utilized in vaccine formulations, including lipid-based nanoparticles, biomimetic nanoparticles, inorganic nanoparticles, polymeric nanoparticles. This section provides a brief overview of their respective advantages and disadvantages.

2.1. Lipid-based nanoparticles

Lipid-based nanoparticles are colloidal nanocarriers ranging from 1 to 100 nm in size[5], composed of ionizable cationic lipids, phospholipids, cholesterol, and polyethylene glycol-derived lipids (PEG-lipids)[6]. They exhibit exceptional cellular penetration capabilities and drug-loading efficiency[7], along with targeted specificity[8]. Currently, lipid nanoparticles represent the most advanced and mature platform for RNA delivery[9], effectively protecting RNA from enzymatic degradation, enhancing cellular uptake, and facilitating cytoplasmic release[10]. A mannose-modified lipid nanoparticle vaccine has been shown to efficiently encapsulate mRNA encoding antigens and cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) agonists, significantly enhancing mRNA gene expression, promoting antigen presentation, and stimulating immune activation[11]. However, lipid nanoparticles may compromise lysosomal membrane integrity, leading to cytotoxicity[12]. Additionally, the structural instability of lipid nanoparticles can result in off-target delivery and low cross-presentation efficiency, adversely affecting vaccine efficacy *in vivo*[13]. Furthermore, the strong immunogenicity of ionizable cationic phospholipids may elicit immune responses due to the components of the nanoparticle lipid formulation[14]. Compared to other nanomaterials, lipid nanoparticles offer advantages in terms of production costs[15].

2.2. Biomimetic nanoparticles

Biological carriers encompass cell membranes, extracellular vesicles, and viruses. Biomimetic nanoparticles are composed of these biological carriers and functional agents, leveraging the inherent properties of biological carriers along with surface modification techniques. As a result, biomimetic nanoparticles exhibit biocompatibility and low toxicity[16]. Furthermore, the proteins present on the extracellular surface confer advantages such as prolonged blood circulation, enhanced targeting capabilities, improved cellular interactions, and gradual drug release[17]. A specific biomimetic nanovaccine HybridDC, incorporates cell membranes derived from genetically engineered dendritic cells (DCs), thereby circumventing several limitations associated with traditional DC vaccines, including poor antigen delivery, weak lymph node (LN) homing, and risks associated with live cell infusion. This approach enhances antigen presentation and stimulates effective anti-tumor immunity[18]. Additionally, a biomimetic nanovaccine coated with membranes from senescent cancer cells demonstrates superior performance in promoting DC internalization, improving LN targeting, and enhancing immune responses compared to live senescent cancer cells. This advancement increases the immunogenic potential of vaccines based on senescent cancer cells while minimizing adverse effects, thereby facilitating the design of biomimetic nanovaccines for cancer immunotherapy[19]. However, the synthesis of biomimetic nanovaccines is more complex compared to other nanovaccines, necessitating intricate production methods for the selection and processing of various nanomaterials, which poses challenges for large-scale manufacturing[20,21].

2.3. Inorganic nanoparticles

Inorganic nanoparticles, including gold, metal oxides, and quantum dots, have unique optical, electrical, and magnetic properties, size dependence, high surface area to volume ratio, and affinity for antigens, which render these materials particularly advantageous for vaccine design[22]. Inorganic nanoparticles can enter the nucleus without the need for transfection agents and can significantly enhance cellular immune responses and specific antibody production[23]. Furthermore, their capacity for surface modification and adjuvant properties has led to their widespread application in the delivery of nanovaccines[24-26]. The nanovaccine rF1-V10@AMMSN, delivered by a novel amino-decorated mesoporous manganese silicate nanoparticle (AMMSN), can be internalized by DCs and promotes DC maturation through the activation of the cyclic GMP−AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway, resulting in the production of type I interferons and enhanced antigen presentation. This represents a valuable strategy against lethal bacterial pneumonia[27]. However, the introduction of inorganic nanoparticles into the body raises certain toxicity concerns due to challenges in clearance and degradation, the potential generation of reactive oxygen species (ROS), chemical instability, and the release of excess free metal ions[28,29]. In addition, inorganic nanomaterials have limited solubility and require surface modification to accelerate their dissolution or maintain stability in an aqueous environment, and when using inorganic materials as carriers or adjuvants, an optimal balance needs to be reached between the surface functionalized of soluble polymers and the effective loading of antigens[20].

2.4. Polymeric nanoparticles

Polymer nanoparticles are primarily composed of polymers, surfactants, and an aqueous phase[30]. These nanoparticles exhibit

excellent biodegradability, the ability to mimic pathogen characteristics, and ease of surface modification, making them ideal carriers for vaccines. The inherent adjuvant-like properties of the polymers also provide a pathway for optimizing nanovaccines[31]. The nasal mucosa poses a barrier to intranasal vaccine delivery, however, by coating influenza vaccine nanoparticles with alternating mucoadhesive cationic chitosan and muco-inert anionic cytosine-phosphate-guanine (CpG) adjuvants, the formulation enhances nasal delivery and interaction with resident immune cells, significantly improving both cellular and humoral immune responses[32]. Nanoparticles containing polycaprolactone (PCL) encapsulating hepatitis B surface antigen (HBsAg) serve as an oral delivery vehicle. Compared to traditional vaccines, this nanoparticle system effectively presents the antigen post-oral administration and elicits a prolonged antibody response, thereby minimizing the required dosage. Nonetheless, during clinical translation, polymer nanoparticles still face challenges such as potential toxicity from degradation products, non-specific immune responses, and instability in antigen release[20].

3. The design principles of nanovaccines

In general, nanovaccines consist of antigens, adjuvants, and nanocarriers. Antigens, usually consisting of attenuated pathogens or proteins isolated from pathogens, are a means by which the immune system recognizes a specific target[33]. Adjuvants are divided into immunostimulants and delivery systems, which prolong the retention time of antigens within the body, enhance the processing and presentation of antigens by antigen-presenting cells (APCs), and amplify the intensity and duration of the immune response[34]. Utilizing nanomaterials as carriers for delivering antigens and adjuvants can enhance the targeted specificity of nanovaccines[35], enhance the immune response[36], and protect the cargo transport[37]. The physicochemical factors of nanovaccines, such as size, shape, and surface charge, can significantly influence their distribution within the body, cellular uptake, and the type and intensity of the immune response[38] (Fig. 1). Therefore, it is essential to develop and design nanovaccines based on their intended purpose and specific requirements, as shown in Table 1[39–57].

3.1. Size

The size of nanoparticles significantly influences their distribution, clearance, and efficacy within the body, due to the presence of various physiological size thresholds and size-related bionano interactions[58]. Nanoparticles in the range of 20–200 nm are more likely to provoke an immune response, whereas smaller nanoparticles benefit from cell-associated migration and lymphatic drainage, resulting in enhanced antigen presentation efficacy[59]. Generally, nanoparticles with an average diameter of less than 100 nm, and particularly those below 50 nm, are more readily internalized by DCs, whereas larger nanoparticles are preferentially internalized by macrophages[59]. Research indicates that interstitial blood flow can effectively transport 25 nm ultrasmall nanoparticles to lymphatic vessels and their associated LN, whereas the transport efficiency of 100 nm nanoparticles is only 10% of that[60]. Smaller particles (<50 nm) can directly enter LN, while larger particles are more effectively retained in LN[61], the diameter of the channels formed by the extracellular matrix (ECM) of stromal cells typically measures around 100 nm, which

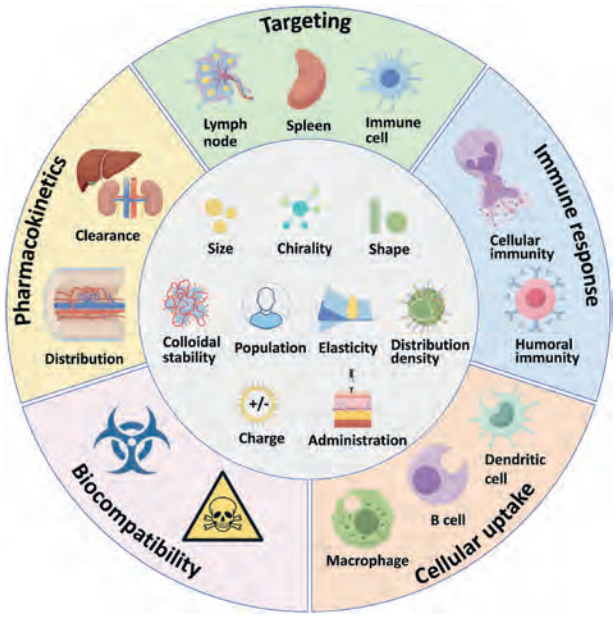

Figure 1 Impact of design principles on nanovaccines. Created by Figdraw.com.

restricts the molecular size that can migrate to lymphatic vessels. Nanoparticles exceeding 100 nm in size encounter difficulties in penetrating lymphatic capillaries[62]. However, it is also critical that nanoparticles do not become excessively small, studies indicate that nanoparticles smaller than 9 nm are more likely to enter vascular capillaries and are rapidly eliminated through urinary excretion[63], and the current study suggests that 10–100 nm is the optimal size range of nanoparticles that can passively enter the LN[64] (Fig. 2A). For instance, loading wasp toxin onto a high-density lipoprotein-mimicking peptide-phospholipid scaffold results in the formation of 10–20 nm wasp toxin-lipid nanoparticle (α-melittin-NP), whose size allowing the nanovaccine to effectively enter lymphatic capillaries and LN, overcoming the limitation of small molecule free wasp toxin preferentially entering the bloodstream and being rapidly metabolized, thus enabling the wasp toxin to exert its immunomodulatory effects within the LN[39]. In addition to influencing the distribution of vaccines within LN, the size of nanoparticle vaccines also affects the uptake and maturation of immune cells. Larger particles (>1 μm) typically elicit a T-helper cell type 1 (Th1) immune response, while smaller particles (<500 nm) tend to induce a Th2 response[59]. Wang et al.[40] designed hydroxyapatite (HA) particles in rod shapes ranging from 100 nm to 10 μm. The results indicated that shorter HA rods, measuring between 100 and 500 nm, enhanced cellular uptake of the antigen and promoted DC maturation. In contrast, longer HA rods, ranging from 500 nm to 10 μm, were found to prolong antigen retention and facilitate DC aggregation. Notably, the HA rods of intermediate size at 500 nm exhibited the strongest capacity for promoting antigen uptake, DC aggregation and maturation, as well as T cell maturation, thereby demonstrating optimal *in vivo* antitumor immune efficacy. However, it is important to note that the nanoparticle size of nanovaccines determined in rodent models may not be directly translatable to non-human primates. Hassett et al.[65] designed lipid nanoparticles with varying sizes ranging from 60 to 150 nm, and studies

Table 1 Overview of nanoparticles referenced.

Nanoparticle characteristic	Nanovaccine name	Parameter studied	Effect observed	Ref.
Spherical; neutral; lipid nanoparticles	α-Melittin-NP	Size	The 10−20 nm α-melittin-NPs promote the activation of $CD80^+CD86^+$ macrophages and $CD80^+CD86^+$ DCs in LNs from 12.3% to 15.1% to 26.3% and 31.4%, respectively, and increase antigen-specific $CD8^+$ T cell responses by 3.6-fold	39
Rod-shaped; immune-targeted delivery system	HA rods	Size	HA rods of 100−500 nm promote antigen uptake and DC maturation, enhance LN targeting of antigen; HA rods of 500 nm to 10 μm prolong antigen retention and increase DC accumulation at the injection site; HA rods of 500 nm combine the advantages of short and long rods, showing the highest $CD4^+$ and $CD8^+$ T cell populations and the best anti-tumor immunity *in vivo*	40
Spherical, ellipsoidal, and rod-like; viromimetic nanoparticles	RLNVax	Shape	RLNVax (rod-like) induced antigen-specific antibody titers about 64 times and 4.6 times higher than those induced by free antigen proteins and spherical nanoparticle vaccines respectively	41
Rod-like (AR 1.5 and 5); cationic; tetraethyl orthosilicate mesoporous silica	MSN-FITC	Shape	Short rod MSN-FITC were mainly trapped in the liver, while long rod MSN-FITC were mainly distributed in the spleen	42
Cage-shaped, star-shaped, rod-shaped; cationic; polymeric nanoparticles	An@AuNPs, An@AuNSs, An@AuNCs	Shape	An@AuNSs (star-shaped) induce the highest antibody titers, approximately 1.84 and 1.36 times higher than An@AuNCs (cage-shaped) and An@AuNRs (rod-shaped), respectively; An@AuNSs significantly promoted $CD4^+$ T cell proliferation, with a proliferation rate about 2.8 times higher than the control group, while An@AuNCs mainly promoted $CD8^+$T cell proliferation; An@AuNSs induced significantly higher levels of IL-6, TNF-α, and IFN-γ compared to the other two groups	43
Spherical; anionic; ascorbyl palmitate-coated nanoparticles	AsP-PC-LNPs	Surface charge	Anionic AsP-PC-LNPs show enhanced tumor targeting compared to neutral AsP-PC-LNPs	44
Spherical; anionic, cationic; superparamagnetic iron oxide nanoparticles	γFe_2O_3/APTS NPs (cationic), γFe_2O_3/DMSA NPs (anionic)	Surface charge	Cationic γFe_2O_3/APTS NPs significantly enhance the cross-presentation ability of DCs compared to anionic γFe_2O_3/DMSA NPs, leading to stronger T-cell activation	45
Finger-like; anionic, cationic; transfersome-based nanovaccines	OVA-SATMNs (cationic), OVA-SCTMNs (anionic)	Surface charge	The IFN-γ level in the OVA-SATMNs group reached 120 pg/mL, significantly higher than the OVA-SCTMNs group (80 pg/mL) and OVA-MNs group (60 pg/mL); the IL-2 level was 100 pg/mL in the OVA-SATMNs group, significantly higher than the OVA-SCTMNs group (70 pg/mL) and OVA-MNs group (50 pg/mL); the LN accumulation was significantly higher in the OVA-SATMNs group compared to the OVA-SCTMNs group and OVA-MNs group	46
Spherical; cationic; polymeric nanoparticles	PEI/CpG/OVA	Surface charge	Cationic PEI/CpG/OVA promote infiltrating $CD8^+$ T cells, IFN-γ and TNF-α levels in tumor tissues compared to the other groups	47
Spherical; cationic; mannose receptor-targeted nanoparticles	Man-PLL-RT/OVA/CpG	Surface charge	Cationic Man-PLL-RT/OVA/CpG significantly enhance the maturation of BMDCs and promote higher secretion of TNF-α and IL-12p70, crucial cytokines for T-cell activation	48
Spherical; neutral; porcine circovirus virus-like particles	Cap-E2 NPs	Distribution density of antigens	The high-density display of antigens on Cap-E2 NPs induce higher levels of E2-specific IgG antibodies, promote a strong Th1-biased cellular immune response and increase CTL activity, indicated by increased antibody avidity, neutralizing antibody titers, levels of IFN-γ and IL-2 and GrB secretion	49
Spherical; neutral; antigen-decorated nanoparticles	NPs displaying HIV-1 Env and influenza HA	Distribution density of antigens	NPs with low antigen density could stimulate B cell activation, enhance humoral immune response, increase antibody titer and the number of antibody secreting cells	50

(*continued on next page*)

Table 1 (*continued*)

Nanoparticle characteristic	Nanovaccine name	Parameter studied	Effect observed	Ref.
Spherical; anionic; LDH nanoparticles	s-BTLC, a-BTLC	Colloidal Stability	The ratio of $CD8^+$/IFN-γ^+ T cells and the level of IFN-γ produced by mouse spleen cells induced by dispersion-stable s-BTLC were about 2 times and 2.6−4.0 times higher than those induced by a-BTLC and other formulations, respectively; the *in vivo* CTL activity induced by s-BTLC was 25.3 ± 1.2%, significantly higher than that of a-BTLC (9.5 ± 1.8%) and other formulations (1.5−5.2%)	51
Spherical; anionic; HBc VLPs	THM-HA@Mn	Colloidal Stability	Dispersion-stable THM-HA@Mn induce significantly higher titers of rHA-specific IgG antibodies and M2e-specific IgG antibodies, which were 7-and 8-fold higher, respectively, than THM-HA; THM-HA@Mn sustain antigen release at the injection site and improve efficiency of LN delivery	52
Spherical; anionic; PEG-*b*-PLA	VPNVax	Chirality	Dextrorotatory VPNVax had an advantage in activating IgG2c, with an IgG2c/IgG1 ratio of 0.85	53
Spherical; anionic; carbon dots	D-OVA (dextrorotatory), L-OVA (levorotatory)	Chirality	The percentage of $CD86^+CD80^+$ BMDCs in D-OVA group (64.0%) was significantly higher than that in L-OVA group (2.94%) and OVA group (2.02%). The secretion of TNF-α in D-OVA group was 7.29 pg/mL, which was significantly higher than that in L-OVA (2.03 pg/mL) and OVA (1.61 pg/mL)	54
Spherical; cationic; polypeptides	P_L-K-OVA (levorotatory), P_D-K-OVA (dextrorotatory)	Chirality	The expression of CD86, CD80, CD40 and MHC II in P_D-K-OVA group were significantly higher than those in P_L-K-OVA group, and the secretion levels of IL-6, IL-12 p70, IFN-γ and TNF-α were 1.9, 3.4, 6.0 and 3.5 times higher than those in P_L-K-OVA group, respectively	55
Spherical; lipid nanoparticles	PLGA nanovaccine	Route of administration	Intravenous injection: induces the strongest Th1 immune response, including high titers of IgG2c antibodies, high levels of IFN-γ, and strong cytotoxic T cell activity; subcutaneous injection: lower levels of IgG2c antibodies and IFN-γ, weaker cytotoxic T cell activity, and lower iNKT cell activation; intranodal injection: similar to subcutaneous injection, with lower levels of IgG2c antibodies and IFN-γ, limited cytotoxic T cell activity, and lower iNKT cell activation	56
Spherical; anionic; HBsAg polymeric nanoparticles	HBsAg polymeric nanovaccine	Route of administration	Oral administration: the lowest levels of IgG and IgA antibodies, as well as IFN-γ and IL-2, indicating the weakest immune effect; nasal administration: IgG and IgA antibody levels are similar to intramuscular injection, but IFN-γ and IL-2 levels are slightly lower than intramuscular injection; subcutaneous administration: IgG and IgA antibody levels are similar to intramuscular injection, but IFN-γ and IL-2 levels are slightly lower than intramuscular injection; intramuscular injection: induce the strongest humoral and cellular immune responses, producing high titers of IgG and IgA antibodies, as well as high levels of IFN-γ and IL-2	57

NP, nanoparticle; DC, dendritic cell; LN, lymph node; HA, hydroxyapatite; AR, aspect ratio; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; OVA, ovalbumin; BMDC, bone marrow-derived dendritic cell; Th, T helper; CTL, cytotoxic T lymphocyte; GrB, granzyme B; HIV-1 Env, human immunodeficiency virus envelope glycoprotein; LDH, layered double hydroxide; rHA, recombinant hemagglutinin; PEG-*b*-PLA, polyethylene glycol-*b*-polylactic acid; MHC, major histocompatibility complex; P_L-K, poly(L-phenylalanine)-block-poly(L-lysine); P_D-K, poly(L-phenylalanine)-block-poly(D-lysine); iNKT, invariant natural killer T cells.

indicated that lipid nanoparticles with an average size of approximately 100 nm were necessary to elicit sustained high antibody titers in mice. Nevertheless, all tested sizes (60−150 nm) induced robust immune responses in non-human primates. This discrepancy may stem from anatomical differences in lymphatic vessels between mice and primates, where the smaller lymphatic vessels in mice may exhibit greater sensitivity to particle size compared to the larger lymphatic vessels in

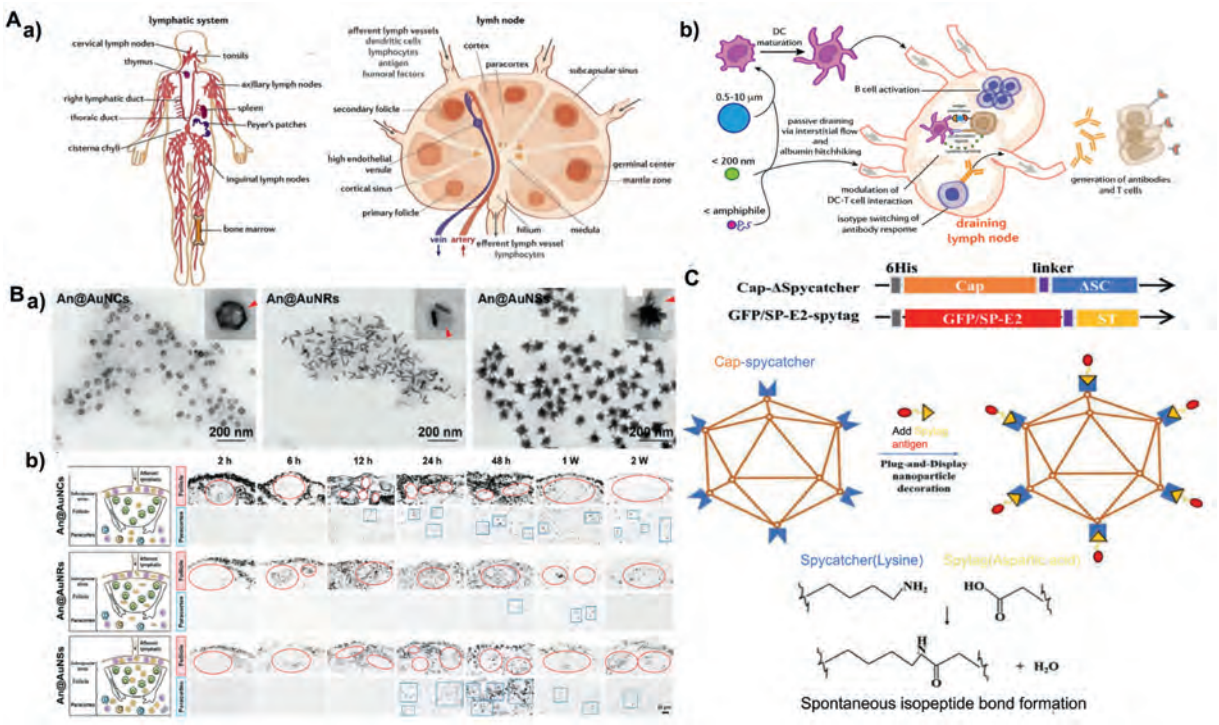

Figure 2 Studies on physicochemical factors of nanovaccines. (A) a) Physiology of the lymphatic system and anatomy of the lymph node. b) Schematic illustration of the influence of size and morphology on lymphatic transportation and illustration of the interplay between innate immune cells in the lymph node. Reprinted with the permission from Ref. 45. Copyright © 2020 American Chemical Society. (B) Research on An@AuNPs with different shapes. a) Transmission electron microscopy characterization of nanovaccines with different shapes. b) Schematic diagram of the shape-reliant An@AuNPs transmission dynamics, and histological images of diverse An@AuNPs shapes entered or retained in lymph nodes within 2 weeks post intradermal footpad injection into C57BL/6 mice. Reprinted with the permission from Ref. 57. Copyright © 2023 Elsevier. (C) Schematic diagram of Cap-SC VLP based antigen display. Reprinted with the permission from Ref. 75. Copyright © 2022 Springer Nature.

primates within the tested size range. Furthermore, upon contact with biological fluids, the size of nanoparticles may differ from measurements taken post-manufacture, and aggregation may occur prior to reaching target cells. Therefore, it is essential to characterize nanoparticle size not only under static conditions but also after interactions with blood and other biological fluids to better reflect their *in vivo* efficacy [66,67].

3.2. *Shape*

The morphology of nanoparticles is a critical determinant of cellular uptake; nanoparticles with a wrinkled surface enhance interactions with the cell membrane, thereby facilitating their internalization by cells[68]. The shape of nanoparticles affects their distribution *in vivo* and their cellular uptake, and most current nanocarriers are spherical, but it has been shown that nanocarriers with non-spherical structures, such as filamentous or worm-like micelles and needle or disc-like structures, may have higher drug-carrying capacity, stronger cellular uptake and more potent immune properties, and may be the next generation of nanocarriers[69]. Compared to traditional spherical vaccines, rod-shaped nanoparticle vaccines with a higher aspect ratio demonstrate enhanced LN drainage efficiency and more effective B cell activation[41]. Huang et al.[42] designed two distinct shapes of fluorescent mesoporous silica nanoparticles (MSNs) with aspect ratios of 1.5 and 5 The results indicated that the short rod-shaped MSNs predominantly localized in the liver, while the long rod-shaped MSNs were found in the spleen, with the clearance rate of short rod-shaped MSNs being faster than that of the long rod-shaped MSNs. The morphology of nanoparticles significantly influences their half-life and interactions within the human body. Ellipsoidal particles may prolong circulation half-life by reducing nonspecific collisions with vascular walls. Due to the alignment of blood flow, elongated liposomes and polymeric filamentous micelles also exhibit extended circulation times[70]. Spherical nanoparticles can elicit cardiopulmonary responses post-injection; however, altering the shape of nanoparticles to rod-like or disc-like forms can delay macrophage recognition of the particles, resulting in a lack of pronounced cardiopulmonary reactions, potentially attributable to the slower clearance rates associated with these morphologies[71]. Nevertheless, elongated nanoparticles may lead to increased cellular membrane loss due to their larger contact surface area with cells[66]. Furthermore, the morphology of nanoparticle vaccines influences the type of immune response elicited. Zhao et al.[43] synthesized caged and star shaped tumour antigenic nanovaccines respectively, and comparisons revealed that star shaped nanovaccines flowed mainly into the follicular region of the LN, captured and retained more repetitive antigenic epitopes, induced stronger humoral immunity, and had a significant prophylactic effect on tumours. In contrast, caged nanovaccines were more retained in the paracortical region, preferentially presenting peptide-MHC-I (p-MHC-I)

complexes and inducing $CD8^+$ T cells, showing significant therapeutic effects (Fig. 2B).

3.3. Surface charge

The surface charge of nanovaccines affects their transport efficiency to target sites and cellular uptake[44], as well as the immune response[45], and positively charged nanoparticles tend to accumulate at the injection site, whereas neutral or negatively charged nanoparticles are more likely to reach LN[72]. Neutral and anionic nanoparticles can diminish serum protein adsorption and inhibit charge interactions with macrophage membranes, thereby prolonging their circulation half-life. Cationic nanoparticles typically exhibit greater interactions with negatively charged cell membranes, resulting in more significant membrane perturbation and enhanced cellular uptake compared to neutral and anionic nanoparticles[73], and cationic nanovaccines accumulating more in LN, activating DC maturation and inducing a Th1 immune response more efficiently than anionic nanovaccines[46]. Guan et al.[47] designed polycationic polyethylenimine (PEI), while Chen et al.[48] developed a mannosylated polylysine backbone incorporating *p*-toluylsulfonyl arginine (Man-PLL-RT), both formulations utilize electrostatic interactions to encapsulate antigens and adjuvants, resulting in positively charged nanovaccines that enhance DC uptake and antigen presentation. However, cationic nanoparticles exhibit toxicity characterized by a dose-dependent response[74]. Research indicates that mice treated with cationic nanoparticles demonstrate increased hepatic enzyme release and weight loss compared to those treated with neutral or anionic nanoparticles[75]. Furthermore, cationic nanoparticles induce more pronounced disruption of plasma membrane integrity, greater mitochondrial and lysosomal damage, and a higher number of autophagosomes relative to anionic nanoparticles[76]. However, anionic nanoparticles may interact with the cell membrane, potentially compromising its integrity[76], while anionic nanoparticles can also affect the functionality of lysosomes and other organelles[77], thereby exhibiting a degree of toxicity.

It is important to note that due to the complex physiological environment *in vivo*, the design of the surface charge of nanovaccines varies according to the route of administration. The physiological pH of blood ranges from 7.2 to 7.4, while the isoelectric point of most serum proteins is below 7, resulting in a net negative charge. Consequently, neutral or anionic nanoparticles can resist protein adsorption for extended periods post-intravenous administration[78], thereby prolonging circulation time and enhancing tumor accumulation *via* the enhanced permeability and retention effect. In contrast, cationic nanoparticles interact electrostatically with negatively charged serum proteins, leading to the formation of a protein corona that diminishes bioavailability and accelerates clearance[79,80]. For intramuscular and subcutaneous injections, it is essential to facilitate the interaction of nanoparticles with target cells; thus, nanovaccines administered *via* these routes can be engineered to possess a certain degree of positive charge at physiological pH to enhance interactions with cell membranes[81]. Oral administration necessitates overcoming the acidic environment of the gastrointestinal tract and the degrading effects of digestive enzymes. Anionic nanoparticles can interact with integrins on the surface of intestinal epithelial cells, thereby enhancing intestinal permeability and improving the absorption efficiency of nanoparticles[82]. Reversible surface charge nanoparticles can utilize changes in pH and redox potential, endogenous enzymes, or exogenous factors to maintain a moderate anionic surface charge during administration, ensuring high mobility in bodily fluids and tissues while enabling the reversal of surface charge at target cells to enhance cellular uptake. pH variation is one of the most commonly utilized stimuli for achieving surface charge conversion in nanoparticles. For example, when administered orally, reversibly charged nanoparticles retain a negative charge in the acidic environment of the stomach, thereby facilitating their passage through the gastric mucus layer and protecting them from degradation by gastric acid. Upon entering the neutral or weakly alkaline conditions of the small intestine or colon, the increase in pH induces a reversal of the surface charge to a positive state, enhancing the interaction of nanoparticles with the negatively charged cell membranes of intestinal epithelial cells, thereby promoting cellular uptake. Similarly, due to the rapid metabolism of tumor cells resulting in elevated lactate production, the pH within the tumor microenvironment (TME) is often lower than that of normal tissues. In tumor targeting, nanoparticles retain a negative charge in the bloodstream, which aids in their stability and evasion from immune clearance. Upon reaching the TME, the decrease in pH triggers a reversal of the surface charge to a positive state, facilitating interactions with the negatively charged cell membranes of tumor cells and enhancing cellular uptake rates[80]. Designing nanoparticles with varying surface charges, or even those capable of charge conversion based on the route of administration, can significantly enhance immunogenic efficacy.

3.4. The distribution density of antigens

The distribution density of antigens within nanovaccines significantly influences the immune response elicited by the vaccine. Nanoparticles with a high antigen density exhibit the highest levels of p-MHC-I on the surface of DCs in mouse bone marrow, thereby inducing a greater number of T cells. Conversely, nanoparticles with a low antigen density demonstrate a higher uptake rate by DCs, resulting in an increased number of $CD8^+$ T cells secreting interferon-γ[83]. In addition, the density of surface antigens on the vaccine is crucial for immune cell receptor cross-linking and activation. Liu et al.[49] developed a modular self-adjuvant polyvalent vaccine platform based on porcine circovirus-like nanoparticles utilizing SpyTag/SpyCatcher technology, which allows for the ordered and repetitive display of antigen epitopes on the nanoparticle surface. This promotes effective recognition and cross-linking of B cell receptors, resulting in a higher level of immune response compared to conventional vaccines, establishing it as a viable multi-antigen delivery system (Fig. 2C). However, high antigen density does not necessarily elicit the optimal immune response. Research indicates that nanovaccines with low antigen density can more effectively stimulate B cells compared to those with high antigen density, resulting in higher antibody titers and an increased frequency of antigen-specific antibody-secreting cells[50].

3.5. Elasticity

The elasticity of nanoparticles reflects their variable characteristics, which can influence cellular uptake[84], blood circulation, phagocytosis, endocytosis, and targeting of nanoparticles[85]. Softer polyethylene glycol (PEG) hydrogel nanoparticles exhibit a

significantly reduced cellular uptake rate in immune cells, endothelial cells, and cancer cells compared to their stiffer counterparts, along with longer distribution and elimination half-lives[85]. Deformable albumin-stabilized emulsions (DASE) possess adaptive deformation capabilities, allowing them to attach between cells, deform, and adjust their size to pass through endothelial gaps, thereby facilitating direct lymphatic transfer. This results in a marked enhancement of LN drainage and antigen accumulation, effectively stimulating cellular immune responses when compared to rigid particles[86]. The reduced lymphatic transport capacity of stiffer nanoparticles is attributed to their diminished ability to traverse narrow lymphatic vessels[61]. However, rigid dimethyldioctadecylammonium (DDA) readily forms a depot at the injection site, demonstrating more effective lymph node drainage and antigen cellular uptake compared to highly fluid dimethyldioleoylammonium (DODA). Therefore, further investigation is warranted to elucidate the impact of nanoparticle elasticity on LN drainage[72,87]. Comparatively, the softer nanoparticles had a lower cellular uptake rate. The lower cellular uptake rate of softer nanoparticles may be due to their ability to deform the cell membrane, as rigid particles are typically more readily enveloped by the membrane, while flexible particles tend to diffuse more easily across the membrane[88]. Additionally, the increased energy expenditure associated with membrane deformation in softer particles contributes to their lower uptake rates[61].

3.6. Colloidal stability

When nanoparticles are introduced into complex biological media containing electrolytes, proteins, lipids, and other components, they are subjected to a range of forces that dictate their behavior in these environments. The aggregation of nanoparticles can significantly influence their pharmacokinetics, toxicity, and biodistribution[89]. The cytotoxicity of nanoscopic calcium phosphate may arise from particle agglomeration, leading to sedimentation onto cellular layers and resulting in extremely high local particle concentrations, which can induce cell death[90]. Zhang et al.[51] synthesized monodisperse (s-BTLC) and aggregated (a-BTLC) LDH nanovaccines, demonstrating that, compared to a-BTLC, a greater quantity of s-BTLC nanovaccine migrated from the injection site to LN after 24 h, thereby promoting a stronger antigen-specific T cell response and more effectively inhibiting melanoma growth than the aggregated a-BTLC nanovaccin. This underscores the impact of colloidal stability on the targeting efficacy of nanovaccines. The presence of stabilizing coatings, such as polyethylene glycol (PEG), can enhance the stability of nanoparticles and reduce protein adsorption on their surfaces. Furthermore, the strength or stability of interactions between the stabilizing coating and nanoparticles is crucial for maintaining colloidal stability, as the composition of the solution can displace stabilizing molecules and trigger aggregation. Additionally, the intermolecular interactions of the coating, the forces between particles, and the components of physiological solutions must also be considered[89]. Numerous investigations have been conducted to enhance the stability of nanovaccines. Manganese oxide (MnOx) synthesized alone precipitates rapidly and exhibits poor stability, whereas the nanovaccine THM@Mn, formed by synthesizing MnOx within recombinant hepatitis B core antigen virus-like particles (HBc VLPs), demonstrates good dispersibility and enhanced colloidal stability[52]. Unlike traditional emulsions that utilize surfactants, the pickering emulsion-based nanovaccine OVA/HSA-MnNE employs antigens and adjuvants as stabilizers, effectively reducing surface tension and enhancing emulsion stability. Furthermore, the lyophilized OVA/HSA-MnNE retains excellent stability upon reconstitution, demonstrating reduced dependence on cold chain logistics during storage and transport. This nanovaccine significantly enhances DC uptake and maturation, promotes antigen accumulation in LNs, and activates the cGAS−STING pathway, thereby eliciting a robust cellular immune response. The innovative approach of utilizing antigens and adjuvants as intrinsic stabilizers in the construction of pickering emulsion-based nanovaccines offers new insights for the design of future subunit vaccines[91]. Additionally, the core−shell structured lipopolyplex (LPP) encapsulating the full-length SARS-CoV-2 Spike protein, known as the nanovaccine SW0123, maintains consistent particle size, mRNA concentration, and encapsulation efficiency after six weeks of storage at 4 °C, exhibiting commendable colloidal stability[92].

3.7. Chirality

The chirality of nanovaccines is a crucial factor influencing their fate within the body, as variations in the chirality of nanomaterials can lead to differing affinities or repulsions with chiral biomolecules, potentially resulting in specific immune responses[93]. Due to the complexity of the cellular microenvironment, traditional nanomaterials still face limitations in practical applications, such as long-term toxicity and biochemical stability. The introduction of chirality may provide new insights into these issues. Chiral nanomaterials exhibit improved biocompatibility and can enhance cellular uptake, thereby prolonging *in vivo* stability in the bloodstream[94]. Chiral nanoparticles demonstrate potential in immunotherapy. For instance, left-handed chiral gold nanoparticles can more effectively activate APCs compared to right-handed and achiral gold nanoparticles. Additionally, chiral nanoparticles can serve as vaccine adjuvants, enhancing the immunogenicity of vaccines[95]. By modifying the chirality of nanovaccines, their immunogenicity can be improved[96]. Huang et al.[53] synthesized three different chiral forms of polylactic acid (PLA)-racemic (DL, 50% L-lactic acid+50% D-lactic acid), levorotatory (L, 100% L-lactic acid), and dextrorotatory (D, 100% D-lactic acid)-by adjusting the ratio of the two optically active monomers. Their research indicates that the dextrorotatory PLA carrier nanovaccine can induce a higher proportion of IgG and has advantages in activating Th1-type immune responses. Liu et al.[54] utilized chiral carbon dots (D-CDs and L-CDs) as carriers and adjuvants in conjunction with ovalbumin (OVA) to construct chiral nanovaccines D-OVA and L-OVA. D-OVA demonstrated superior immunoactivation and antitumor efficacy compared to L-OVA in both *in vitro* and *in vivo* experiments. This study represents the first investigation into the development of chiral CD nanovaccines for cancer immunotherapy, offering a novel strategy for the advancement of multifunctional nanovaccines. Additionally, two types of chiral peptides, poly(L-phenylalanine)-block-poly(L-lysine) (PL-K) and poly(L-phenylalanine)-block-poly(D-lysine) (PD-K), were employed as adjuvants to synthesize nanovaccines PL-K-OVA and PD-K-OVA. PD-K-OVA exhibited enhanced efficacy in activating DCs, promoting antigen cross-presentation, and inducing adaptive immune responses, along with significant anticancer effects in the B16-OVA melanoma model[55]. However, the majority of chiral nanomaterials remain at the animal testing or theoretical stage, highlighting the need for more advanced synthesis and design of chiral nanomaterials, as well as a deeper understanding of chiral substance-biointerface interactions to

facilitate the application of chiral nanomaterials in the vaccine domain[94].

3.8. The route of administration and the target population

The route of administration significantly influences the interaction of nanovaccines with bodily fluids and the immune system, affecting pharmacokinetics and ultimately determining the accumulation of immune cells and organs targeted by the nanovaccines[97]. The biodistribution of intravenously administered nanoparticles is size-dependent; studies have constructed mesoporous silica nanoparticles of varying sizes (25, 50, and 150 nm) and found that smaller nanoparticles rapidly enter the bloodstream post-injection, while larger nanoparticles tend to accumulate in the liver and spleen. Following intraperitoneal injection, regardless of size, nanoparticles preferentially accumulate in abdominal and thoracic LNs, with their distribution over time being size-dependent. Larger nanoparticles predominantly localize in axillary LNs and the spleen, whereas smaller (25 nm) nanoparticles are primarily found in the inguinal LNs[98]. Intramuscular injection generally favours more direct lymphatic delivery, and by intramuscular, intradermal or subcutaneous injection, nanoparticles are expected to be drained directly from the injection site into the lymphatic system or to be taken up by APCs and transported to LNs[99]. Compared to intravenous administration, both intramuscular and subcutaneous injections result in prolonged antigen presence at the injection site, allowing for more effective drainage into the lymphatic system. During subcutaneous administration, antigens accumulate in LNs near the injection site, while intramuscular injections lead to accumulation in distal LNs[97]. In studies involving lipid nanoparticle-encapsulated mRNA nanovaccines, it was observed that the intradermal route resulted in the longest half-life of antigen expression, whereas the intravenous route exhibited the shortest[100]. The immune responses elicited by nanovaccines vary across different administration routes. Among all routes, subcutaneous, intradermal, and intraperitoneal injections are comparatively more likely to induce Th1 responses[101]. Subcutaneous administration of nanoparticles significantly enhances the levels of pro-inflammatory cytokines in draining lymph nodes, with no notable impact on serum cytokine levels. In contrast, intravenous injection results in an elevation of pro-inflammatory cytokine levels in serum, likely due to the activation of splenic DCs following the intravenous delivery of nanoparticles[102]. Furthermore, the route of nanoparticle administration is associated with the differentiation of immune cells, as studies indicate a correlation between intravenous injection and the differentiation of specific $CD8^+$ T cells into TCF1 stem cell-like cells[103]. A nanovaccine co-delivering an antigen and an invariant natural killer T (iNKT) cell agonist was studied to assess the impact of different administration routes on immune responses. The findings indicate that following subcutaneous or intradermal injection, the nanovaccine predominantly accumulates in LN rich in iNKT17 cells. In contrast, after intravenous injection, the nanovaccine primarily targets the liver and spleen, leading to the highest serum interferon-γ levels, T-cell cytotoxicity, and Th-1 type antibody responses, demonstrating a more pronounced advantage in anti-tumor therapy[56]. Additionally, the administration of Hepatitis B surface antigen (HBsAg)-loaded nanovaccines *via* various routes, including oral, subcutaneous, intranasal, and intramuscular injections, was evaluated for antibody titers and cytokine levels. The results revealed that a single intramuscular injection of the nanovaccine elicited a stronger humoral and cellular immune response[57]. Therefore, it is essential to tailor the delivery route of nanovaccines based on specific requirements, for example, nanovaccines designed for tumor treatment are primarily administered *via* intravenous injection, while those aimed at preventing infections are mainly delivered through intramuscular injections or subcutaneous injections[104]. Additionally, the development of vaccines must take into account the target population, such as the elderly who experience immunosenescence[105], children with compromised immune systems[106] and other patients with immune deficiencies. It is crucial to avoid compromising therapeutic efficacy during the vaccine development process.

Therefore, the design and development of nanovaccines must adhere to relevant principles, aligning with the desired preventive or therapeutic outcomes. However, not all factors need to be considered when designing a nanovaccine. For subunit and peptide nanovaccines, the primary considerations are particle size and distribution, as these are the main factors affecting immunogenicity. In contrast, for mRNA or DNA vaccines, the initial focus should be on the vector to ensure effective expression of the nucleic acids at the target site[104].

4. The application of nanovaccines

In contrast to conventional vaccines, nanovaccines utilize nanocarriers for the targeted delivery of vaccines, enabling the precise targeting of various antigens, adjuvants, or immunomodulators to specific subsets of immune cells. Nanovaccines exhibit remarkable modifiability, offering a range of design strategies to enhance the protective efficacy, immunogenicity, and cross-presentation of antigens, while also achieving targeted delivery and controlled release[107]. This approach offers the flexibility to modulate immune responses and is poised to become a significant intervention strategy for cancer, infectious diseases, and autoimmune disorders[108]. Numerous studies have documented the application of nanovaccines in disease prevention and treatment, and this section presents a concise overview of the disease categories where nanovaccines are utilized (Fig. 3).

4.1. Cancer

In order to ensure the effectiveness of vaccines, tumor vaccines need to highly specific targeting of tumor cells, optimizing the material that delivers the antigen can enhance the effectiveness of the vaccine[109], for example, a DNA nanodevice-based vaccine designed by Liu et al.[110] integrates a low pH-responsive DNA locking strands outside the nanostructure, which allows the vaccine to be opened in the lysosomes of APCs, promoting antigen presentation and T cell immune responses. Such nanovaccines can reduce the side effects of immunotherapy due to the targeted delivery of antigens and reduce the distribution in non-tumor tissues. At the same time, the design is highly flexible and can be adjusted according to different tumors, which can be widely used in the treatment of a variety of cancers. Employing diverse nanomaterials to encapsulate tumor associated antigens or tumor specific antigens (TSAs), cancer nanovaccines stimulate the immune system to generate antibodies, activate various immune cells, and provoke an anti-tumor immune response, which have the potential to mitigate the adverse effects and risks associated with traditional chemotherapy and postoperative treatments[15]. Currently, there is a wealth of preclinical research focused on the application of

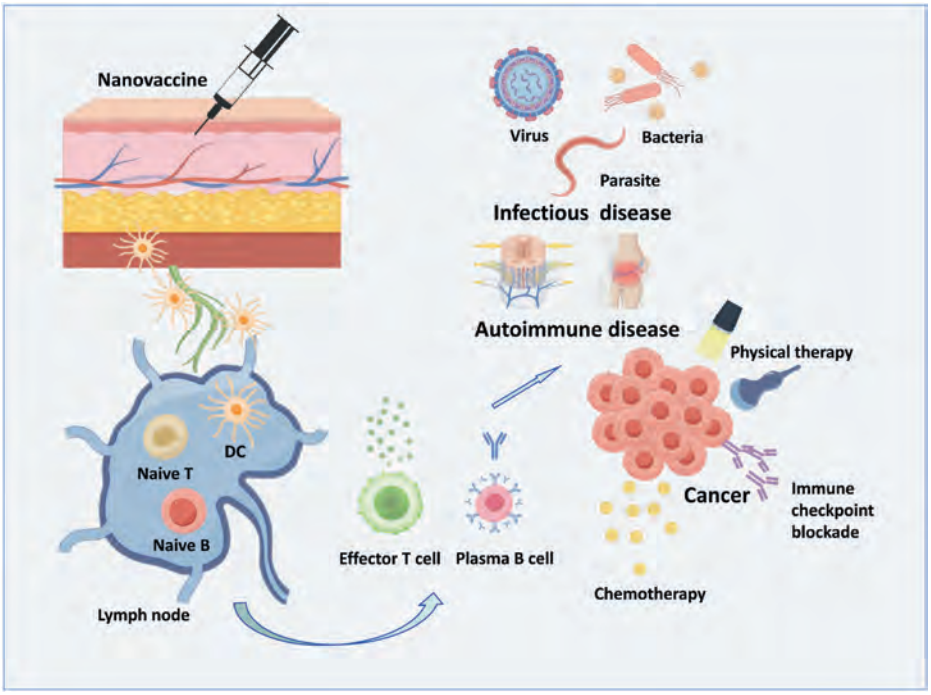

Figure 3 Recent advances in applications and combination therapies of the nanovaccines. DC, dendritic cell. Created by Figdraw.com.

nanovaccines in cancers with high global prevalence, including lung cancer[111], breast cancer[112-117], colorectal cancer[118-121], hepatocellular cancer[122-124], and gastric cancer[125,126], as shown in Table 2[111-113,115–122,124-126]. And lipid-based nanovaccines are the most extensively researched category within the domain of cancer nanovaccines, as shown in Table 3. Recently, there has been a growing interest in personalized cancer treatment, accompanied by significant advancements in nanovaccine research. For instance, the nanovaccine ASPIRE developed by Liu et al.[127] delivers antigenic signals tailored to tumor antigens, facilitating a broad spectrum of personalized immunotherapies for various cancer types by promoting antigenic self-presentation and reversing immunosuppression (Fig. 4A). Wang et al.[128] developed a viral-mimetic nanovaccine strategy, cGAMP@vEVs, derived from engineered tumor cell-derived extracellular vesicles through viral infection. This approach exhibits dual targeting capabilities for LNs and tumors, allowing for the simultaneous incorporation of personalized and broad antigen libraries, as well as various immunoadjuvants. The preparation of personalized nanovaccines can effectively utilize abundant tumor cells obtained from resected tumor tissues or biopsy samples, thereby efficiently eliciting anti-tumor immunity. Notably, the promise of personalized immunotherapy lies in its capacity to elicit tumor-specific immune responses tailored to individual patients without necessitating the identification of each tumor antigen. The nanovaccine DEX-P&A2&N proposed by Zuo et al.[129] specifically enhances the recruitment and activation of DC in mice with *in situ* hepatocellular carcinoma, independent of tumor antigen recognition. This advancement improves tumor antigen cross-presentation and elicits tumor-specific T cell responses, marking the first proof-of-principle study to demonstrate that personalized immunotherapy can be achieved without the need for tumor antigen recognition, suggesting that by modifying the tumor-targeting component, it can be adapted for use in other tumor types (Fig. 4B). Furthermore, personalized immunotherapy for cancer relies on the initial presentation of tumor antigens to DC and macrophages. However, the presence of anti-phagocytic signals on the surface of tumor-derived extracellular vesicles rich in tumor antigen molecules, allows them to evade phagocytosis by DCs and macrophages. Ding et al.[130] demonstrated that iron oxide hydroxide nanocomposites can effectively mask these anti-phagocytic signals without compromising the immune response, thereby promoting the activation and maturation of DCs and the reprogramming of macrophages, which significantly inhibits tumor growth and metastasis. This iron oxide hydroxide nanocomposite can be broadly applied to patient-specific tumor-derived extracellular vesicles, overcoming the prevalent phagocytic barriers and enhancing the anti-tumor immune response. Current tumor nanovaccines primarily elicit immune responses specifically targeting antigens. The cancer cell membranes extracted from apoptotic cancer cells carry a complete membrane protein matrix, which maximizes the immunomodulatory properties of the cancer cell membrane. The surface of apoptotic cancer cells is adorned with calreticulin, facilitating recognition and phagocytosis by immune cells[131] (Fig. 4C). Induced pluripotent stem cells membranes share a significant array of tumor-associated proteins with tumor cell membranes. Utilizing induced pluripotent stem cells membranes as a repository for tumor antigens transcends the limitations of traditional approaches that rely on specific tumor antigens, circumventing antigen-specific tolerance and activating anti-tumor memory T cell and B cell responses, representing a promising strategy for the development of universal cancer vaccines[132] (Fig. 4D).

Currently, physical therapy, immune checkpoint blockade therapy, and chemotherapy are increasingly utilized in cancer treatment[133,134]. The integration of these therapies with nanovaccines can significantly enhance the anti-tumor immunity and offer innovative strategies for cancer management[135]. Currently,

Table 2 Preclinical studies of nanovaccines for cancers.

Application	Nanovaccine name	Antigen	Adjuvant	Immunomodulatory action	Effect	Ref.
Lung cancer	CpG-NP-Tag	Tumor antigen	CpG ODN	Co-deliver tumor antigens and CpG, encapsulated in PLGA nanoparticles, inducing local mucosal effector and resident memory T cells in the lungs, enhancing IFN-γ and granzyme B production by lung-derived $CD8^+$ T cells, promoting lung resident memory T cells	Significantly reduce tumor volumes and lung tumor colonization	111
Breast cancer	MUC1 mRNA nanovaccine	MUC1 mRNA	−	Utilizes LCP nanoparticles as a carrier to target mannose receptors on DCs, delivering MUC1-encoding mRNA to DCs in LNs to activate and expand tumor-specific T cells	Significantly reduce tumor volumes	112
Breast cancer	$CaCO_3$@TCL/CpG	TCL	CpG	Employ pH-sensitive $CaCO_3$ nanoparticles loaded with TCL and the immune adjuvant CpG, neutralizing the acidic TME by consuming lactate, increasing the M1/M2 macrophage ratio, activating DCs, and recruiting cytotoxic T cells to enhance antitumor immune responses	Significantly inhibit tumor growth with noticeable tumor volume reduction	113
Breast cancer	NIGel-Vax	4T1 tumor-extracted proteins	PEI-4BImi-Man	Combine protein antigens with PEI-4BImi-Man adjuvant to form nanoparticles, which are then encapsulated in a hydrogel made of ODEX and 4-arm PEG-ONH_2, achieving spatiotemporal control of antigen release to enhance DC activation and antigen presentation in LNs	Achieve a 92% tumor suppression rate and 33% cure rate in breast cancer postoperative models	115
Breast cancer	Neo-NVs	Neoantigen	R848	Use SpyCatcher003-modified norovirus S protein nanoparticles as a carrier, combined with tumor neoantigens *via* SpyTag003 technology, efficiently targeting LNs, activating DCs, inducing antigen-specific T cell immune responses, and reshaping the TME	Significantly reduce tumor volumes; inhibit postoperative tumor recurrence and lung metastasis when combined with anti-PD-1 mAb	116
Breast cancer	Au/CuNDs-R848	Tumor antigen	R848	Combine PTT and photothermal-enhanced CDT using Au/Cu nanodots; the generated photothermal effect and Fenton reaction induce ICD, releasing tumor-associated antigens and DAMPs to activate DCs, promote T cell infiltration, and suppress immunosuppressive MDSCs, transforming "cold tumors" into "hot tumors"	Significantly reduce both primary and distant tumor volumes, with enhanced survival rates	117
Colorectal cancer	banNVs	Neoantigen	R848,CpG	Co-deliver the peptide neoantigen Adpgk with two adjuvants (R848 and CpG) *via* nanotechnology, significantly enhancing the immunogenicity of the neoantigen, activating TLR signaling pathways, enhancing DC activation and antigen presentation	Significantly inhibit tumor growth, with a survival rate of 40% after 39 days; achieve a 70% complete regression rate in tumors when combined with anti-PD-1 mAb	118
Colorectal cancer	PCNPs	Neoantigen	CpG ODN	Self-assembly of peptide neoantigen and CpG ODN *via* electrostatic interactions to form nanocomplexes, enhancing the maturation of DCs, improving antigen cross-presentation efficiency, and activating cytotoxic T	Significantly inhibit tumor volumes below 200 mm^3 in the preventive model and below 500 mm^3 in the therapeutic model	119

Table 2 (*continued*)

Application	Nanovaccine name	Antigen	Adjuvant	Immunomodulatory action	Effect	Ref.
				cells to significantly prolong the survival of tumor-bearing mice		
Colorectal cancer	NP-TCL@APS	TCL	APS	Co-loading of TCL and APS into PLGA nanoparticles to promote DC maturation, enhance $CD8^+$ T cell responses, inhibit tumor growth, and exhibit good biocompatibility and immune activation	Achieve the tumor inhibition rate of 92.3% and survival rate of 62.5% at 62 days	120
Colon cancer	TCLN	TCL	EH	Combining anti-angiogenic treatment with TCL-loaded polydopamine nanoparticle vaccine to improve the tumor microenvironment, enhance immune responses, and significantly inhibit tumor growth, thereby prolonging the survival of mice	Significantly reduce tumor volumes; extend survival time with some mice living over 60 days	121
Hepatocellular cancer	LDHs-cGAMP	Tumor antigen	cGAMP	Utilize LDHs to load cGAMP, activating the cGAS-STING pathway to enhance type I interferon responses, promote DC maturation, and increase the activity of tumor-infiltrating T cells to significantly inhibit the progression of liver cancer	Significantly reduce tumor volumes; extend survival time with a median survival increase from 17 to 31 days, and some mice surviving over 45 days	122
Hepatocellular cancer	MF@SOR	Tumor antigen	MIL-100 (Fe)	Induce pyroptosis and activate the cGAS−STING pathway to release DAMPs and pro-inflammatory cytokines, promoting DC maturation and T cell activation, effectively inhibiting the recurrence and metastasis of hepatocellular carcinoma	Significantly reduce tumor volumes; extend survival time with 4 mice surviving over 60 days; decrease tumor recurrence and metastasis rates	124
Gastric cancer	PNVAC	Neoantigen	Montanide ISA 51	Co-encapsulation of personalized neoantigens and adjuvants in nanoparticles to improve antigen presentation efficiency, enhance $CD4^+$ and $CD8^+$ T cell-mediated immune responses, and prolong the disease-free survival of patients with gastric/gastroesophageal junction cancer	Significantly reduce tumor volumes with some mice achieving complete tumor regression; extend survival time with about half of the mice surviving over 60 days	125
Gastric cancer	MG7	MG7 mimotope peptide	CpG ODN	Coencapsulate the gastric cancer-specific antigen MG7 mimotope peptide and CpG ODN in a nanoemulsion, significantly enhancing the immune response; the small size, high stability, and low cytotoxicity of the nanoemulsion enable efficient uptake by antigen-presenting cells, inducing stronger cellular and humoral immune responses	Achieve the tumor inhibition rate of 82.5%	126

CpG, cytosine−phosphate−guanine; ODN, oligodeoxynucleotide; PLGA, poly(lactic-*co*-glycolic acid); IFN, interferon; MUC, mucin; −, not applicable; LCP, lipid/calcium/phosphate; DC, dendritic cell; LN, lymph nodel; TCL, tumor cell lysate; $CaCO_3$, calcium carbonate; TME, tumor microenvironment; PEI-4BImi-Man, polyethyleniminebenzoimidazole-4-carboxylic acid-2-isothiocyanatoethyl-α-D-mannopyranoside; ODEX, oxidized dextran; PEG-ONH, polyethylene glycol-*O*-nitrophenyl hydrazine; anti-PD-1 mAb, anti-programmed death-1 monoclonal antibody; PTT, photothermal therapy; CDT, chemodynamic therapy; ICD, immunogenic cell death; DAMPs, damage-associated molecular patterns; MDSCs, myeloid-derived suppressor cells; APS, astragalus polysaccharides; EH, endostar loaded in alginate hydrogel; cGAMP, cyclic GMP−AMP; LDHs, layered double hydroxides; cGAS, cyclic GMP−AMP synthase; STING, stimulator of interferon genes.

Table 3 Clinical studies on lipid-based nanovaccines for cancers.

Application	Nanovaccine name	Phase	Current status	Trial number	Objective
pHGG and GBM	RNA-LP	I/II	Recruiting	NCT04573140	Demonstrate the manufacturing feasibility and safety, and to determine the MTD of RNA-LP vaccines in adult patients with newly diagnosed GBM and in pediatric patients with newly diagnosed HGG
Glioblastoma	RNA-LP	I	Recruiting	NCT06389591	Demonstrate the manufacturing feasibility and safety, and to determine the MTD of RNA-LP vaccines in adult patients with recurrent glioblastoma
Intracranial malignancies	RNA-LP	I/II	Active, not recruiting	NCT05660408	Investigate the manufacturing feasibility, safety and immunologic activity of RNA-LP vaccine in patients with recurrent pulmonary or unresectable osteosarcoma and recurrent pHGG
NSCLC	BLP25	II	Completed	NCT00828009	Determine the safety of BLP25 liposome vaccine and bevacizumab after definitive chemoradiotherapy and consolidation chemotherapy in patients with newly diagnosed, unresectable stage IIIA or IIIB nonsquamous cell NSCLC
HPV-OPSCC	PDS0101	I/II	Recruiting	NCT05232851	Study the effectiveness of PDS0101 alone or in combination with pembrolizumab in patients with HPV-OPSCC that has spread to nearby tissue or lymph nodes
Cervical cancer	PDS0101	II	Active, not recruiting	NCT04580771	Evaluate the safety and toxicity profile of delivering PDS0101 with standard-of-care chemoradiation in patients with locally advanced cervical cancer
Melanoma	Tyrosinase/gp100 peptide vaccine	II	Completed	NCT00003274	Study the effectiveness of tyrosinase/gp100 peptide vaccine in treating patients who have stage II melanoma that can be removed by surgery
Solid tumors	ONT-10	I	Completed	NCT01556789	Evaluate the safety and immunogenicity of repeat dose vaccination with ONT-10 in patients with previously treated stage III or IV solid tumors
Ovarian, breast cancer	ONT-10	I	Completed	NCT02270372	Investigate the efficacy of ONT-10 combined with two different doses of varlilumab in the treatment of ovarian or breast cancer
NSCLC	L-BLP25	I/II	Completed	NCT00960115	Evaluate the safety of L-BLP25 and its effects on survival time in patients with stage III unresectable NSCLC
Melanoma	Lipovaxin-MM	I	Completed	NCT01052142	Determine whether Lipovaxin-MM is safe and effective in improving the body's ability to destroy cancer cells in patients with metastatic melanoma
Breast, ovarian and prostate cancer	DPX-0907	I	Completed	NCT01095848	Determine the safety and immunogenicity profile of two different doses of the vaccine DPX-0907 to treat breast, ovarian and prostate cancer
Follicular lymphoma	Autologous tumor cell vaccine	I	Completed	NCT00020462	Study the effectiveness of vaccine therapy plus interleukin-2 in treating patients who have stage III, stage IV, or recurrent follicular lymphoma

pHGG, pediatric high-grade gliomas; GBM, glioblastoma; MTD, maximum tolerated dose; HPV-OPSCC, human papillomavirus-associated oropharynx cancer; NSCLC, non-small cell lung cancer.

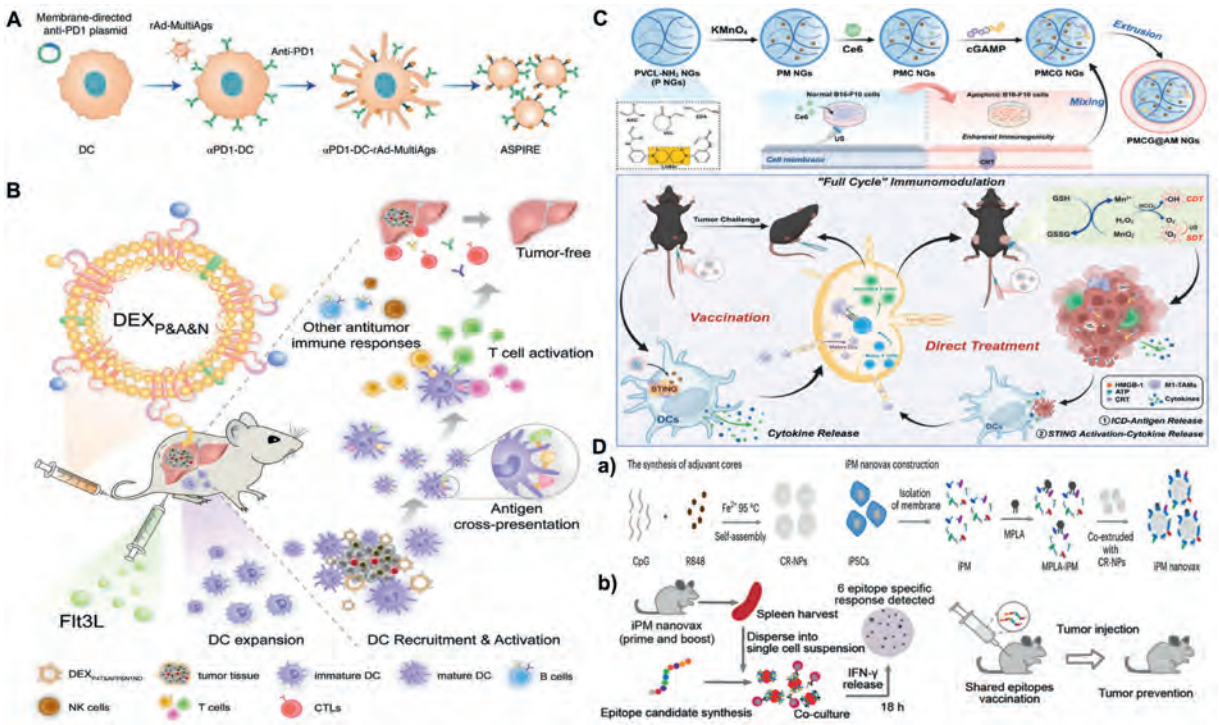

Figure 4 Researches on nanovaccines for personalized cancer therapy. (A) Schematic illustration of the process for preparing ASPIRE vaccine. Reprinted with the permission from Ref. 127. Copyright © 2022 Springer Nature. (B) The mechanism underlying the antitumor immunogenicity of universal DEX vaccines. Reprinted with the permission from Ref. 129. Copyright © 2022 Springer Nature. (C) Synthesis of PMCG@AM NGs for tumor prophylaxis and direct tumor treatment *via* a full-cycle immunomodulation. Reprinted with the permission from Ref. 131. Copyright © 2025 Elsevier. (D) a) Schematic illustration of the integrated nanovaccine technology comprising iPM nanovaccine. b) The shared epitope-specific responses evoked by iPM nanovaccine. Reprinted with the permission from Ref. 132. Copyright © 2025 Springer Nature.

the encapsulation of immune checkpoint inhibitors within nanocarriers to alleviate T cell suppression, thereby inducing tumor cell death, as well as the delivery of chemotherapeutic agents that directly induce tumor cell apoptosis, has entered clinical trials or practical application[15].

Incorporating photosensitizers or acoustic sensitizers into nanovaccines can enhance their immunotherapeutic efficacy by enabling photodynamic therapy, photothermal therapy, or acoustic therapy in response to specific external stimuli. Wang et al.[136] constructed a nanovaccine, Dex-HDL/ALA-Fe_3O_4, loaded with the photosensitizer Fe_3O_4 nanoenzymes, which can ROS species at the tumor site upon laser irradiation, inducing immunogenic cell death (ICD) and facilitating the release of various tumor antigens. Abundant TAA promotes DC maturation and $CD8^+$ T cell activation, which finally induces robust anti-tumor immunity and inhibits tumor metastasis and recurrence. Similarly, the nanovaccine BCNCCM developed by Huang et al.[114] was loaded with black phosphorus-Au, which produces a photothermal effect under laser exposure, triggering ICD, with the released TSAs working synergistically with the adjuvant to exhibit vaccine-like properties. It can not only inhibit the growth of primary and metastatic tumors, but also prevent tumor recurrence by inducing systemic immune memory, providing a new strategy for photo-immunotherapy of metastatic tumors. Additionally, Wang et al.[137] prepared a nanovaccine, BMT@LA, loaded with the acoustic sensitizer black mesoporous titanium dioxide (BMT). Ultrasound stimulation prompted the generation of ROS by BMT at the tumor site, inducing intracellular oxidative stress and DNA double-strand breaks, ultimately leading to tumor cell apoptosis. This ultrasound-induced sonodynamic/gas/immune combination therapy model offers more possibilities for future clinical cancer treatment.

Immune checkpoint inhibitors are crucial in tumor immunotherapy[138], and their combination with nanovaccines can enhance immune responses, inhibit tumor growth, recurrence, and metastasis, while establishing robust anti-tumor immune memory. The synergistic effect of combining nanovaccines with immune checkpoint inhibitors has been shown to surpass the efficacy of single-agent treatments, with relevant preclinical trials already conducted. Zheng et al.[116] combined the nanovaccine Neo-NVs with anti-PD-1 monoclonal antibodies, demonstrating that the combination treatment group promoted cytotoxic T-lymphocyte infiltration within tumors, reversed immunosuppression in tumor-draining LN, and generated a greater number of memory $CD8^+$ T cells compared to the control group. This resulted in a reduction in both tumor mass and the number of lung nodules in models of postoperative recurrence of *in situ* breast tumors and metastasis, indicating a stronger capacity to inhibit tumor recurrence and metastasis. Furthermore, the nanovaccine can enhance tumor cell sensitivity to immune checkpoint inhibitors. For instance, Li et al.[139] developed a nanosystem, mEHGZ, which functions as a nanovaccine, leading to improved ICD effects and activation of the immune microenvironment, thereby increasing the responsiveness of tumor cells to anti-PD-L1 antibodies. As demonstrated in the research conducted by Kim et al.[140], the sequence and timing of administering nanovaccines alongside

Table 4 Preclinical studies of nanovaccines for infectious diseases.

Application	Nanovaccine name	Antigen	Adjuvant	Immunomodulatory Action	Effects	Ref.
Influenza	MHF	Influenza epitope	−	Induce cross-reactive neutralizing antibodies, mediate ADCC, provide long-lasting protection through cellular immunity	Reduce H3N2 influenza virus load by approximately 90% and H1N1 influenza virus load by about 50%	144
Tuberculosis	TMC-AdHP-MST@TLR1/2	MST	TLR-1/2	Facilitate pH-responsive release of antigens and adjuvants, enhance cellular and humoral immunity, activate memory T cells robustly	Reduce the pulmonary load of M. tuberculosis by about 75%	145
Tetanus	TTFC-mi3	TTFC antigen	−	Augment DC uptake and activation, elicit rapid and potent protective immunity without aluminum adjuvants	Improve mouse survival rate by 90%	146
Helicobacter pylori	Alginate/pCI-neo-UreH	*H. pylori* antigen	−	Activate Th1 and Th17 cell immune responses, enhance mucosal and systemic antigen-specific antibody responses	Reduce the *H. pylori* load in gastric tissues by about 20 times	147
Alveolar echinococcosis	Nanovesicle vaccine	*E. multiloculars* antigen	−	Activate DCs, induce specific T/B cell interactions	Reduce the number and size of hydatid cysts by 80% and 70% respectively	148
Toxoplasmosis	SAPNs	*Toxoplasma* antigen	Flagellin, GLA-SE	Activate $CD8^+$ T cells to produce IFN-γ, enhance TLR4 and TLR5 activation	Reduce the number of *T. gondii* cysts in brain tissues by about 70%	149

−, not applicable; ADCC, antibody-dependent cell-mediated cytotoxicity; MST, mycobacterium smegmatis; TLR, toll-like receptor; TTFC, tetanus toxin fragment C; DC, dendritic cell; Th, T helper; GLA-SE, TLR4 ligand-emulsion; IFN, interferon.

immune checkpoint inhibitors significantly influence the efficacy of immunotherapy. Administration of a PD-1 receptor inhibitor one week following the final nanovaccine dose resulted in a heightened frequency of interferon and granzyme B-producing $CD8^+$ tumor-infiltrating lymphocytes, leading to a more effective suppression of tumor growth compared to simultaneous treatment protocols. Conversely, commencing PD-1 receptor inhibitor therapy two weeks after the last nanovaccine dose resulted in compromised tumor-growth inhibition. This clearly indicates that both the sequence and timing of nanovaccine and immune checkpoint inhibitor administration are critical factors affecting therapeutic efficacy, which is pertinent for future combination therapy investigations. The combination of cancer nanovaccines and immune checkpoint inhibitors has been the subject of relevant clinical studies. In a recent clinical trial (NCT05232851), the efficacy of the combination therapy involving the nanovaccine PDS0101 and pembrolizumab is being evaluated to determine its impact on reducing tumors in patients with HPV-associated oropharyngeal cancer that has metastasized to adjacent tissues or lymph nodes. It is hypothesized that the administration of PDS0101 in conjunction with pembrolizumab may lead to the preoperative elimination of a greater number of tumor cells and potentially decrease the volume of normal tissue requiring resection. Another clinical study (NCT02270372) investigated the efficacy and safety of the nanovaccine ONT-10 in combination with two different doses of nivolumab for the treatment of ovarian or breast cancer. Additionally, a clinical trial (NCT00828009) assessed the safety of the combination of the nanovaccine BLP25 and bevacizumab in patients with newly diagnosed, unresectable stage IIIA or IIIB non-squamous non-small cell lung cancer following definitive chemoradiotherapy and consolidation chemotherapy.

The integration of chemotherapy with nanovaccines can amplify the synergistic anti-tumor effects through various mechanisms. The ECM formed by tumor-associated fibroblasts hinders the penetration of chemical and biological therapeutic agents within tumors, thereby limiting their therapeutic efficacy. The nanovaccine FAP(PEP)-SLNP developed by Shin et al.[141] enhances the accumulation of chemotherapeutic drugs by reducing ECM production in the TME and improving drug penetration within the tumor, which is expected to become a suitable platform for various combination therapies. One reason for the poor efficacy of immunotherapy in ovarian cancer is the lack of known vaccine-effective antigens. Chemotherapy, one of the most commonly used treatments for ovarian cancer, can produce chemotherapy-associated antigens during the treatment. Zhou et al.[142] demonstrated that the nanovaccine NP-TP1@M-M could utilize chemotherapy-induced apoptosis as an adjuvant, enriching TSAs post-chemotherapy, thereby promoting DC maturation and activating the immune response, which provides an effective treatment strategy for ovarian cancer. Another approach to combination therapy involves loading chemotherapeutic agents onto

Table 5 Clinical studies on nanovaccines for respiratory viruses.

Application	Nanoaccine name	Nanoaccine platform	Phase	Current status	Trial number	Objective
RSV	LNP CL-0059/CL-0137	LNP	I/II	Active, not recruiting	NCT05639894	Assess the safety and immunogenicity profiles across the dose-level groups with two different LNPs and different doses of RSV vaccine encapsulated in one of the LNPs
hMPV/RSV	hMPV/RSV mRNA Vaccine	LNP	I/II	Recruiting	NCT06686654	Evaluate the safety and immunogenicity of hMPV/RSV mRNA vaccine candidate encapsulated in a LNP based formulation for the prevention of LRTD caused by hMPV/RSV among adults aged 60 years and older
RSV	RSV-F	PNP	II	Completed	NCT01704365	Evaluate the immunogenicty and safety of an RSV-F protein nanoparticle vaccine, without aluminum, in healthy women of child-bearing potential
Influenza	UFluA	Ferritin-NP	I	Completed	NCT05155319	Evaluate the safety, tolerability and immunogenicity of UFluA vaccine candidate at two dose levels and two schedules in healthy adult (18−45-year-old, inclusive) male and non-pregnant female subjects
Influenza	NanoFlu	Recombinant trivalent nanoparticle	I/II	Completed	NCT03293498	Assess the safety and tolerability of Tri-NIV with Matrix M1™ adjuvant in healthy older adults ≥60 years of age
Influenza	FluMos-v1	Tetravalent nanoparticle	I	Completed	NCT04896086	Evaluate the safety and tolerability of the investigational vaccine alone or with adjuvant in healthy adults
Influenza	H2HA-Ferritin	Ferritin-NP	I	Completed	NCT03186781	Evaluate the safety and tolerability of H2HA-Ferritin either alone or in prime-boost regimens
SARS-CoV-2	BNT162b2	LNP	II/III	Completed	NCT04368728	Evaluate the safety, tolerability and efficacy of BNT162b2 in healthy individuals
SARS-CoV-2	mRNA-1273	LNP	III	Completed	NCT04470427	Evaluate the efficacy, safety, and immunogenicity of mRNA-1273 to prevent SARS-CoV-2 for up to 2 years after the second dose of mRNA-1273
SARS-CoV-2	PepGNP-Covid19	GNP	I	Completed	NCT05113862	Evaluate the safety and reactogenicity of two intradermal injections of two different doses of the PepGNP-Covid19 administered to healthy volunteers
SARS-CoV-2	ChulaCov19	LNP	I/II	Completed	NCT04566276	Evaluate the safety, tolerability, and reactogenicity of escalating doses of the ChulaCov19 vaccine in healthy adults aged 18−55 years and in elderly adults aged 56−75 years
SARS-CoV-2	PepGNP-COVID19	GNP	I/II	Not yet recruiting	NCT05633446	Investigate the safety and immunogenicity of one dose *vs* two doses of a T-cell priming next-generation vaccine against Coronavirus disease
SARS-CoV-2	SARS-CoV-2 rS	SARS-CoV-2 rS NP	I/II	Completed	NCT04368988	Evaluate the safety and immunogenicity of SARS-CoV-2 rS nanoparticle vaccine with or without Matrix-M adjuvant
SARS-CoV-2	GBP510	PNP	I/II	Completed	NCT04742738	Assess the safety, reactogenicity and immunogenicity of GBP510 adjuvanted with Alum in healthy younger and older adults
SARS-CoV-2	mRNA-1273	LNP	I	Completed	NCT04283461	Evaluate the safety and reactogenicity of a 2-dose vaccination schedule of mRNA-1273, given 28 days apart, across 5 dosages in healthy adults
SARS-CoV-2	Moderna COVID-19 Vaccine	LNP	III	Completed	NCT04811664	Evaluate the efficacy of the Moderna COVID-19 vaccine against SARS-CoV-2 infection, as well as its effect on peak nasal viral load, in adults aged 18−29

RSV, respiratory syncytial virus; LNP, lipid nanoparticle; hMPV, human metapneumovirus; LRTD, lower respiratory tract disease; PNP, protein nanoparticle; NP, nanoparticle; GNP, gold nanoparticle.

Table 6 Preclinical studies of nanovaccines for autoimmune diseases.

Application	Nanovaccine name	Antigen	Adjuvant	Immunomodulatory Action	Effects	Ref.
Multiple sclerosis	MSN-MOG-Ce	MOG	–	Induce tolerogenic APCs with low expression of costimulatory molecules, generates $Foxp3^+$ Tregs in the spleen, reduces CNS-infiltrating APCs and autoreactive $CD4^+$ T cells	Reduce clinical scores and enhance therapeutic effects	165
Multiple sclerosis	LNP-Dex-MOG	MOG	–	Utilize intrinsic ROS-scavenging properties of LNPs to suppress APC activation, induce tolerogenic DCs and antigen-specific Tregs, reduce CNS inflammation and demyelination by suppressing autoreactive T cells and APCs	Prevent the occurrence of multiple sclerosis, reduce clinical scores and improve symptoms	166
Type 1 diabetes	NPITE + Ins	Proinsulin	AhR agonist	Decrease the activation of insulin-specific effector T cells, increase the differentiation of $FoxP3^+$ Tregs, inhibit NF-κB activation and proinflammatory cytokine production	Suppress the development of autoimmune diabetes in 8-week-old non-obese diabetic mice	167
Rheumatoid arthritis	NEs@CitP/Rapa	Multiepitope citrullinated peptide	Rapamycin	Induce a tolerogenic phenotype in DCs, promote the activation of Tregs	Significantly reduce paw thickness and clinical scores in collagen-induced arthritis mice, alleviating joint inflammation and cartilage degradation	168
Rheumatoid arthritis	REGvac	Collagen II	Vitamin D3, TGF-β1, GM-CSF	Reduce DC maturation, decrease expression of MHC II and costimulatory molecules (CD40, CD80, CD86), increase the IL-10/IL-12 ratio	Significantly reduce paw inflammation, cartilage degradation, and restore gait parameters in collagen-induced arthritis mice	169
Systemic lupus erythematosus	DART	Antigen peptides	miR-23b	Improve plasma cytokine levels, glomerulonephritis and joint lesions in MRL/lpr mice, decrease expression of proinflammatory cytokines (IL-1β, IL-6), increase expression of anti-inflammatory cytokines (IL-10) in joints of DART-treated mice	Mitigate symptoms of systemic lupus erythematosus in MRL/lpr mice, including normalizing plasma cytokine levels, reducing glomerulonephritis and joint lesions	170

MOG, myelin oligodendrocyte glycoprotein; APC, antigen-presenting cell; Tregs, regulatory T cells; CNS, central nervous system; ROS, reactive oxygen species; LNP, lipid nanoparticle; DC, dendritic cell; AhR, aryl hydrocarbon receptor; NF-κB, nuclear factor kappa-B; TGF, transforming growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; MHC, major histocompatibility complex; IL, interleukin; miR, microRNA.

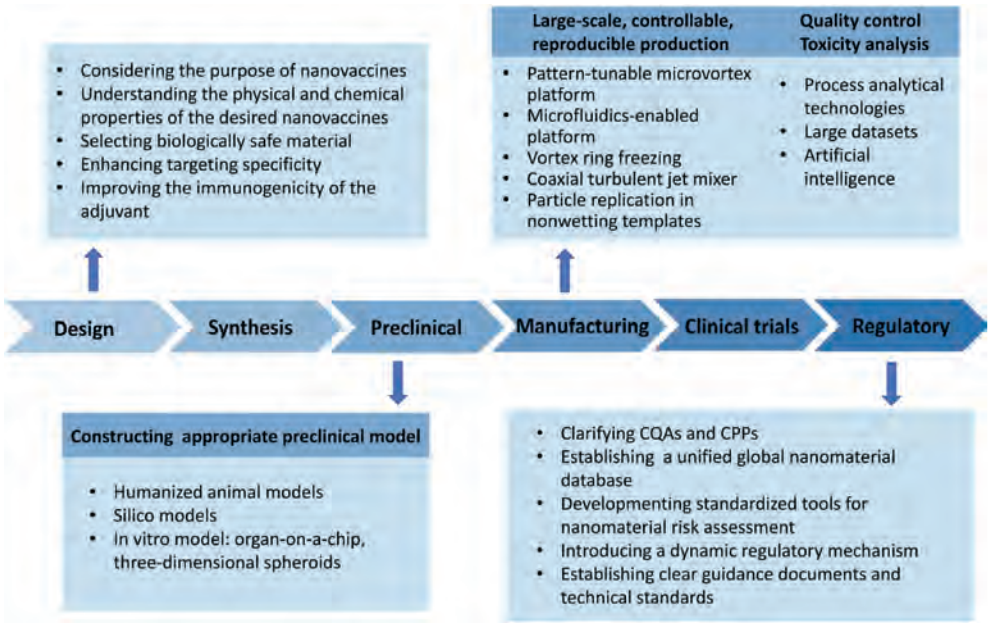

Figure 5 Strategies to expedite the clinical application of nanovaccines. CQAs, critical quality attributes; CPPs, critical process parameters.

nanovaccines and releasing them at targeted sites. For instance, Huang et al.[143] incorporated doxorubicin (DOX) into the nanovaccine FMDM@PNGs, which responsively released DOX in a simulated TME, thereby achieving a DOX-mediated therapeutic effect. Additionally, an ongoing Phase II clinical trial (NCT04580771) is investigating the efficacy and safety of the liposomal vaccine PDS0101 in combination with chemotherapy and radiotherapy for patients with stage IB3-IVA cervical cancer.

Therefore, the combination of nanovaccines and the above treatment methods can enhance the therapeutic effect of cancer. However, it is essential to thoroughly comprehend the side effects associated with combination therapies prior to their implementation.

4.2. *Infectious diseases*

Infectious diseases significantly affect global health security, and nanovaccines present a viable solution against various infectious agents, including viral, bacterial, and parasitic pathogens, as shown in Table 4[144-149]. These nanovaccines offer a distinct advantage over conventional vaccines by fostering long-lasting immunity[150]. Research has particularly focused on their application in respiratory viruses, such as respiratory syncytial virus[151], severe acute respiratory syndrome coronavirus[152] and influenza virus[144], with preclinical studies showcasing remarkable therapeutic efficacy and ongoing clinical trials, as shown in Table 5. For instance, a clinical trial (NCT04784767) led by Ober Shepherd et al.[153] demonstrated that the nanovaccine SpFN/ALFQ successfully elicited the production of conjugated and neutralizing antibodies against the SARS-CoV-2 variant and other sarbecoviruses, exhibiting strong immunogenicity and safety profiles. BNT162b2 and mRNA-1273 have demonstrated safety and efficacy against SARS-CoV-2, achieving protection rates of 95% and 94.1%, respectively, in clinical trials (NCT04368728, NCT04470427)[154,155]. The two-dose NVX-CoV2373 vaccination regimen exhibited an 89.7% efficacy in preventing SARS-CoV-2 infection among adult participants, with notable effectiveness against the B.1.1.7 variant[156]. A Phase I clinical trial (NCT03186781) evaluated the safety and efficacy of the ferritin nanoparticle influenza vaccine platform H2HA-Ferritin, revealing its ability to induce a broad neutralizing antibody response. In addition to its favorable immunogenicity, the platform's design allows for rapid initiation based solely on the hemagglutinin sequence, indicating potential applications in pandemic influenza preparedness and the development of universal influenza vaccines[157]. Furthermore, nanovaccines targeting bacterial and parasitic infections, including *Mycobacterium tuberculosis*[145], *Mycobacterium tetani*[146], *Helicobacter pylori*[147], *Echinococcus multilocularis*[148], *Plasmodium*[158], *Toxoplasma*[149], *Leishmania*[159] are now available, offering innovative strategies for the prevention and management of these diseases. Notably, the nanovesicle vaccine developed by An et al.[148] represents the first proposed nanovaccine capable of completely eradicating *Echinococcus multilocularis* by activating DC and promoting the reciprocal activation of specific T and B cells to combat infections. In the context of the current threat posed by antibiotic resistance due to conventional antibiotic therapies, nanomaterials have the potential to restore the antibacterial efficacy of traditional antibiotics by optimizing pharmacokinetics, facilitating antibiotic internalization, enhancing membrane permeability, and modifying the microenvironment of biological membranes. This represents one of the significant advantages of their application in bacterial and fungal infectious diseases[160]. Mucosal vaccines possess significant advantages in eliciting immune responses at both mucosal and systemic levels, effectively preventing pathogen invasion at the initial entry sites. Furthermore, the administration of vaccines *via* mucosal routes, such as oral or intranasal immunization, can markedly enhance patient compliance and delivery efficiency. Currently, the majority of mucosal nanovaccines have been developed primarily for the prevention of viral and bacterial infections[161]. For instance, a pH-sensitive polymeric nanovaccine, when delivered intranasally, has been shown to induce robust antigen-specific $CD8^+$ memory T cell responses in the airways and lungs, with protection against infection in mouse models

Table 7 The approaches to improve the immunogenicity of nanovaccines.

Nanoparticle/nanoplatform name	Approach	Effectiveness	Ref.
RGO-PEG	Develop nanocarriers	Improve targeting specificity, enhance immune response	199
Mn-LDH	Develop nanocarriers	Enhance immune response, activate APCs	200
SiLNPs	Develop nanocarriers	Improve targeting specificity, facilitate antigen presentation	201
PD LNPs	Develop nanocarriers	Enhance immune response, activate APCs	202
Glucosylated nanovaccines	Modify nanovaccine structures	Improve targeting specificity, activate APCs	203
Hr 8-conjugated nanovaccines	Modify nanovaccine structures	Improve targeting specificity, facilitate antigen presentation	204
ApoE3-incorporated biomimetic nanoparticle	Modify nanovaccine structures	Improve targeting specificity, activate APCs	205
Polysaccharide-based nanovaccines	Develop self-adjuvanting nanovaccines	Enhance immune response	208
AM@AEvs-PB	Develop self-adjuvanting nanovaccines	Enhance immune response, activate APCs	209
OVA amyloid-like fibrils	Develop self-adjuvanting nanovaccines	Enhance immune response, facilitate antigen presentation	210
UCMSs	Develop nanocarriers with adjuvant effects	Enhance immune response, activate APCs	211
HPLNP	Develop nanocarriers with adjuvant effects	Enhance immune response, facilitate antigen presentation	212
MnHARis	Develop nanocarriers with adjuvant effects	Enhance immune response, activate APCs	213
Poly-γ-glutamic acid nanopar-ticles	Develop nanocarriers with adjuvant effects	Enhance immune response, activate APCs	214
FUC-HTCC	Develop nanocarriers with adjuvant effects	Enhance immune response, activate APCs	215
CS/O-HTCC	Develop nanocarriers with adjuvant effects	Enhance immune response, activate APCs	216
ADA-modified metal-organic framework nanocarriers	Develop nanocarriers with adjuvant effects	Enhance immune response, activate APCs	217

APCs: antigen-presenting cells; ApoE3: apolipoprotein E3; OVA: ovalbumin; ADA: alginate dialdehyde.

lasting up to nine weeks[162]. Immune memory is a fundamental characteristic for assessing vaccine efficacy and durability, serving as the basis for the long-term effects of vaccines in preventing infections. Nanotechnology offers advantages in promoting the induction of lasting immunity against infectious diseases, primarily targeting adaptive immune memory to prevent infections. Although trained immunity may not exhibit the same specificity and duration as adaptive immune memory, both aim for a more rapid and robust response to infections or reinfections[163]. Optimizing potency, enhancing quality, and prolonging the durability of immune responses remain focal points for future research in nanovaccine technologies aimed at combating infectious diseases[150].

4.3. Autoimmune diseases

Autoimmune diseases arise from dysfunctions in the immune system, leading to inappropriate responses against self-antigens and subsequent organ damage. The ideal therapeutic approach for autoimmune diseases is to eliminate spontaneous immune responses directed at self-antigens while preserving responses to foreign antigens. Therapeutic nanovaccines designed to induce immune tolerance align with this objective[164]. At present, nanovaccines are mainly used in multiple sclerosis[165,166], type 1 diabetes[167], rheumatoid arthritis[168,169] and systemic lupus erythematosus[170], as shown in Table 6[165-170]. Nguyen et al.[165] developed a therapeutic nanovaccine, MSN-MOG-Ce, which incorporates cerium oxide nanoparticles that effectively scavenge ROS, diminish T-cells and APCs in the central nervous system, and restore antigenic immune tolerance. This approach has shown promise in recovering a mouse model of autoimmune encephalomyelitis, a relevant experimental model for human multiple sclerosis, from severe paralysis. Similarly, the lignin nanoparticle-based nanovaccine LNP-MOG, created by Phan et al.[166], also demonstrates the capacity to scavenge ROS, thereby alleviating multiple sclerosis through a comparable mechanism. By leveraging various material platforms capable of scavenging ROS, tailored therapeutic nanovaccines can be developed utilizing different autoantigens for the treatment of other autoimmune disorders.

5. The clinical development challenges and principles for nanovaccines

Since the development of liposomes in the 1960s, nanoparticles have begun to find applications in the biomedical field[171]. Prior to

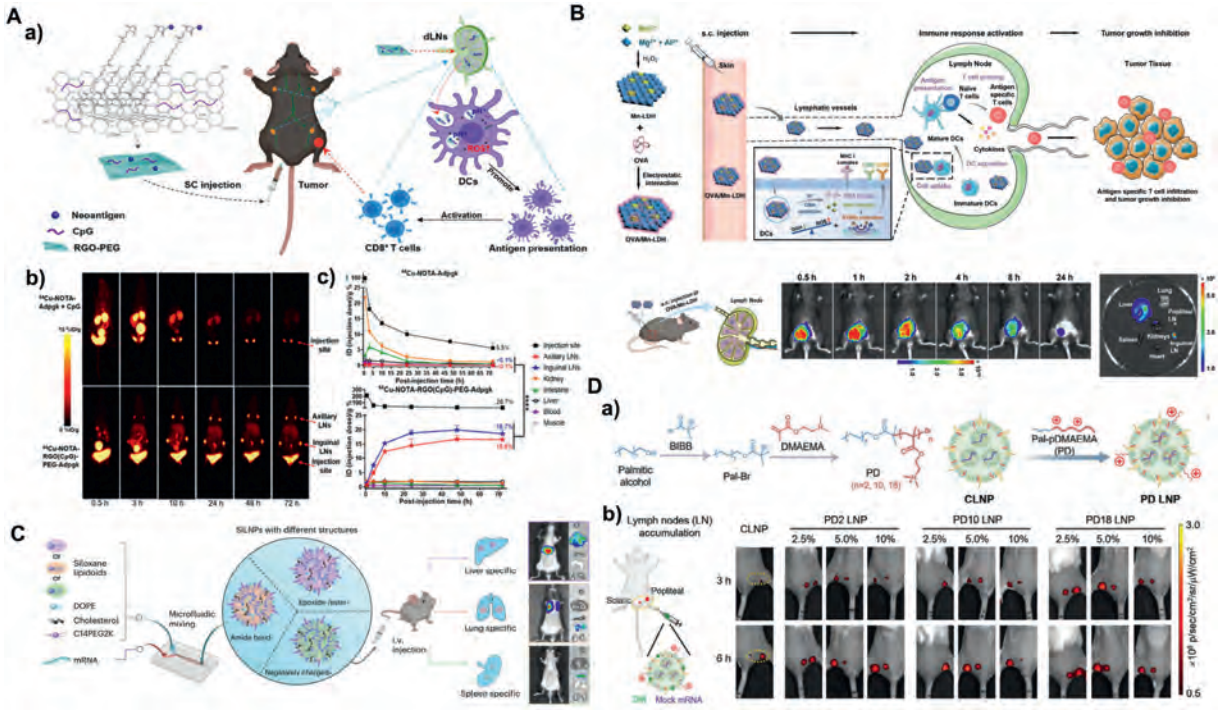

Figure 6 Researches on nanocarriers capable of targeted delivery of nanovaccines. (A) RGO-PEG nanoplatform for lymph node-targeted delivery. a) Schematic illustration of RGO(CpG)-PEG-neoantigen for lymph node-targeted delivery. b) Serial positron emission tomography images of C57BL/6 mice after subcutaneous administration of nanovaccines. Inguinal and axillary lymph nodes and injection site are indicated by red arrowheads. c) Time-radioactivity curves of injection site, inguinal and axillary lymph nodes, kidney, intestine, liver, blood, and muscle. Reprinted with the permission from Ref. 199. Copyright © 2020 American Chemical Society. (B) OVA/Mn-LDH nanovaccine for efficient intracellular antigen delivery. Reprinted with the permission from Ref. 200. Copyright © 2024 John Wiley and Sons. (C) Schematic illustration of SiLNPs for tissue-specific mRNA delivery. Reprinted with the permission from Ref. 201. Copyright © 2025 Springer Nature. (D) PD LNPs for enhanced cytosolic mRNA delivery. a) Fabrication of PD polymers synthesis and PD LNP formation for enhanced cytosolic mRNA delivery. b) *In vivo* fluorescent images of DIR-labeled PD LNPs in lymphnodes at 3 and 6 h post footpad administration. Reprinted with the permission from Ref. 202. Copyright © 2024 John Wiley and Sons.

the 21st century, the publication volume concerning nanovaccines was quite limited, primarily focusing on the preliminary design of nanocarriers. However, since 2000, there has been a steady increase in related research, with a diversification of nanocarrier types. The rise of cancer immunotherapy, advancements in nanotechnology, and the outbreak of the SARS-CoV-2 pandemic have led to an explosive growth in nanovaccine research, yielding promising results in preclinical studies, with several nanovaccines now entering clinical research phases. Currently, several nanovaccines have been commercialized. For instance, the SARS-CoV-2 vaccines BNT162b2 and mRNA-1273 utilize lipid nanoparticles (LNPs) to encapsulate mRNA, thereby safeguarding it from degradation and enhancing cellular uptake[172]. This exemplifies the feasibility and efficacy of LNP-mRNA technology. The malaria vaccine RTS,S/AS01 employs an adjuvant AS01 composed of liposome-encapsulated 3-*O*-desacyl-4′-monophosphoryl lipid A (TLR4 agonist) and QS-21 (saponin), which was piloted in endemic countries in 2019[173]. Similarly, the recombinant zoster vaccine Shingrix, which includes the adjuvant AS01B, has also been approved for market release. The human papillomavirus vaccine Gardasil 9 utilizes virus-like particles to deliver antigens and has been approved for use, recognized as a safe and effective preventive vaccine[174,175]. This underscores the promising development prospects of nanovaccines in the vaccine domain. However, the clinical translation is significantly hindered by the limitations and drawbacks associated with nanovaccines. Therefore, it is essential to implement strategies to address these challenges to expedite the clinical application of nanovaccines (Fig. 5).

5.1. *Challenges*

The structure of nanovaccines is intricate, involving nanocarriers, adjuvants, and antigens, all of which possess the potential for biotoxicity. Once nanoparticles enter the human body and the bloodstream, they may accumulate in certain organs and potentially obstruct the body's excretory systems[176]. Furthermore, the necessity for repeated administration of nanovaccines can exacerbate toxicity risks. While nanocarriers demonstrate advantages in enhancing immune responses, their development is hindered to some extent by the potential biotoxicity of certain types, particularly inorganic and polymeric nanocarriers. For instance, silica nanoparticles can induce lipid peroxidation and damage to cell membranes, resulting in a dose-dependent decrease in cell viability[177,178]. Quantum dots can directly harm cell membranes, leading to apoptosis and necrosis[179,180], while iron oxide nanoparticles can cause cytotoxicity through oxidative stress pathways[181]. Cationic micelles and liposomes have been associated with renal impairment and DNA damage in the liver and spleen[182]. Furthermore, the protein corona of nanomaterials significantly

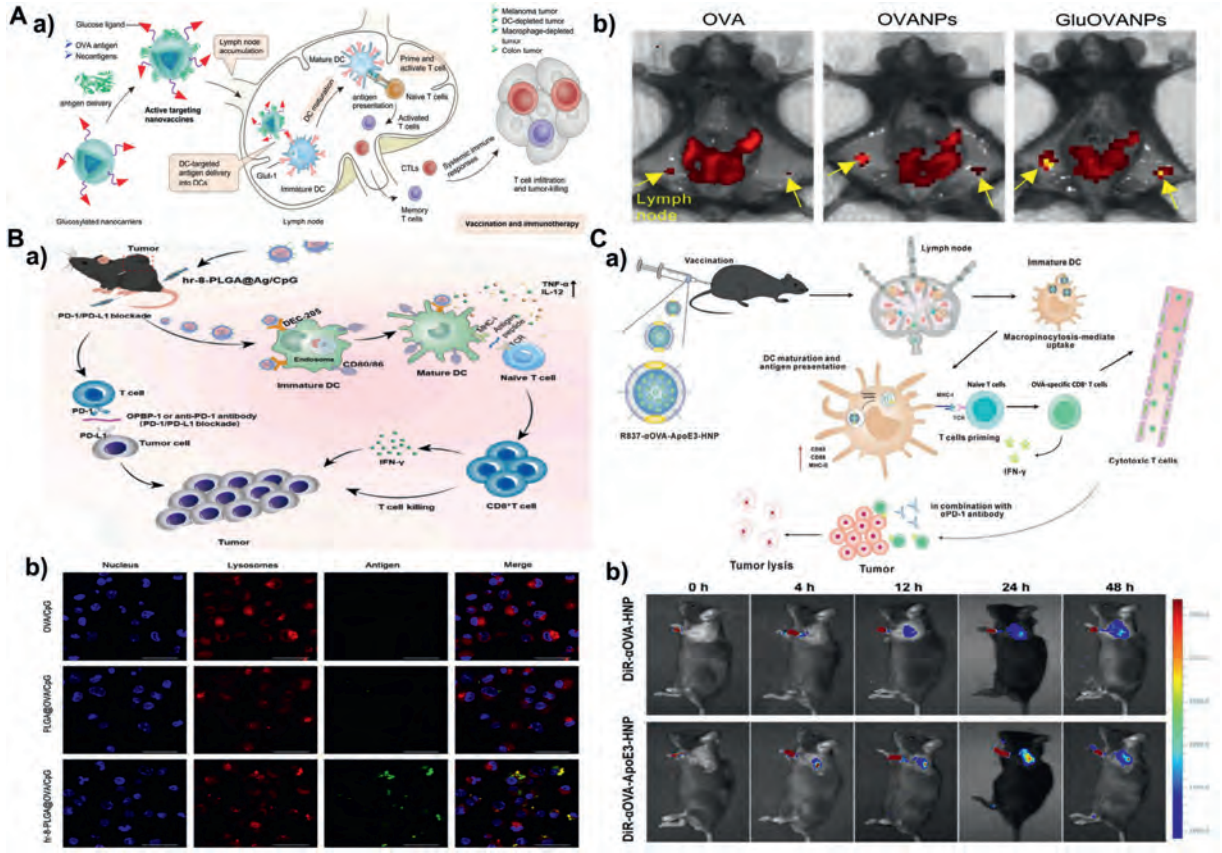

Figure 7 Schematic illustration of nanovaccines modified according to the surface receptors of target cells and the transport pathways of substances. (A) a) Scheme of dendritic cell-targeted antigen delivery by glucosylated nanovaccines for precision tumor immunotherapy. b) *In vivo* imaging system images of Cy5-labeled OVA, OVANPs, and GluOVANPs in mice. Reprinted with the permission from Ref. 203. Copyright © 2024 American Chemical Society. (B) a) Schematic illustration of structure of the hr-8-modified nanovaccine hr-8-PLGA@Ag/CpG for efficient delivery to dendritic cells. b) Confocal laser scanning microscopy images of BMDCs treated with OVA/CpG, PLGA@OVA/CpG and hr-8-PLGA@OVA/CpG for 2 h. Cell nuclei were stained with Hoechst33342 (blue), lysosomes were stained using LysoTracker (red), and OVA-FITC encapsulated in nanoparticles was shown in green. Reprinted with the permission from Ref. 204. Copyright © 2024 Elsevier. (C) a) Schematic illustration of ApoE3-modified biomimetic nanoparticle for efficient delivery to antigen-presenting cells *via* the macropinocytosis pathway. b) The fluorescence signals of C57BL/6 mice after subcutaneous administration of DiR-αOVA-HNP or DiR-αOVA-ApoE3-HNP at footpad. Reprinted with the permission from Ref. 205. Copyright © 2020 Elsevier.

influences drug release capacity, stability, tissue distribution, and pharmacokinetics. Variations in the types of nanomaterials and their surface modifications result in distinct compositions of the protein corona[183]. Some adjuvants also cause biotoxicity, for instance, the aluminum adjuvant approved for human vaccines elicits a weak cellular immune response and may cause adverse effects such as erythema and allergic reactions. Additionally, the mechanisms of action for some adjuvants remain insufficiently understood, leading to their irrational use and associated side effects. Additionally, it has been demonstrated that mRNA nanovaccines delivered *via* lipid nanoparticles may exhibit potential biotoxicity, leading to damage in the liver and spleen, as well as immunopathogenic effects by elevating pro-inflammatory cytokines in the body, activating the complement system, and triggering hypersensitive responses[184]. Beyond the inherent biotoxicity of nanovaccines and their complex interactions with the human body, the larger surface area-to-volume ratio of nanomaterials makes them particularly prone to endotoxin binding[185], and contamination during the production of nanovaccines is also a significant contributor to biotoxicity.

Reducing potential biotoxicity through the interactions between substances, without compromising the efficacy of nanovaccines, represents a strategy to enhance the biocompatibility of these formulations. The nanovaccine CS-TEMPO-OVA, synthesized by combining organic nitrogen oxides, particularly 4-carboxy-TEMPO, with chitosan, has demonstrated improved biocompatibility of 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO) and extended circulation time in the bloodstream. It exhibits favorable biocompatibility at both cellular and organoid levels, addressing safety concerns[186]. Through solid-phase synthesis, peptide antigens are conjugated with hydrophobic amino acids to generate fully defined molecular entities. Under aqueous conditions, these molecules self-assemble into various nanoparticles and chain aggregates. Mice immunized with entities containing 15 leucine residues can effectively clear bacterial loads from target organs without triggering the release of inflammatory mediators, indicating a highly biocompatible vaccine delivery system[187]. Duan et al.[188] developed an innovative mRNA nanovaccine delivery system by coating the natural anionic polymer sodium alginate (SA) onto cationic liposomes containing 2,3-

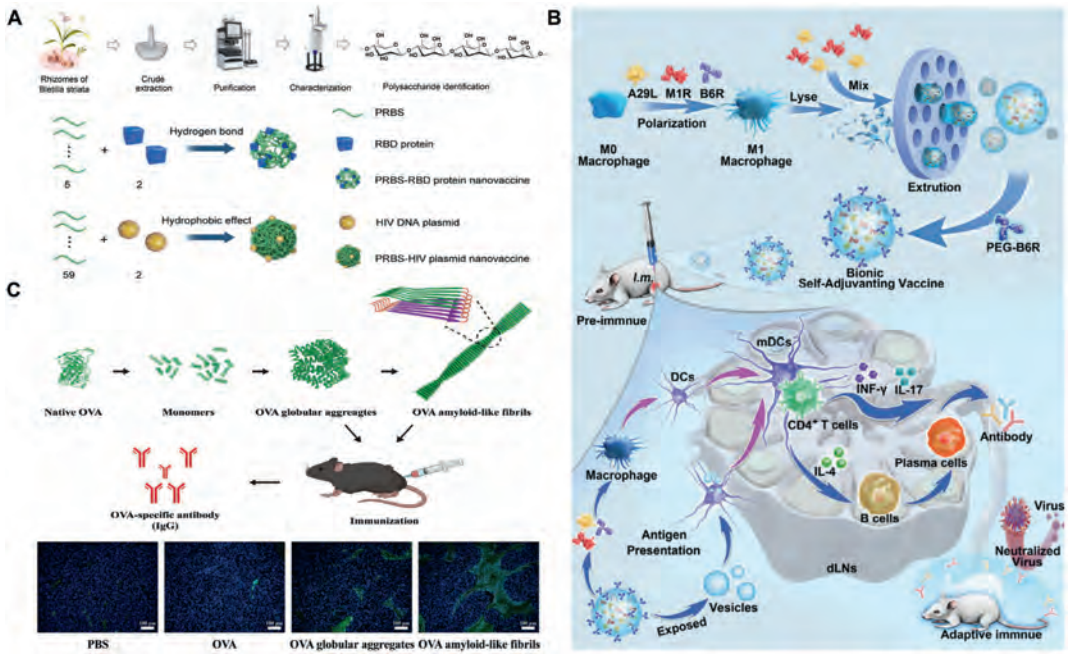

Figure 8 Schematic illustration of self-adjuvanting nanovaccines. (A) Schematic diagram for the assembly of PRBS-based nanovaccines. Reprinted with the permission from Ref. 208. Copyright © 2022 Springer Nature. (B) Schematic illustration of the rational design of programmable macrophage vesicle based nanovaccine AM@AEvs-PB. Reprinted with the permission from Ref. 209. Copyright © 2024 John Wiley and Sons. (C) Schematic diagram of OVA assemblies with different structures and terminal deoxynucleotidyl transferase dUTP nick end labeling staining of tumor after treatment. Reprinted with the permission from Ref. 210. Copyright © 2025 John Wiley and Sons.

dioleylpropyl-trimethyl ammonium chloride (DOTAP) and mRNA, resulting in the formulation of the nanovaccine SA@DOTAP-mRNA. The findings indicated that SA@DOTAP-mRNA significantly mitigated cytotoxicity and hemolysis, did not induce substantial organ damage, and exhibited biocompatibility compared to the uncoated SA group. This advancement provides a pathway for future investigations into anionic polymers with similar properties to further diminish the biotoxicity associated with mRNA vaccines.

Enhancing the biosafety of nanovaccines is a pivotal step toward facilitating their widespread clinical application. In the development of nanovaccines, the initial phase involves selecting biologically safe material. Researchers are investigating novel biocompatible and biodegradable materials, such as poly(lactic-*co*-glycolic acid) (PLGA), liposomes, and natural biopolymers, which can safely degrade and be eliminated by the body after fulfilling their intended function, thereby reducing long-term burdens[189]. The interaction of nanomaterials with the human body can lead to potential toxicity, for instance, the protein corona on the surface of nanomaterials can influence biological behavior. Designing nanomaterials with controlled protein interactions is essential for enhancing biosafety without compromising efficacy[190,191]. Therefore, a clear understanding of the pharmacokinetic characteristics and appropriate dosing concentrations of nanovaccines is necessary prior to their design, as well as a comprehensive understanding of the interactions between nanovaccines and the human body to minimize adverse reactions and improve therapeutic outcomes. Establishing suitable biological models to evaluate the interactions between nanovaccines and the human body is a critical area for future exploration[15]. The safety analysis of nanomaterials requires precise characterization, however, current technologies often overlook key features such as size, surface charge, stability, and drug release[192], leading to inconsistencies between preclinical and clinical outcomes. Furthermore, regulatory agencies require reliable data on nanomaterials, but the lack of standardized methods hinders their approval, necessitating the development of advanced, reliable, and standardized techniques for the characterization of nanomaterials[193]. Even when nanomaterials share identical fundamental chemical compositions, inferring the risk of one nanomaterial from another is deemed unreasonable. Various authoritative bodies advocate for a methodical safety and risk assessment of nanomaterials on a case-by-case basis[194]. The existing frameworks still rely on traditional drug evaluation methods, which fail to accommodate the unique characteristics of nanomaterials, complicating the assessment of safety and efficacy[171]. There is a pressing need to develop new approaches to evaluate the long-term toxicity and ecological impact of nanomaterials[195,196], as well as to establish emerging, flexible approaches that align with the rapid advancement of nanomaterials[193]. To ensure the long-term safety and efficacy of nanomaterials, post-market surveillance is essential[171,197]. Additionally, enhancing communication among academia, industry, and regulatory agencies is crucial for establishing clear characterization methods and safety thresholds for nanomaterials, ensuring alignment with regulatory expectations and incorporating feedback to streamline the approval process[198].

5.2. *Principles for preclinical development of nanovaccines*

5.2.1. *Improving the immunogenicity of nanovaccines*

The primary objective of developing nanovaccines is to elicit a robust immunogenic response, thereby enhancing the preventive and therapeutic efficacy of traditional vaccines. Consequently, augmenting the immunogenicity of nanovaccines is one of the key

principles that must be achieved during their preclinical development. The targeted specificity of nanovaccines amplifies the subsequent cascade of immune responses while minimizing off-target toxicity. Since the introduction of adjuvants in vaccines, numerous classical adjuvants have been extensively utilized in vaccine development and formulation. However, some adjuvants have limited capacity to enhance immune responses, necessitating the consideration of adjuvant effects in the design of nanovaccines. Currently, in addition to employing novel adjuvants and dual adjuvants, self-adjuvanting nanovaccines and nanocarriers with adjuvant properties demonstrate unique advantages in enhancing immune responses. Designing nanovaccines that specifically target immune organs and immune cells, while employing adjuvants with superior immunogenic enhancement, can significantly improve the preventive and therapeutic outcomes of nanovaccines, thereby facilitating their clinical translation, as shown in Table 7[199-205,208-217].

Developing nanocarriers capable of targeted delivery of nanovaccines is one approach to improve the specificity. For example, a highly modular and biodegradable platform, polyethylene glycolylated reduced graphene oxide nanosheets (RGO-PEG), showed rapid, efficient, and sustained accumulation in LN, facilitating direct delivery of antigen and adjuvant, achieving >100-fold improvement in targeted delivery to LNs compared with soluble vaccines. It can also guide antigen processing and presentation to T cells and induce specific T cell responses, which may be an effective delivery platform for individualized cancer vaccination[199] (Fig. 6A). The nanovaccine developed by Liu et al.[200] utilizes manganese-based layered double hydroxide (Mn-LDH) nanoparticles, an effective delivery system to deliver the antigen OVA to DCs, improving the anti-tumor effect (Fig. 6B). Xue et al.[201] developed a novel nanoparticle platform for organ-specific mRNA delivery, known as siloxane-incorporated lipid nanoparticles (SiLNPs). Their research demonstrated that siloxane groups can enhance the cellular internalization of mRNA-LNPs and improve their intracellular escape capabilities, thereby increasing mRNA delivery efficiency. The varying lipid structures can dictate the organ-targeting properties of SiLNPs, enabling selective delivery to the liver, lungs, and spleen (Fig. 6C). A polyvalent stimulator of interferon genes activating polymer (PD), synthesized from tertiary amine units and a biodegradable alkyl chain, when combined with lipid and mRNA to form lipid nanoparticles PD LNPs, significantly enhances the delivery of mRNA vaccines to lymph nodes, representing an effective mRNA cancer vaccine delivery system[202] (Fig. 6D). Receptor-mediated endocytosis can improve the targeting of nanovaccines. Surface glycosylated nanovaccines can bind to glucose transporter 1 (Glut-1) on DCs, resulting in maximal accumulation in LNs and thereby enhancing intracellular delivery of antigens (Fig. 7A). This indicates that Glut-1 serves as a reliable target for antigen delivery to DC[203]. Similarly, the nanovaccine hr-8-PLGA@Ag/CpG, formulated by Zheng et al.[204], consists of the anti-hydrolysis peptide hr-8 targeting the DC endocytic receptor DEC-205, along with PLGA-encapsulated antigen (Ag) and CpG adjuvant, effectively targeting DCs ultimately localizing the OVA antigen to lysosomes (Fig. 7B). In addition to improving the targeting of nanovaccines through receptor-mediated endocytosis, macropinocytosis can enhance the uptake of exogenous antigens. The biomimetic nanovaccine R837-αOVA-ApoE3-HNP, which incorporates apolipoprotein ApoE3, is effectively internalized by DC *via* macropinocytosis, significantly advancing DC maturation and antigen presentation. This suggests that macropinocytosis may function as an efficient mechanism for facilitating the uptake of nanovaccines by DC[205] (Fig. 7C). It is clear that the targeted modification of nanovaccine structures, based on the surface receptors of target cells and the transport pathways of substances, is an effective strategy to enhance the targeting capabilities of nanovaccines. The modification of both target cells and the surface of nanovaccines provides a method to improve the targeting specificity of nanovaccines by creating binding sites on the surface of target cells. For example, the surface modification of lymphatic endothelial cells with azide groups generates binding sites for dibenzocyclooctyne-modified liposomes, which significantly enhances the targeting specificity of the encapsulated antigens and adjuvants to LN. Compared to groups lacking azide groups, this strategy results in a more robust T cell response and improved survival rates, representing a strong approach for delivering vaccine components to LN to strengthen anti-tumor immunity[206].

Self-adjuvanting nanovaccines currently demonstrate advantages in enhancing immune responses, simplifying vaccine components, and improving vaccine safety, potentially minimizing or even eliminating the need for additional adjuvants[207]. Both polysaccharide-protein nanovaccines targeting SARS-CoV-2 and polysaccharide-DNA nanovaccines against Human Immunodeficiency Virus induce stronger antigen-specific antibodies and cellular responses *in vivo* than traditional vaccines through polysaccharide-mediated immune activation without adjuvant. Significantly enhanced antibody and T cell immune responses, polysaccharides can co-assemble with different types of antigens and activate a variety of key immune cells, which has the potential to develop potent self-adjuvant nanovaccines[208] (Fig. 8A). A programmable macrophage vesicle based bionic self-adjuvanting vaccine (AM@AEvs-PB), developed by loading macrophage-derived vesicles stimulated by monkeypox virus antigens with mature virion-associated intracellular proteins and modified enveloped virion antigens, facilitates antigen delivery and prolongs retention through pre-activated macrophage-derived vesicles. In addition, the activated vesicles and Monkeypox virus antigens can cooperate to stimulate innate immunity and further coordinate adaptive immunity, providing an effective method for Monkeypox virus immunization[209] (Fig. 8B). The assembly of the model antigen OVA into amyloid-like aggregates, due to its unique self-adjuvanting structural properties, improves antigen stability, facilitates antigen internalization, enhances transport to lymphoid organs, and induces robust humoral immune responses and immunological memory. Compared with most current strategies that rely on the incorporation of molecular adjuvants and complex synthetic vectors into vaccine formulations, this minimalist self-adjuvant vaccine strategy introduces a one-step engineering technology that can guide the design of other protein antigen-based vaccines and has broad prospects in immunotherapy[210] (Fig. 8C). The nanocarriers endowed with the effect of adjuvant can also enhance the immunogenicity of nanovaccines. Large-pore mesoporous silica-coated upconversion nanoparticles (UCMSs), with a size of less than 100 nm, can effectively load photosensitizers and antigens, facilitating efficient vaccine delivery *in vivo* while serving as a novel immunoadjuvan, which avoids the limitations of current immune adjuvants, such as poor cellular uptake ability and biocompatibility, too large particle size, single function, and unsatisfactory efficacy[211]. A hybrid polymer lipid nanoparticle (HPLNP) can prolong the retention time of antigens at the injection site and enhance lymphatic drainage of the antigens, thereby boosting both humoral and cellular immune responses, and with advantages of good biocompatibility, simple

preparation, low cost, and the ability to enhance both humoral and cellular immune responses, it is an effective adjuvant for protein vaccines such as HBsAg-VLP[212]. Risedronate-functionalized manganese-hydroxyapatite micro-nanoparticles (MnHARis) have demonstrated the ability to elicit a stronger humoral immune response compared to aluminum adjuvants, indicating their potential as effective adjuvants for enhancing vaccine immunogenicity[213]. Nanoparticles based on poly-γ-glutamic acid (γ-PGA) are readily taken up by DCs, promoting cytokine secretion and improving T cell activation, thus exhibiting unique adjuvant properties[214]. Fucoidan-*N*-(2-hydroxy-3-trimethylammonum)propylchitosan nanoparticles (FUC-HTCC NPs) exhibit a stronger humoral immune response compared to CpG and may serve as a potential adjuvant for the anthrax vaccine adsorbed, facilitating rapid induction of protective immunity[215]. Curdlan sulfate/*O*-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride (CS/O-HTCC) enhances the activation of APC, upregulates the production of inflammatory factors and cytokines, induces cross-presentation, and activates type I interferon-related genes. This leads to a more robust systemic and mucosal immune response, positioning it as a multifunctional adjuvant to improve immune responses[216]. The ZIF-7/8-ADA nanoparticles, coated with alginate dialdehyde (ADA) on the surface of aminated ZIF-7/8 nanoparticles, induce a superior Th1/Th2-type immune response compared to alum adjuvant and can function as an adjuvant to enhance both humoral and cellular immune responses against Pseudorabies virus infection[217].

Further research is necessary to develop more targeted and cost-effective universal nanoparticle vaccine delivery platforms, thereby enhancing the specificity of antigen and adjuvant delivery to improve immune responses. Furthermore, the development of adjuvants characterized by robust immunogenicity and minimal adverse effects will be a pivotal focus for the clinical advancement of nanoparticle vaccines. Exploring the targets and mechanisms of adjuvants may facilitate the synergistic use of dual adjuvants to further enhance immunogenicity in nanoparticle vaccines, representing a promising direction for future research. Self-adjuvanting nanovaccines not only enhance immune responses but also eliminate the need for supplementary enzymes or chemical catalysts, thereby streamlining the vaccine formulation process and further improving biosafety, this underscores the significant potential for ultimate translation and application in the field of nanovaccines. The development of nanocarriers with adjuvant properties also demonstrates advantages in augmenting immune responses.

5.2.2. Construction of appropriate preclinical model

Extensive research has been undertaken on nanovaccines within preclinical models, resulting in significant progress. However, the results observed in clinical trials frequently diverge from those in preclinical investigations. This discrepancy may stem from the limitations of the preclinical models selected during preclinical trials in accurately simulating human diseases, as these models often fail to encapsulate the complexity and pathogenesis of human conditions. Currently, mice are widely utilized as preclinical models for studying the human immune system, however, there are differences between the innate and adaptive immunity of mice and humans[218], which may result in discrepancies between preclinical trial outcomes and clinical trial results.

Preclinical models play a crucial role in the development of nanovaccines, as they facilitate the exploration of administration routes, dosage concentrations, therapeutic efficacy, and biosafety. Preclinical trials are an indispensable phase that must not be overlooked before advancing to clinical trials. Therefore, it is crucial to develop preclinical models that are more closely related to human diseases, enhancing the efficacy of preclinical trials to ensure the successful clinical translation of nanovaccines. The incorporation of human genes, cells, and tissues can significantly enhance the clinical relevance of animal models. Numerous investigations have already been conducted on humanized animal models[219,220]. Some researchers propose that human cell lines cultured *in vitro* can be transplanted into immunodeficient animals, or that gene editing techniques can be utilized to introduce human genes into animals, substituting their endogenous genes to create human disease models. By employing patient-derived tissue xenografts, it is feasible to develop animal models that are homologous to the patient, thereby enabling more precise individualized treatment of diseases[15,221]. For instance, human peripheral blood lymphocyte (Hu-PBL) models, established through intravenous or intraperitoneal injection of human peripheral blood mononuclear cells (PBMC) in immunodeficient mice, allow for the detection of human T cells, B cells, NK cells, and DC, which can be utilized for evaluating the efficacy of cancer vaccines[222,223]. Additionally, co-transplanting human embryonic thymus/liver tissues and $CD34^+$ hematopoietic stem/progenitor cells into mice leads to the regeneration of a substantial population of human lymphoid cells, including T cells, B cells, and DC, resulting in the production of high levels of human IgM and IgG antibodies, thereby mediating a robust immune response. This serves as a powerful model system for studying human immune function[224]. Furthermore, optimizing silico models that can be integrated with *in vitro* and *in vivo* models, along with refining advanced *in vitro* models that incorporate human tissues, such as organ-on-a-chip and three-dimensional spheroids, represents another strategy to enhance the accuracy of preclinical trials[225]. For example, human lymphoid follicle chips containing B cells, T cells, and autologous antigen-presenting DCs can be employed to investigate the immune responses of humans to vaccines and adjuvants *in vitro*, the formation of ectopic lymphoid follicles and antigen-induced immune responses *in vivo*, thereby enabling patient-specific assessments of the efficacy and safety of vaccines, adjuvants, and immunotherapeutic agents, potentially serving as a more effective preclinical tool[226]. It is important to note that while preclinical models that more accurately simulate the pathogenesis of human diseases are continuously being developed, each preclinical model has its own advantages and disadvantages. Selecting or developing preclinical models that can precisely reflect the progression of human diseases and the mechanisms of vaccine action, in accordance with the diseases applicable to nanovaccines, is a fundamental principle in the preclinical development of nanovaccines.

5.2.3. Development of advanced technologies

The small-scale production of nanovaccines can reduce variability and ensure the feasibility of research, however, large-scale manufacturing is ultimately necessary to facilitate their clinical application. The production process of nanovaccines often involves multiple steps and technologies, resulting in high production costs, which pose challenges to the stability and reproducibility of nanovaccine mass production. The uncontrollable quality of nanovaccine production can lead to discrepancies between different production batches, ultimately resulting in unpredictable biological characteristics of the nanovaccines. This unpredictability may manifest as unforeseen side effects during

clinical trials, hindering the clinical application of nanovaccines[15,227].

The clinical translation of nanovaccines encounters challenges related to production costs and scalability. One of the key principles of preclinical trials for nanovaccines is the ability to achieve large-scale production of the developed nanovaccines. Advanced technologies capable of large-scale nanoparticle production are already in existence. A pattern-tunable microvortex platform allows for the mass production of lipid-polymer hybrid nanoparticles (LPH) while providing control over the size, uniformity, and reproducibility of the nanoparticles[228]. An innovative microfluidics-enabled platform facilitates the large-scale production of eutectic gallium indium (EGaIn) nanoparticles in aqueous media[229]. Additionally, techniques such as vortex ring freezing[230], coaxial turbulent jet mixer[231], particle replication in nonwetting templates (PRINT)[232] enable the mass production of nanoparticles. Some of these methods also enable the manipulation of nanoparticle properties, including size and shape, thereby laying the groundwork for the production of nanovaccines and propelling their clinical trials and industrial manufacturing. However, the rapid and accurate synthesis of nanoparticles with varying properties in a reproducible manner remains a significant challenge, impeding the large-scale screening of these nanoparticles[15]. Moving forward, the development of technologies and platforms that can enable the rapid and precise large-scale production of nanoparticles, ensuring their reproducibility, controllability, and environmentally friendly low-cost processes, will be vital in advancing the clinical translation of nanovaccines.

In addition to advancing novel technologies for the large-scale synthesis of nanoparticles, the progression of emerging methodologies for toxicity assessment and quality assurance is vital for the clinical translation of these nanomaterials. The US Food and Drug Administration (FDA) promotes the adoption of Process Analytical Technologies (PAT), which utilize sophisticated analytical techniques such as near-infrared spectroscopy, Raman spectroscopy, and mass spectrometry to monitor product quality in real-time. By obtaining continuous and instantaneous data throughout the manufacturing process, PAT can swiftly detect and address issues, thereby lowering production costs, optimizing manufacturing workflows, minimizing inter-batch variability, and enhancing both production efficiency and product quality[192]. The integration of extensive datasets generated from organ-on-a-chip models with artificial intelligence can facilitate the development of predictive screening models that support data-driven nanoparticle design, thereby mitigating the randomness and uncertainty inherent in nanoparticle fabrication[233,234]. By leveraging artificial intelligence to forecast how variations in the physicochemical properties of nanoparticles influence their behavior in biological systems, precise therapeutic effect evaluations and real-time treatment modifications can be realized, ultimately refining nanoparticle design[171].

5.2.4. Overcoming regulatory challenges

Nanovaccines encounter regulatory challenges similar to those faced by other nanomedicines. The global regulation of nanomedicines is marked by a significant lack of consistency and harmonization, with prominent regulatory authorities such as the European Medicines Agency (EMA) and the FDA employing distinctly different nomenclature and regulatory frameworks[235]. To ensure quality control, it is imperative to delineate the critical quality attributes (CQAs) specific to nanomedicines, which generally encompass predictive physicochemical properties, drug characteristics, and formulation parameters. Researchers should define CQAs based on their comprehension of nanomedicines, establishing a framework for validating the performance of the final manufactured product[225]. To mitigate the escalating development costs and regulatory hurdles, the FDA and the International Conference on Harmonization strongly advocate for the implementation of Quality by Design (QbD) principles in drug development, manufacturing, and regulation. QbD encourages the pharmaceutical industry to employ risk management and science-based production principles to achieve an understanding of processes and products, thereby ensuring product quality. QbD necessitates the identification of CQAs and the elucidation of critical process parameters (CPPs), which are often the primary focus of process review[236]. To reduce regulatory risks and identify potential issues that may lead to non-approval, it is advisable for researchers to seek regulatory expertise early, familiarize themselves with regulatory guidelines, and engage with regulatory agencies throughout the development process, prior to clinical development, and before submission for review[237]. Simultaneously, researchers must select appropriate regulatory pathways based on specific circumstances, certain nanomedicines require the submission of an investigational new drug (IND) application or a Biological License Application (BLA)[238], while some nanomedicines derived from existing drugs may be classified as a generic formulation, potentially exempting them from IND and BLA submissions[239]. To enhance the efficiency and effectiveness of nanomaterial regulation, Ma et al.[194] propose the establishment of a unified global nanomaterial database that integrates definitions, classifications, applications, and risk assessments of nanomaterials from various regions, thereby facilitating regulatory coordination and information exchange. Furthermore, the development of standardized tools and methodologies for nanomaterial risk assessment could streamline evaluation processes across different regulatory agencies, ensuring that risk assessments are comparable and consistent on a global scale. Additionally, to adapt to innovations in nanotechnology and maintain relevance and scientific validity, a dynamic regulatory mechanism that evolves with technological advancements could be introduced. Through interdisciplinary collaboration, a comprehensive understanding of the behavior and potential impacts of nanomaterials can lead to more effective regulatory strategies to address their complexities. Finally, to resolve the current ambiguities within existing regulations, clear guidance documents and technical standards need to be established, including explicit definitions and regulatory requirements for inadvertently produced nanomaterials, ensuring a more direct interpretation of the regulations. Moreover, under the current regulatory framework, seeking approval for additional indications or novel applications of nanomaterials is expected to improve their clinical translation[234].

5.2.5. Formulating strategies in advance

The creation and preparation of nanovaccines encompass several stages, with many currently stagnating in clinical trial phases, significantly impacting their future development. Some of these delays stem from inadequate prior considerations, necessitating the formulation of strategic plans during the preclinical phase, along with thorough feasibility analyses. Initially, a safety feasibility assessment is required. This involves studying the biological behavior of nanomaterials, developing and selecting new biocompatible materials, and establishing a comprehensive safety

evaluation system[196]. It is essential to enhance fundamental research to understand the mechanisms of action, distribution, and metabolic pathways of nanovaccines through *in vitro* and *in vivo* preclinical experiments, as well as to assess their toxicity, safe dosage ranges, appropriate vaccination intervals, and routes of administration. During the clinical trial phase, it is crucial to establish clinical benchmarks for target indications early on, including inclusion/exclusion criteria for participants, recruitment stratification, primary and secondary endpoints, and ethical considerations for the trials, conducting non-inferiority trials with toxicity as a secondary endpoint[225,240], while closely monitoring adverse reactions in subjects and focusing on well-defined outcomes[192]. Additionally, technical feasibility must be addressed. As previously mentioned, developing technologies capable of large-scale, reproducible production of nanoparticles, along with quality control and real-time monitoring, will enhance the stability of the preparation process and further improve the production efficiency and quality of nanoparticles. The development of environmentally friendly production methods has the potential to significantly minimise solvent waste and pollution, thereby achieving sustainable production while concomitantly reducing costs[241]. It is imperative to consider economic feasibility as a pivotal factor in the decision-making process. The synthesis of nanovaccines is optimised through the streamlining of synthesis steps and the reduction of raw material consumption. In addition, conducting cost analyses prior to design and production ensures that material and process costs remain manageable[225]. A comprehensive needs assessment should evaluate the existence of effective preventive or therapeutic alternatives for the target disease, determining whether the development of nanovaccines can yield superior preventive or therapeutic outcomes or result in fewer adverse effects compared to existing solutions, as well as their competitive advantage and market potential. It is imperative to promote and disseminate the efficacy of nanovaccines to enhance public awareness and understanding, which is critical for their acceptance and commercialisation. In order to mitigate the risks associated with investment in nanovaccines, companies must carefully formulate investment strategies and seek partnerships or alternative funding sources[194], while governments can increase policy and financial support to expedite the market entry of nanovaccines.

6. Conclusions

Nanovaccines are engineered to overcome the limitations of traditional vaccines, offering flexibility and control in their design, enabling customization according to specific needs. They possess the ability to engage with various immune cell types and provide multiple benefits, such as targeted specificity, improved antigen presentation, and effective immune regulation. These characteristics render them potent tools in the prevention and treatment of cancer, infectious diseases, and autoimmune conditions. Currently, relying solely on cancer nanovaccines for complete tumor suppression remains unachieved. Given the immunosuppressive characteristics of cancer cells and the TME, the synergistic application of nanovaccines with physical therapies, chemotherapies, immune checkpoint inhibitors, or other treatment modalities can significantly enhance immune responses, generating synergistic effects and improving vaccine efficacy[242]. Personalized cancer nanovaccines, designed based on tumor specificity and individual patient immune profiles, genetic makeup, and overall health status, can enhance therapeutic efficacy while minimizing off-target effects, thereby contributing to improved safety[243,244]. Nanovaccines for infectious diseases must possess rapid response capabilities and scalability for mass production; establishing a universal nanovaccine platform and enabling the delivery of multivalent antigens can adapt to the rapid mutations of viruses. High global vaccination rates are crucial for preventing the spread of infectious diseases; however, the limitations of ultracold-chain systems and the pain and fear associated with injections pose challenges to the widespread adoption of many vaccines[245]. Developing nanovaccines that can be stably stored at room temperature or lower, thereby reducing reliance on cold chain transport, can lower the high costs and infrastructure demands of cold chain distribution, enhancing the accessibility of nanovaccines, and intranasal or sublingual vaccines can improve patient compliance[2].

Key considerations in the advancement of nanovaccine research and development include the compatibility of different components, the efficacy of preclinical studies, the practicality of large-scale production and storage, and the scalability of clinical applications. Researchers suggest that the seven components of the DELIVER framework-design, experimental, manufacturing, preclinical, clinical, regulatory, and business-should be thoroughly investigated during the initial stages of nanodrug design and development, including nanovaccines[225]. Collaboration among academia, industry, and regulatory bodies can streamline the transition from preclinical success to clinical efficacy. Enhancing the biocompatibility of nanovaccines, constructing preclinical models that reflect changes in the human immune system, developing advanced technologies that facilitate large-scale, reproducible production, and implementing quality control and toxicity analysis, as well as dynamic regulatory measures that evolve with nanovaccine development, are critical for clinical translation.

Looking ahead, the urgent priority is to overcome existing challenges and advance clinical translation by integrating multidisciplinary approaches from materials science, immunology, bioengineering, clinical medicine, and ethics to develop safe, effective, and widely accessible nanovaccines.

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Author contributions

Gao Yuhong and Hua Sijia conceptualized and wrote the manuscript. Dong Xiulin revised the manuscript. Zhang Xiaofeng and Zhang Kun supervised the whole process. Yang Jianfeng acquired

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Conflicts of interest

The authors have no conflicts of interest to declare.

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