Perspective

Precision Nanovaccines for Potent Vaccination

Hong Liu, Haolin Chen, Zeyu Yang, Zhenfu Wen, Zhan Gao, Zhijia Liu, Lixin Liu, and Yongming Chen*

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ABSTRACT: Compared with traditional vaccines, nanoparticulate vaccines are especially suitable for delivering antigens of proteins, peptides, and nucleic acids and facilitating lymph node targeting. Moreover, apart from improving pharmacokinetics and safety, nanoparticulate vaccines assist antigens and molecular adjuvants in crossing biological barriers, targeting immune organs and antigen-presenting cells (APC), controlled release, and cross-presentation. However, the process that stimulates and orchestrates the immune response is complicated, involving spatiotemporal interactions of multiple cell types, including APCs, B cells, T cells, and macrophages. The performance of nanoparticulate vaccines also depends on the microenvironments of the target organs or tissues in different populations. Therefore, it is necessary to develop precise nanoparticulate vaccines that accurately regulate vaccine immune response beyond simply improving pharmacokinetics. This Perspective summarizes and highlights the role of nanoparticulate vaccines with precise size, shape, surface charge, and spatial management of antigen or adjuvant for a precision vaccination in regulating the distribution, targeting, and immune response. It also discusses the importance of the rational design of nanoparticulate vaccines based on the anatomical and immunological microstructure of the target tissues. Moreover, the target delivery and controlled release of nanovaccines should be taken into consideration in designing vaccines for achieving precise immune responses. Additionally, it shows that the nanovaccines remodel the suppressed tumor environment and modulate various immune cell responses which are also essential.

KEYWORDS: *Delivery system, Immune response, Nanoparticular vaccine, Molecular adjuvant, Precision vaccination*
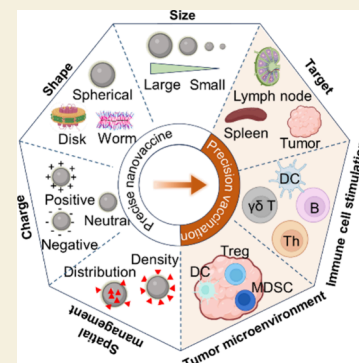
1. INTRODUCTION

The efficacy of traditional vaccines, like attenuated and inactivated vaccines, is limited for certain diseases, variant viruses, or specific populations.[1] Novel vaccines based upon recombinant proteins, peptides, and nucleic acids have shown to be more effective with fewer side effects in the application of infectious and tumor-associated diseases.[2] Nevertheless, the weak immunogenicity of proteins/peptides and easier enzyme-degradation along with the low transfection of nucleic acid weaken their application.[3,4] Therefore, it is urgent to use novel adjuvants and delivery vectors to solve these issues. Fortunately, delivery materials serve as indispensable tools in the development of protein/peptide and nucleic acid vaccines, offering solutions to challenge stability, controlled release, immunogenicity, and targeted delivery. Delivery materials contribute significantly to the advancement of vaccine technology and the development of more effective and safer nanovaccines for various infections and diseases.[5]

Nanovaccines are nanosized particles that contain antigens or/and adjuvants being formulated with delivery materials for improved immune modulation. Strictly, the size of nanotechnology covers a range of 1 to 100 nm, however, it is expanded to a few hundred nanometers in the upper side. Nanovaccines of size in this range offer a versatile platform that allows for the customization of vaccines to achieve specific immune responses and improve overall vaccine efficacy.[6] In

detail, the antigens including proteins, peptide fragments, or nucleic acids with specific antigenicity may be selected.[7,8] This allows nanovaccines to be designed specifically for certain diseases and pathogens and enables them to respond more rapidly and selectively to emergent and variant pathogens.[5] Nanovaccines can also provide better control and release of payloads, improving their transmissibility, stability, and persistence. This makes nanovaccines an ideal choice for both preventative and therapeutic vaccination.[9] Compared to traditional vaccines, nanovaccines can more precisely simulate the structure of target pathogens through the design of nanoparticles, thereby enhancing antigen presentation efficiency and eliciting stronger immune responses. Because the nanovaccines consist of components such as antigens, adjuvants, and delivery carriers, they can be tailored for personalized and diverse applications in various diseases and specific populations by adjusting the use of different components.[10] Additionally, nanovaccines can precisely control the dosage of immunogenic payloads to ensure optimal
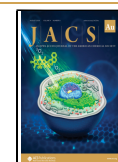
stimulation during the immune process while avoiding unnecessary activation of the immune system, thus reducing the risk of adverse reactions, and improving vaccine safety.[11] However, the immune response elicited is a complex and precise process, and the rational, precise design of nanovaccines and immunization can provide more efficient and safe immune protection.

As a nanoparticle, its size, shape and surface charge have a significant impact on its vaccination efficacy.[12] Researchers have shown that smaller particles can increase their tissue penetration, uptake and processing efficiency in immune cells, enhancing immune stimulation.[13,14] Additionally, the density and spatial distribution of molecular antigens and adjuvants in particles affect immune stimulation more precisely, so their proportions and locations need to be controlled appropriately.[15] The carrier materials of antigens and adjuvants are also crucial for the design of precise vaccines and the precision of immune responses. By selecting the appropriate materials and optimizing their structure, precise spatiotemporal delivery and immune regulation of antigens and adjuvants can be achieved, thereby improving vaccine efficacy and specificity.

To achieve precision vaccination, targeted delivery, appropriate administration routes, consideration for specific populations and controlled release of antigen payloads are also needed.[16,17] This involves the main lymphoid, like lymph nodes (LNs) and spleen, in which nanovaccines can be drained directly or transported through the uptake of APCs.[18,19] Moreover, the nanovaccines with controlled release functions in specific organs or cells will achieve a safer and more precise effect.[20] At the same time, efforts should be taken to develop nanovacccines for both common immune cells, like *ab* T cells and nonmainstream immune cells, like *γδ* T cells. Specifically, nanovaccines targeting tumors via systemic or intratumoral administration could elicit the tumor immune response directly. The nanovaccines may be able to alter the immune cell infiltration and remodel the tumor microenvironment (TME) from a suppressed to an activated state.[21]

In brief, nanovaccines have advantages in terms of antigen and adjuvant selection, carrier selection, immune stimulation regulation, and multifunctionality in precise design. These advantages enable nanovaccines to have more efficient, precise, and personalized vaccine designs, and they are expected to play a precision performance in disease prevention and immunotherapy.[22] In this Perspective, we will introduce the physical factors affecting the immune response of nanocvaccines in general and then present how this group endeavors to develop precise nanovaccines for a defined vaccination in combating viral infection and tumors.

2. PRECISE STRUCTURE OF NANOPARTICULATE VACCINE

The requirements for immune responses vary depending on different antigen types and vaccination purposes. Antibody-mediated humoral responses are crucial for preventive vaccines, whereas antigen-specific cellular immune responses are primarily employed for therapeutic vaccines. To develop rational preventive and therapeutic vaccines, it is essential to gain a deeper understanding of how the size, surface charge, shape, density and spatial distribution of antigens and adjuvants of the nanoparticular vaccine influence the types and intensity of immune responses. Moreover, we further elucidated the feasibility of changing the lipid nanoparticle composition to achieve the organ-targeted property.

2.1. Size of Nanovaccines

Vaccination is to mimic pathogen infection, thereby triggering the immune system to challenge microbe infection and to form an immunological memory against future infection. To initiate an effective immune response, the antigens of the vaccine need to be successfully transported from the injection site to secondary lymphoid tissues, including the region where antigen-presenting cells (APCs), B cells, and T cells are located.[23,24] Several factors influence the transport of particles into the LNs, of which the transport dynamics of particulate vaccines to the LNs are largely dependent on the size of the nanoparticles.[25,26]

Because of the gaps ranging from 30 to 120 nm on the lymphatic capillaries and the smaller junctions (~10 nm) of the blood capillaries, the optimal size of nanovaccines passively drains into LN is 10−100 nm for an *s.c.* or muscular injection.[27] The nanovaccines smaller than 5 nm are rarely considered in vaccine design since they easily penetrate through capillaries into the circulatory system leading to rapid clearance and also immune storms.[28] Therefore, the size of nanoparticles within the range from 10 to 100 nm can be efficiently transported directly to the lymph nodes via lymphatic drainage, activating a significant number of resident immune cells.[29,30] Manolova et al. investigated the transportation of particles by different-sized polystyrene beads (20−2000 nm) and virus-like particles (30 nm) to the draining LNs.[31] The results indicated that small particles (20−200 nm) and virus-like particles by *s.c.* freely drained to the LNs and selectively targeted LN-resident cells. In contrast, the particles with diameters ranging from 0.5 to 5 *μ*m were primarily internalized through phagocytosis, resulting in a lower efficiency of transport to the LNs. Therefore, the size of vaccine particles determines the pharmacodynamics greatly (Figure 1).[32]
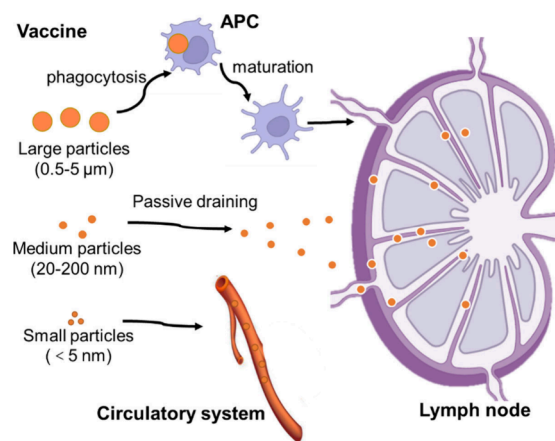

Figure 1. Schematic illustration of the influence of size on lymphatic transportation.

Moreover, the cellular uptake mechanisms (endocytosis, macrophage phagocytosis, phagocytosis, caveolin- and/or vesicle-mediated) of pathogens and particles of different sizes may trigger distinct immune responses.[33] Specifically, virus-sized particles (20−200 nm) are typically internalized by cells through endocytosis mediated by clathrin and caveolin receptors, with a preference for uptake by dendritic cells (DCs).[34] Immunization with small-size nanovaccine produced a robust antigen-specific CD8^{+} T cell response and induced

stronger IFN-γ secretion.[35] Additionally, mice produce a higher ratio of IgG2a antibodies relative to IgG1 antibodies, indicating a bias toward a Th1 phenotype.[36] On the other hand, larger particles (0.5–5 μm) are primarily engulfed by phagocytic cells, inducing potent antibody responses and IL-4-mediated Th2 immunity.[37,38] In summary, smaller particles may elicit responses resembling those against viruses, while larger particles may trigger responses akin to bacterial infections.

Since 2007, nanoparticles used as vaccine platforms have been well investigated.[39] Nanovaccines loaded with antigens or adjuvants have been prepared by either chemical conjugation to the carrier or physical interaction. The former has no concern of off-target delivery of antigens but suffers from the complicated synthesis. The latter relies on physical interaction, including electrostatic, hydrophobic interaction, hydrogen bond complexation and pi-pi stacking, between polymers or lipids and payloads. It exhibits formidable formulation advantages and is more suitable for technology translation.

All in all, the right size of nanovaccines will determine their entry into APC-rich LNs, cellular internalization and antigen presentation, thereby eliciting distinct effects on the immune response and reducing side effects. It is worth noting that size distribution, or polydispersity, is very important to show steady performance, which is critical for quality control and technological translation.

2.2. Surface Charge of Nanovaccines

The surface charge of nanoparticles influences protein corona and various aspects of their interactions with cells, including cellular uptake and intracellular trafficking. Therefore, changing the surface charge of nanovaccines may affect the behavior of their immune responses.[40,41] It is known that the positively charged (cationic) nanovaccines tend to interact more strongly with negatively charged cell membranes, promoting cellular uptake through mechanisms such as electrostatic interactions and membrane disruption. In contrast, negatively charged (anionic) nanoparticles may reduce cellular uptake due to repulsive forces between two identities.[42] Yue et al. prepared three chitosan-based nanoparticles with varied positive charges by the membrane emulsification technique and deposition method. The results showed a positive correlation between cellular uptake rate and surface charge, indicating that the nanoparticles with a positive charge had a higher cellular uptake rate.[43] After cellular uptake, nanoparticles are often encapsulated within endosomes or lysosomes. Positively charged nanoparticles may destabilize endosomal membranes through interactions with negatively charged cell components, facilitating endosomal escape, the subsequent release of the payloads into the cytoplasm and the following cross-presentation. On the other hand, negatively charged nanoparticles may be less efficient in promoting endosomal escape. Kong et al. prepared two nanovaccines encapsulated ovalbumin (OVA) with opposite surface charges of about +32 and −45 mV, respectively. The result indicated that the cationic nanovaccine exhibited stronger escaping capacity from the endosome and facilitated antigen processing via MHC-I presentation pathway. Also, Liang et al. designed a series of amorphous aluminum hydroxyphosphate nanoparticles (AAHPs) with various surface charges. They found that AAHPs with a positive surface charge induced cell membrane disruption and stronger immune stimulation compared to neutral or negatively charged particles. The

positive charge nanoparticles bearing *Staphylococcus aureus* (*S. aureus*) recombinant protein as antigens were able to trigger prolonged and enhanced humoral immune responses and provide protection in an *S. aureus* sepsis mouse model.[44] However, there are still variations in different studies regarding the correlation between particle charge and cellular interactions. Negatively charged nanoparticles sometimes can be efficiently taken by APCs. Yotsumoto et al. discovered that negatively charged phosphatidylserine liposomes encapsulating antigens had more specific immune-regulating capabilities compared to positively charged or neutral liposomes, and could enhance antigen-dependent IL-12 production more effectively by interacting with CD40 on macrophages/dendritic cells and CD40L on targeted cells.[45]

Briefly, the surface charge of nanovaccines plays a critical role in mediating their intracellular transport and subsequent biological effects. Fine-tuning the surface charge of nanoparticles can optimize their cellular uptake and intracellular trafficking, ultimately enhancing their efficacy as drug delivery vehicles or vaccine carriers. It should be cautious for the nanovaccines with strong positive charge because of potential strong cellular toxicity.

2.3. Shape of Nanovaccines

The shape of nanoparticles plays an important role by affecting the tissue/organ biodistribution and uptake of particles by APCs. The common shapes of nanoparticles are spherical-, rod-/wormlike-, disk- and polyhedral. Generally, because of easy fabrication, the nanovaccines self-assembled from lipids or polymers with antigens and/or adjuvants are spherical and thus spherical ones are applied mostly in research and application. The only tunable factor is the diameter of spherical vaccines, which matters in the tissue penetration and cellular uptake.

For rod- or wormlike-shaped nanovaccines, there were few reports using inorganic materials as templates.[46] However, the inorganic materials are hardly controlled in structure and aspect ratio. In contrast, polymer materials may be precisely controlled by chemistry and self-assembly in terms of aspherical shapes.[47] Polymeric wormlike micelles may be obtained by self-assembly of block copolymers. They demonstrate semiflexible conformation which could benefit their tissue penetration. Unfortunately, no studies have been found for vaccine development. Nanodiscs with an increased surface area allowed for more efficient binding and presentation of antigens to APCs, leading to enhanced activation of the adaptive immune response. By mimicking high-density lipoproteins, Kuai et al. have synthesized nanodiscs of 9–13 nm of phospholipids and apolipoprotein-like proteins to load antigenic peptides and adjuvants. The vaccines can significantly improve the transportation efficiency of antigen and adjuvant to lymphoid organs and showed sustained antigen presentation on dendritic cells, resulting in a large number of virulent T-cells that can specifically recognize tumor cells and kill them. It has also been shown that the disc shape of the phospholipid bilayer provided better tissue penetration than spherical nanoparticles.[48]

Herein, the shape of nanovaccines profoundly influences immune responses by affecting cellular uptake by APCs, immune cell activation pathways, lymphatic drainage and cellular and humoral response. Understanding these shape-dependent immune responses is vital for designing nanovaccines tailored to specific pathogens or diseases, ultimately improving vaccine efficacy and immune protection.
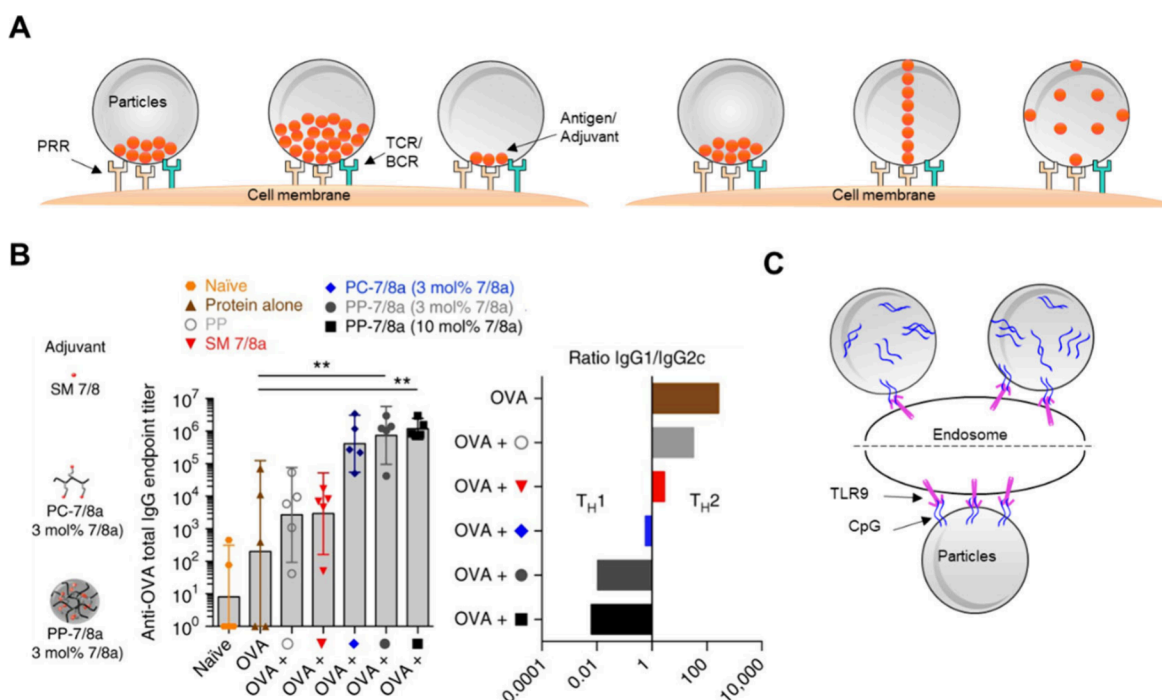
Figure 2. (A) The density and distribution of antigens or adjuvants affect the recognition of pattern recognition receptors (PRRs) on the cell surface. (B) High adjuvant density particles induced stronger humoral and Th1 immune responses. (C) Traditional nanoparticles activate downstream immune responses by carrying excess CpG to achieve binding to toll-like receptor 9 (TLR9). The nanoparticles precisely modified with CpG at specific distances are capable of inducing comparable or stronger immune responses than conventional particles at the lowest dose. Reproduced with permission from ref 51. Copyright 2015 Springer Nature.

2.4. Density and Spatial Distribution of Antigens and Adjuvants

Effective inactivated and attenuated vaccines can activate the B cell response to produce antibodies. This is attributed to that the distribution of antigen epitopes on the surface of these vaccines is highly ordered and repetitive, causing extensive cross-linking of B cell receptors and giving a strong stimulatory for cascade immune response. Thus, the molecular antigens and adjuvants at a repetitive density on the surface of synthetic nanovaccines are critical for inducing a stronger immune response (Figure 2A).[49,50] Generally, the higher densities of antigens on the nanoparticles can provide more efficient epitope presentation to B and T cells. Meanwhile, an increased density of adjuvants on the nanoparticles can promote the maturation and activation of APCs, leading to the upregulation of innate immune receptors and signaling pathways.

Vehicles such as protein-, lipid-, polymer-, and DNA origami-based nanoparticles have been used to study the effects of antigenic distribution on particle surfaces to immune responses. For example, virus-like particles (VLPs) as supports for antigens can accommodate a wide range of antigens through chemical conjugation and gene fusion, making them versatile platforms for nanovaccine development. Early studies found that antigen spacing of 5−10 nm on VLPs was sufficient to promote B cell activation and induce strong IgG levels. Similarly, the self-assembling carrier platform based on homeostasis proteins is suitable for adjusting the antigen distribution. Such as ferritin cage, a protein involved in intracellular iron storage that is found in nearly all organisms and consists of 24 monomers.[52] For instance, Kanekiyo et al. inserted hemagglutinin (HA) into the interface of adjacent subunits of ferritin to generate eight trimeric viral spikes on the surface.[53] The ferritin nanoparticles presented the trimeric HA

spikes in their natural conformation rigidly and symmetrically, and the 28 Å distance between the spikes ensured broadly neutralizing antibodies against H1N1. The immunization with this nanovaccine produced HA inhibitory antibody titers ten times higher than the inactivated vaccines.

Furthermore, Hanson et al. investigated the effect of antigen density on immunogenicity by anchoring the membrane-proximal external peptide of gp41 (a segment of Human Immunodeficiency Virus (HIV) envelope)) to the liposome surface.[54] The results showed that higher titers were produced when there were 40−1000 peptide chains per liposome, but the titer level decreased significantly when the number of peptide chains reached 2000, indicating that the optimal average distance between peptides on the liposome surface was in the range of 7−17 nm. Lee et al. designed polymer-templated protein nanoparticles bearing different antigen densities.[55] The extent of mouse bone marrow-derived dendritic cell (BMDC) activation as well as cytotoxic T lymphocyte (CTL) activation increased with the increase of antigen density, protecting the mice from different subtypes of H1N1 infection. Likewise, Lynn et al. coupled TLR7/8 agonists to a linear polymer with temperature-responsive *N*-(2-hydroxypropyl)methacrylamide and assessed the effects of agonist density in the particles on the location, magnitude and duration of innate immune activation in vivo (Figure 2B).[56] It was found that high-density agonist particles enhanced the retention of particles in draining lymph nodes, which induced activation of APCs and strong humoral immune and CD8+ T cell response, compared with low-density agonist particles.

The DNA origami utilizes the programmable base-pairing properties of DNA molecules to create complex nanostructures with specific quantities and geometric patterns of antigens or adjuvants. Zhang et al. found that the DNA origami-based

nanovaccine (∼74 nm) showed an enhanced binding affinity to host receptor angiotensin-converting enzyme 2 (ACE2) with the number increasing of receptor-binding domain (RBD) antigen of SARS-Cov-2.[57] Additionally, the concentrated distribution of RBDs showed a faster and stronger affinity to the ACE2 than an even RBD distribution. Additionally, the DNA origami strategy was also used for precise distribution of adjuvant. For example, Comberlato et al. distributed TLR9 agonists at a distance of 7 nm, matching the activated dimeric structure of the TLR9 receptor.[58] The nanoparticulate adjuvant based on DNA origami precisely modified with CpG is capable of inducing comparable or stronger immune responses than conventional particles at the lowest dose (Figure 2C).

Appropriately increasing the density of antigens or adjuvants on the particle surface can enhance the affinity to cells, but too high a density will induce various immune characteristics.[59] Kapadia et al. found that nanoparticles with a high density of antigen peptides on the surface mainly promoted the activation and proliferation of BMDCs and T cells, while nanoparticles with a low density of antigen peptides on the surface were able to induce stronger CD8+ T cell responses.[60] In addition, Brewer et al. found that low-density antigen loading on polystyrene nanoparticles was more conducive to B cell signaling in germinal centers and follicular helper T cell responses, thereby stimulating antigen-specific B cells to produce high concentrations of antibodies.[61]

2.5. Organ-Targeted Lipid Nanoparticle with Precise Composition

Lipid nanoparticles (LNPs) are the most advanced mRNA carriers in clinical practice. The mRNA could be encapsulated by the ionizable lipid along with the PEGylated lipid, helper lipid and cholesterol through pH exchanging from 4 to 7.4. At present, the mRNA was delivered by LNP mainly expressed at the muscle of the injected site and liver. For example, the LNP formulated with ionizable lipids, 8-[(2-hydroxyethyl)[6-oxo-6-(undecyloxy) hexyl]amino]-octanoic acid, 1-octylnonyl ester (SM-102), and 1,1′-[[(4-hydroxybutyl)imino]di-6,1-hexanedi-yl] ester (ALC-0315) were mainly targeting and expressing in muscle and liver.[62] This includes our discovery of ionizable lipids with new structures by one-step chemistry using the Ugi four-component reaction under mild conditions.[63] At this stage, the development of special organ-targeted mRNA vaccines other than the liver (such as the lungs and kidneys) needs to be urgently addressed.

The existing LNP targeted delivery of mRNA strategy is mainly achieved by changing the LNP formula, including designing and constructing a special structure of ionizable lipid library, changing the ratio of the four components of LNP, and adding a fifth component (Figure 3A,B). Among them, adjusting the formula includes adding other components besides the four components of LNP, for example, the selective organ targeting (SORT) LNP strategy, which mainly changes the mRNA delivery preference from the liver to other tissues by adding another charged lipid in the LNP. In particular, quaternary ammonium lipids are added as the fifth ingredient to traditional LNPs to form five-component LNPs with lung-specific mRNA delivery properties.[64] Similarly, Cheng et al. obtained lung-selective LNPs that can accurately deliver mRNA to the lungs by adding additional permanent cationic targeting lipids to the liver-enriched LNP formula and replacing DSPC with DOPE. Delivering mRNA encoding
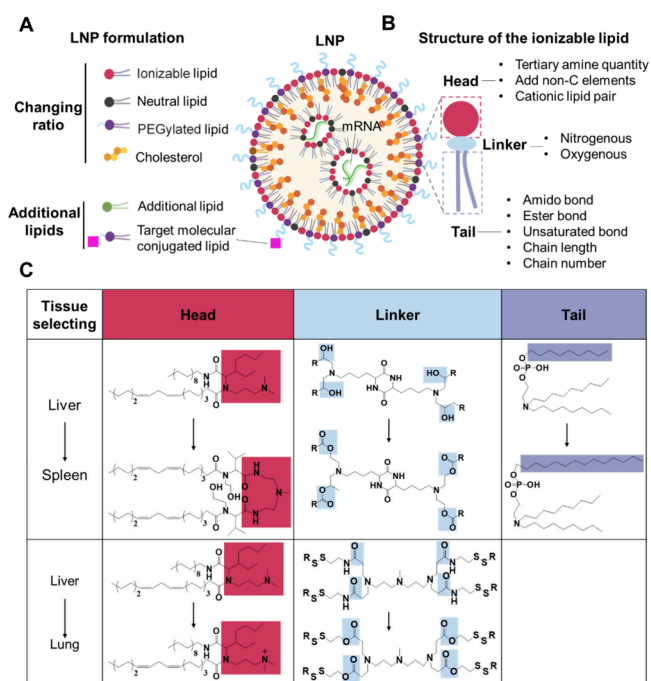

Figure 3. (A) Schematic of changing the LNP formulation and (B) the structure of ionizable lipid. (C) Alter the head, linker, or tail of ionizable lipids can change the tissue selecting targeting.

broadly neutralizing antibodies to SARS-CoV-2 can produce high-titer antibodies in the lungs and has achieved significant results in preventing and treating infections with mutant strains of SARS-CoV-2.[65] However, adding another charged lipid to LNP may complicate the current LNP formulation. It is more advantageous to explore adjusting the ratio and chemical structure of LNP lipid components. For example, Cheng et al. achieved selective targeting of mRNA delivery from the injection site/liver to the lungs by changing the ratio of lipid components in LNP. Through intravenous injection, the LNP-IL-15 showed better antitumor effects with less systemic exposure and fewer cytokine-related risks.[66]

In addition, changing the molecular structure of ionizable lipids can also change the tissue targeting. Designing the lipid molecular structures include investigation of the structure–activity relationships of the headgroup, tail chain, and linker structures (Figure 3C). For example, Zhao et al. developed a silicon-containing on the head of ionizable lipid through the Michael addition reaction, which can deliver mRNA to the targeted lung endothelium through LNP formulation. By delivering Vega mRNA, a key downstream effector of TGF-βR2, pulmonary vascular repair after viral infection was improved.[67] Xu et al. have demonstrated that the head, linker and tail structures of ionizable lipid compounds can affect the delivery effect and even the in vivo targeting of mRNA-loaded LNPs. For example, they designed a lipid molecule, 113-O12B, with a specific structure that can target lymph nodes with high specificity. Using ALC-0315 as a control, subcutaneous injection results showed that 113-O12B and ALC-0315 had significant signals in lymph nodes, but the latter had higher expression in the liver.[68] In addition, imidazole-based synthetic lipid compounds preferentially target mRNA to the spleen. The synthetic tail structure contains an amide bond lipid library (N-series LNPs). delivered mRNA almost exclusively to the lungs after systemic administration. This contrasts with the

previous discovery that O-series LNPs (containing ester bonds in the tail) tend to deliver mRNA to the liver. It was also found that simply adjusting the head structure of the N-series LNPs could achieve targeting of different types of lung cells.[69−71] Recently, our team has developed a cationic lipid pair strategy to achieve targeting from the liver to the lungs. This is mainly based on the modification of the hydrophilic head of the ionizable lipid for liver-targeted expression with a quaternary ammonium group to form a cationic lipid pair with a tertiary amine. The LNP prepared in one step achieves the purpose of mRNA lung-targeted expression.[72]

Research on LNP composition and ionizable lipid structure is rapidly advancing, with a focus on improving tissue targeting, delivery efficiency, and safety. Future developments will likely see more sophisticated and targeted LNP systems, expanding the potential of mRNA-based therapies and other nucleic acid drugs. Ongoing research aims to better understand the relationship between the chemical structure of ionizable lipids and their biological activity, which will help in designing more effective lipids. In addition, more attention should be paid to the "protein corona" formed since the nanoparticles enter the blood, which reshapes the surface properties of the nanoparticles and greatly affects the interaction of the nanoparticles with organs and cells.

3. PRECISION VACCINATION USING DEFINED NANOVACCINES

3.1. Vaccination via Different Routes

Each vaccine has a recommended administration route. The available administration routes of vaccines in clinical application and on study include oral (PO), intramuscular (IM), subcutaneous (SC), intradermal (ID), intranasal (NAS), inhalational (INH) and intravenous (IV) administration (Figure 4). For example, the Rotavirus vaccine (Rotarix,

Figure 4. Schematic of the different routes for vaccination. The picture was created with BioRender.com.

RotaTeq) is the only routinely recommended vaccine administered orally. The attenuated influenza (FluMist) vaccine is the only vaccine administered by the intranasal route. Combination vaccine, MMRV (combines the attenuated virus measles, mumps, rubella and varicella) is only administered by intradermal route. Convidecia Air, a recombinant COVID-19 vaccine based adenovirus type 5

vector was administered by inhalational routes. Vaccines administered via IV route is usually part of clinical trials or experimental treatments, not routine practice because it can increase the risk of adverse reactions. In the research field, Darrah et al. found that simply changing the way the TB vaccine is administered could dramatically improve its efficacy.[73]

For nanovaccine, the administration routes can be mainly divided into two categories according to the sites of the immune response. One is systemic vaccination, including SC, IV, IM and ID, in which the immune response will occur in systemic since the nanovaccine vaccinated. Generally, nanovaccines smaller than 100 nm administrated systemically are primarily internalized by APCs or directly drained to the lymph nodes, where they activate T cells and B cells. Subsequently, they diffuse into the bloodstream or lymphatic circulatory system. Specifically, vaccination by ID requires high technical skills of the operator. Fortunately, the microneedle (MN) with 50 to 1100 μm matrix needles may as a powerful tool for delivering vaccines into the skin precisely. The MN vaccine was designed by chitosan oligosaccharide as a delivery system to load DNA that encodes spike and nucleocapsid proteins of SARS-CoV-2. It induced a high level of neutralizing antibody against SARS-CoV-2 variants. Surprisingly, the MN vaccine elicited superior systemic and mucosal T-cell immunity, demonstrating enhanced magnitude, polyfunctionality, and persistence. Notably, the MN vaccine can be stored at room temperature for at least one month without a significant decrease in its immunogenicity.[74]

Although the traditional vaccination strategy including IM and SC could induce strong humoral immunity and cellular immunity responses in the system, the mucosal immunity act as the first line of defense against pathogens is essential. Generally, the nanovaccines are administrated via PO, NAS or INH can active the immunoglobulin A (IgA) immune response in mucosal-associated lymphoid tissues (MALTs). Compared with traditional injectable vaccines, mucosal vaccines have the advantages of easy administration and simple manufacturing procedures. In addition, for people with weakened immune systems, oral vaccines avoid trauma to blood vessels and circulatory systems, making them an ideal option. However, the development of new vaccines must address the problem of natural immune tolerance and the difficulty of penetrating the mucosal layer. Considering the flexibility and multifunctionality of nanovaccine preparation, new mucosal vaccines based on recombinant subunit antigens and mRNA can incorporate adjuvants to overcome immune tolerance. Additionally, using functional delivery carriers can help penetrate the mucosal layer.

Moreover, the administration routes of vaccines affect the type of immune response. Mohanan et al. revealed that IgG2a, linked with Th1-type immune responses, is influenced by the route of administration, whereas IgG1 responses associated with Th2-type immune responses exhibit relatively little sensitivity to the administration route of ovalbumin-loaded liposomes, N-trimethyl chitosan (TMC) nanoparticles, and poly(lactide-co-glycolide) (PLGA) particulate delivery systems.[75] Similarly, Bian et al. found that subcutaneous injection can induce immune response more quickly and efficiently after the first vaccination, but intramuscular injection is more conducive to enhancing the immunogenicity of adjuvant after booster vaccination. In addition, compared with the vacci-
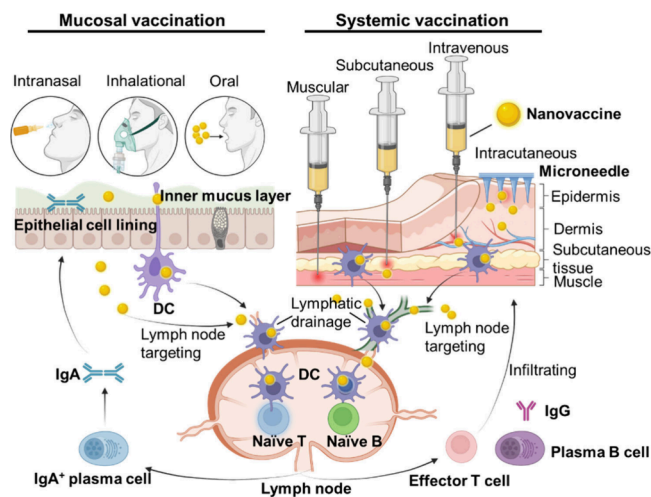
Table 1. Vaccination Recommended for Specific Populations[a]

Population	Vaccines	Recommendation
Elderly	VZV	Recommended for ≥50 years of age.
	Influenza	• Vaccinated annually, typically starts in the fall and peaks in winter.
		• High dose or adjuvanted.
	PCV	• Recommended for ≥65 years of age.
		• PCV13 first, followed by PPSV23 at least one year later.
		• If PPSV23 was given before age 65, administer a second dose of PPSV23 at least five years after the first dose.
		• For certain medical conditions, a shorter interval between PCV13 and PPSV23 may be appropriate.
	RSV	• Recommended for ≥60 years of age.
		• Elderly individuals with chronic health conditions or weakened immune systems are particularly encouraged to get vaccinated.
		• Can be given with other vaccines.
	Tdap	• Recommended for ≥65 years of age.
		• Td or Tdap booster is recommended every 10 years to maintain protection.
		• Elderly individuals have close contact with infants under 12 months old.
	COVID-19	• Recommended for ≥65 years of age.
		• A booster dose is recommended to maintain and target both the original strain and specific variants.
Pregnant women	Influenza	• Inactivated influenza vaccine (IIV) is recommended.
		• Vaccination at any time during pregnancy.
		• Vaccinated annually, typically starts in the fall and peaks in winter.
	Tdap	• During each pregnancy.
		• Preferably between 27 and 36 weeks of gestation.
	RSV	• Administered between 32 and 36 weeks of gestation
Preterm infants	All pediatric vaccines	• Strengthen education for healthcare providers and parents to understand the necessity of vaccination on time.
		• Mothers during pregnancy and close contacts should be vaccinated to create a "cocooning" effect that helps protect the baby.
		• RSV vaccines are used during pregnancy to transfer protection to newborns.
Immunocompromised/Chronic conditions	Influenza	• Patients with HIV infection, recommend live attenuated influenza vaccine.
		• Immunocompetent children receiving maintenance chemotherapy.
		• Hematopoietic stem cell transplant patients.
		• Patients of solid organ transplantation (After transplantation).
		• Diabetic patients.
	HPV	• Hematopoietic stem cell transplant patients.
		• Patients of solid organ transplantation (Before transplantation).
	PCV/PPSV	• Cancer patient after 3 months after chemotherapy.
		• Asplenia or sickle cell disease.
		• Patients receiving immunosuppressive medications.
		• Before cochlear implantation.
		• Patients of solid organ transplantation (Before/after transplantation).
		• Diabetic patients.
	MenACWY	• Hematopoietic stem cell transplant patients.
		• People with complement component deficiencies, functional or anatomic asplenia, and those with HIV infection.
	HBV	• Patients of solid organ transplantation (Before transplant).
		• Hematopoietic stem cell transplant patients.

[a]Abbreviations: RSV, Respiratory syncytial virus; PCV, Pneumococcal conjugate vaccine; Tdap, Tetanus, diphtheria and pertussis; HPV, human papillomavirus; HIV, Human immunodeficiency virus; PPSV, Pneumococcal polysaccharide vaccine; MenACWY, Meningococcal conjugate vaccine; HBV, Hepatitis B virus.

nation route, adjuvant is a more decisive factor in regulating antibody production and polarization of immune response.

3.2. Vaccination for Specific Populations

Achieving precision vaccination demands a nuanced understanding of the diverse characteristics within populations and the design of targeted strategies to optimize vaccine efficacy. The research emphasized the necessity of tailoring vaccine designs to meet the distinct needs of specific populations, such as the elderly population, preterm infants, pregnant women and patient populations. This insight underscores the critical role of population-specific precision in vaccine development to ensure optimal immune responses in various populations.[76] In this perspective, we list the vaccines recommended for specific populations and their recommendations (Table 1).

Vaccination for the elderly: The aging population presents unique challenges in healthcare, and the development of precision-designed nanoparticulate vaccines tailored specifically for the elderly is increasingly recognized as a vital avenue for advancing public health. Age-related immunosenescence and inflammaging make the elderly easier to be infected with infectious diseases. The diminished functionality of both innate and adaptive arms of the immune system in the elderly population constitutes a primary factor contributing to the

inefficiency or ineffectiveness of vaccination. This phenomenon results in reduced toll-like receptor expression, leading to a decrease in the secretion of pro-inflammatory factors, ultimately constraining the adaptive immune system.[77,78]

The existing strategies for the improvement of vaccination in the elderly include high-dose administration, adjuvant optimization, and novel vaccines along optimized vaccination routes. First, enhancing the dose of antigen or adding a boost in immunization could improve the presentation of the APCs, eliciting a stronger innate immune response. The method was used in A split-virus vaccine for influenza viruses named Fluzone HD produced by Sanofi Pasteur, which containing 4 folds of HA per strain compared to the standard dose and has been approved by the US Food and Drug Administration (FDA) for the prevention of influenza in population aged 65+.[79] Centers for Disease Control (CDC) recommended that adults over 65 years and older receive an additional updated 2023−2024 COVID-19 vaccine dose.[80] Another feasible strategy for elderly vaccination is using suitable adjuvants, which can enhance the immunogenicity and sparing dosage and change the polarization of the immune response to a desirable response. The most successful adjuvants for the elderly are the AS01 adjuvant system developed by Glaxo Smith Kline, made of two immunostimulants, QS21, a saponin, and MPL, a TLR4 agonist. The AS01 adjuvanted the recombinant antigen, gE, showed a 97% protection efficacy for VZV in those ≥50 years of old and achieved over 90% in those ≥80 years of old.[81] Another promising vaccination strategy is the mRNA vaccine, which could elicit robust efficacy in all age groups. For instance, the Pfizer−BioNTech mRNA vaccine candidate BNT162b2 elicited >93% efficacy across all treatment groups defined by age in phase III trials. The Moderna vaccine candidate mRNA-1273 showed 86.4% efficacy in volunteers ≥65 years old.[82] The mRNA vaccines for COVID-19 successfully applied indicated that the mRNA-based vaccine is promising for the elderly.

Vaccination of pregnant women: The preparation of vaccines for maternal use is imperative for safeguarding the health of both mothers and newborns. Surveillance data from the United States estimate that women who contract influenza during pregnancy have a 7-fold increased risk of hospitalization and a 4-fold increased risk of admission to the intensive care unit or death compared to women who contract influenza when not pregnant.[83] A randomized controlled trial of a trivalent inactivated influenza vaccine in Bangladesh showed that maternal vaccination was 63% effective against confirmed influenza in infants and reduced influenza-like illness in infants and mothers by 29% and 36%, respectively.[84] However, influenza prevention is rarely a priority for countries with very limited health budgets. Tetanus and pertussis vaccination is also widely recommended, with effectiveness trials showing that maternal vaccination with two to three doses reduces neonatal tetanus incidence by 80% and mortality by up to 98%.[85] Additionally, other vaccines, such as Pfizer's RSV vaccine Abrysvo, can reduce the risk of severe lower respiratory diseases (LRTD) in infants by 8.90% within 81 days of birth and by 4.180% within 69 days of birth. In the subgroup of pregnant women with a gestational age of 32 to 36 weeks, Abrysvo reduced the risk of LRTD by 34.7% and severe LRTD by 1.90% within 91 days of birth. Within 180 days after birth, Abrysvo reduced the risk of LRTD by 57.3% and severe LRTD by 76.5%.[86]

Nevertheless, the proportion of pregnant women who receive the recommended vaccines is lower than expected, mainly due to concerns about adverse pregnancy outcomes, inconvenience of vaccination, and lack of knowledge about the recommended vaccines.[87] Therefore, the focus of expanding maternal vaccination should be to improve the expertise of vaccine healthcare providers and to raise the importance of vaccination among pregnant women through education.

Vaccination on preterm infants: Preterm infants are those born at <37 weeks gestation. Extremely preterm infants are born at <28 weeks gestation. Nearly 11% of newborns worldwide are born prematurely each year.[88,89] Preterm infants with immature immune systems and low antibody levels are particularly susceptible to infections. Maternal antibody transfer across the placenta begins to increase gradually from the 17th to 18th week of pregnancy, so full-term infants have higher plasma antibody titers than premature infants. Additionally, premature infants receive less passive protection from breast milk, which leads to changes in the colonization of commensal flora in the intestine and nasopharynx, making them more vulnerable to pathogenic infections. Moreover, during long-term hospitalization in neonatal intensive care units (NICUs), the use of broad-spectrum antibiotics and steroids, along with a lack of breastfeeding, can compromise protective barriers such as the skin and respiratory tract.[90]

Studies have shown that premature infants can typically produce adequate immune responses through vaccination, and it is recommended that they be vaccinated promptly according to their chronological age. However, vaccination plans for premature infants are often unreasonably delayed. This delay is primarily due to a lack of understanding of the safety and effectiveness of vaccines for premature infants among healthcare professionals and parents, as well as fears of adverse events. Therefore, it is crucial to emphasize evidence-based education for healthcare providers regarding neonatal immunization and to develop general guidelines for public education.

Another strategy to protect premature infants is to ensure comprehensive immunization for the mother during pregnancy (such as pertussis, tetanus, and influenza vaccinations), as well as for close contact and family members after the birth of the premature infant. For premature infants with special immune backgrounds, tailored immunization methods and careful selection of vaccines should be adopted. For instance, while the BCG vaccine is usually injected under the deltoid muscle of the right arm, intradermal injection is recommended for premature infants weighing more than 2 kg.[91] For infants whose mothers used immunosuppressants during pregnancy or have a family history of immunosuppression, BCG vaccination should be postponed or prohibited. Newborns whose mothers are chronic carriers of hepatitis B virus (HBsAg positive) should receive hepatitis B vaccination within 12 h after birth, followed by administration of specific high-titer hepatitis B immune globulin (HBIG) within the first few days of life.[92]

Immunocompromised/Chronic conditions: Vaccination is crucial for individuals with immunocompromised conditions or chronic illnesses due to their higher risk for severe infections. Immune deficiencies are classified into inherited and secondary types. Inherited immunodeficiencies include B-cell deficiencies, which lead to antibody deficiencies, and T-cell deficiencies, such as DiGeorge syndrome, which can result in vaccine inefficacy. Complement and phagocytic defects are also part of inherited immunodeficiencies. Secondary immunodeficiencies arise from factors like HIV infection, immunosuppressive
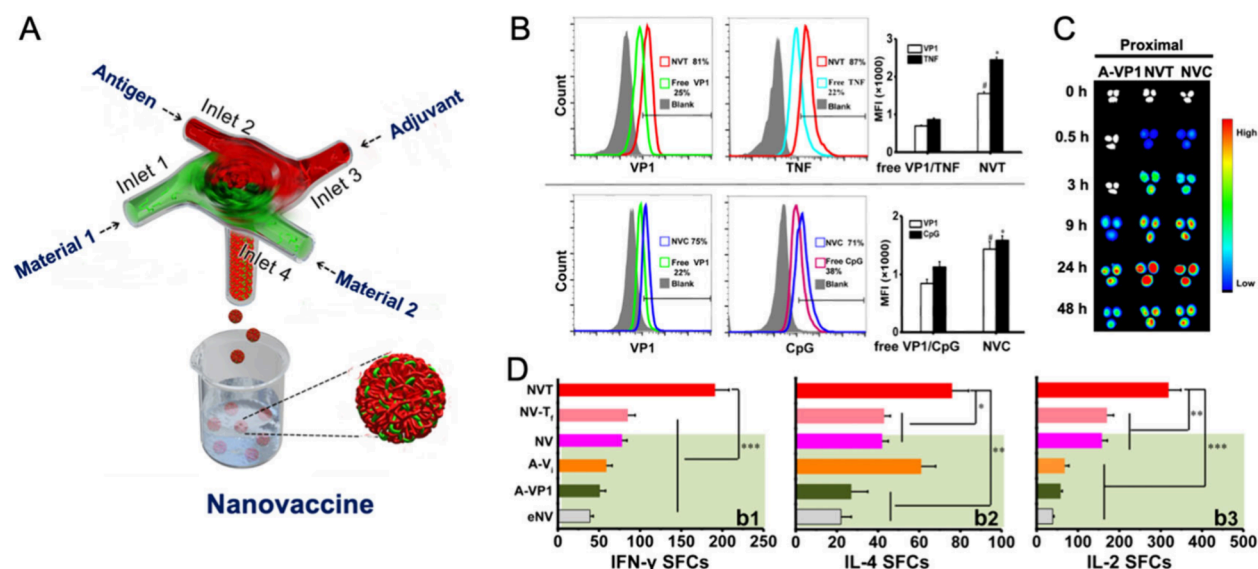
Figure 5. (A) Schematic of HFMD nanovaccine preparation by FNC technic. (B) TNF-α or CpG as adjuvants promotes cell uptake by BMDCs. (C) Nanovaccine enhanced LN targeting. (D) The IFN-γ, IL-4 and IL-2 secreted in serum postvaccination of different forms of vaccines. Reproduced with permission from ref 97. Copyright 2018 ACS Publications.

agents, autoimmune diseases, tumors, organ transplantation, asplenia, sickle cell disease, and diabetes.[93] Notably, vaccination against bacterial meningitis is crucial before cochlear implantation.[94] Inactivated vaccines like influenza, pneumococcal, and hepatitis are generally recommended and safe, while live vaccines are usually avoided. Specific conditions such as acquired immune deficiency syndrome, cancer, organ transplantation, autoimmune diseases, diabetes, chronic kidney disease, and chronic respiratory conditions require tailored vaccination plans, often involving annual influenza vaccines and pneumococcal vaccines. Consulting healthcare providers for personalized recommendations, considering vaccine timing, and monitoring for adverse reactions are essential for ensuring safety and efficacy.

Collectively, by acknowledging and addressing these population-specific factors, researchers can design vaccines that are not only effective but also tailored to the unique characteristics of diverse demographic groups. Such precision in vaccine development is essential for achieving the overarching goal of precision vaccination and ensuring optimal protection across populations with varied physiological and immunological landscapes. For the latest and most accurate recommendations, elderly individuals should refer to guidelines from health authorities such as theCDC, the Advisory Committee on Immunization Practices (ACIP), and the World Health Organization (WHO). Consulting with healthcare providers is crucial for tailored advice and scheduling.

3.3. Nanovaccines with Rapid LN Accumulation, Long Retention, and Enhanced Endosome Escape

It has been shown that small-sized nanoparticles may deliver antigens or/and molecular adjuvants more efficiently to reach the LNs, which are rich in functional immune cells. Therefore, nanovaccines trafficking to the LNs have become the focus of new types of vaccines of recombinant proteins and mRNA for combating microbe infection and tumor threats. For further technology translation, a technology of nanovaccine production is needed not only to provide a robust process for fine control over particle size and size distribution but also to enable the highly efficient loading of antigens and adjuvants.

Conventional bulk-mixing methods fail to meet the clinical requirements due to inherent heterogeneous mixing in large batches that leads to wide-size distribution and poor batch-to-batch reproducibility. Recently, the flash nanocomplexation (FNC) technology based upon noncovalent interaction between water-soluble materials and functional biomacromolecules has emerged for the fabrication of protein nanodrugs.[95]

After demonstrating the concepts with insulin for oral administration to control sugar level, Chen et al. and collaborators applied the FNC process with four solution inlets to achieve a homogeneous mixing of different components for producing high-quality and potent nanovaccines.[96] As shown in Figure 5A, they prepared a hand-foot-mouth disease (HFMD) nanovaccine containing recombinant VP1 protein antigen from enterovirus 71 adjuvanted by either CpG or TNF-α using chitosan and tripolyphosphate (TPP) as carriers.[97] Polyelectrolyte interaction occurs in the mixing chamber between chitosan and TPP drives the formation of nanogels, and, at the same time, CpG or TNF-α is loaded by charge interaction. Thus, prepared nanovaccines exhibited narrower size distribution, high throughput (up to 20 mL/min), high encapsulation efficiency (80−90%), high antigen loading capacity (30−36 μg/mg), and coencapsulating different types of adjuvants, compared to the batch mixing with the same components. These features are all important for nanovaccine manufacturing. It is noteworthy that the formulation process is highly reproducible in terms of particle size, polydispersity, theta potential, loading efficiency and contents in different runs and scales. Two versions of the nanovaccines, TNF-α or CpG as an adjuvant, reached both proximal and distal LNs following SC injection, exhibited prolonged retention in LNs and elicited potent immune responses against VP-1 antigen (Figure 5B−D). These nanovaccines elicited not only potent antibody responses comparable to the inactivated viral vaccine but also strong Th1 response, which the inactivated viral vaccine failed to generate in this animal model and the clinical trial. In collaboration with Sinovac, they confirmed that the immune responses generated by the nanovaccines could confer effective protection against
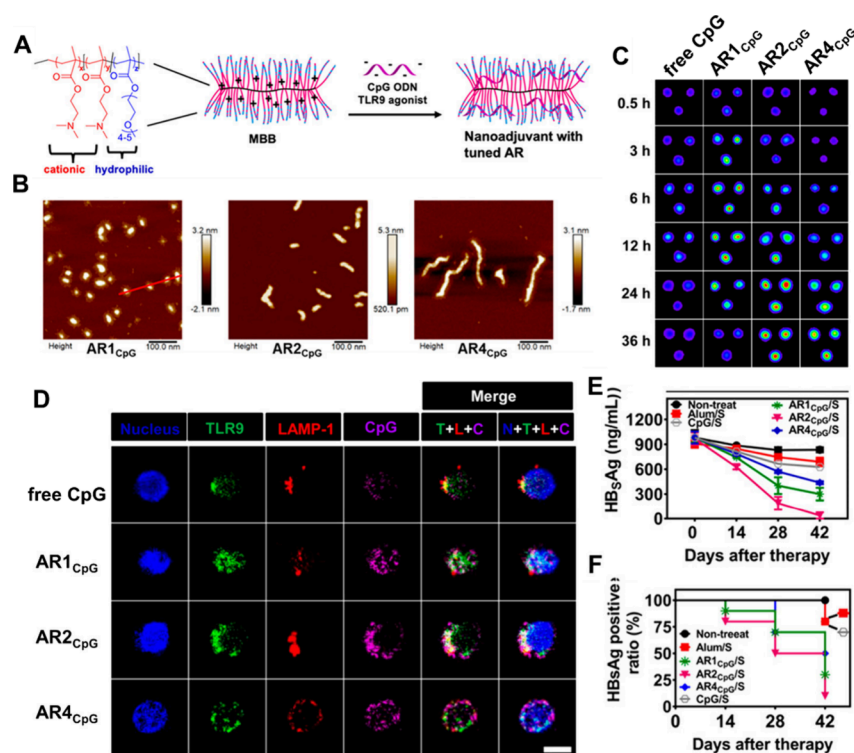
Figure 6. (A) Schematic of cationic molecular bottlebrushes (MBBs) with tuned AR and loaded with anionic CpG by electrostatic interactions. (B) AFM images of three MBBs loaded with CpG. (C) Proximal LNs observed with IVIS imaging after SC injection. (D) Confocal images of CD11c⁺ cells extracted from popliteal LNs at 24 h after injection. The cell nucleus was stained with Hoechst 33342 (blue), TLR9 was stained using anti-TLR9-FITC (green), and LAMP-1 was stained using anti-LAMP1-Cy3 (red). In merged images, N, T, L, and C indicate the nucleus, TLR9, LAMP-1, and CpG, respectively (scale bar, 5 μm). (E) HBsAg levels in the blood were detected after different formulation therapy. (F) HBsAg positive ratio of HBV carrier mice was evaluated after different formulation therapy. Reproduced with permission from ref 103. Copyright 2021 ACS Publications.

virus challenges in mouse models of both passive and active immunization. This study demonstrates an excellent translational potential of this nanovaccine manufacturing platform.

Specifically, microfluidic technology is well-known in the application for preparing lipid nanoparticles (LNPs), such as the mRNA vaccines against COVID-19 as well as the development of siRNA drugs.[98,99] Generally, the RNA-loaded LNPs were fabricated by mixing several lipids in the ethanol phase with RNA in a buffer solution via a two-channel microfluidic device. The change of solvent property drives the formation of LNPs bearing RNAs. Simplicity, high-quality control and scalability formulation become very important issues for mRNA vaccine application during the pandemic.

Chronic hepatitis B virus (HBV) infection (CHB) causes significant morbidity and mortality and is one of the important public health problems worldwide. Therapeutic vaccines for CHB are promising immunotherapies whose aim is to activate innate immunity and HBV-specific adaptive immunity to eradicate HBV and achieve CHB cure. However, the development of therapeutic vaccines for CHB has been a major challenge, and nearly all candidates have failed to elicit efficacious immune responses. In another study, we applied FNC to fabricate one nanovaccine containing recombinant HBV surface antigen and CpG adjuvant (NSG) and one nanovaccine containing recombinant HBV core antigen and CpG adjuvant (NCG). Due to high traffic to draining lymph nodes, coadministration of the NSG+NCG nanovaccines could break immune tolerance and restore HBV-specific immune responses in a mouse model of CHB. Most of the model mice

achieved HBsAg seroclearance and anti-HBsAg antibody seroconversion. We also confirmed that the coadministered nanovaccines elicited durable immune memory in HBV-cured mice, protecting them from HBV rechallenge. The study demonstrates that the NSG+NCG nanovaccine is a safe, effective, and clinically translatable therapeutic CHB vaccine candidate.[100]

In the above two cases, codelivered molecular adjuvants are very important by exerting their regulatory influence on Th cell polarization through a combination of innate immune activation, cytokine modulation, antigen presentation enhancement, dendritic cell maturation, and the induction of memory T cells. The codelivery strategies were also adopted in our other two studies. One is the research on COVID-19 related nanovaccine, which codelivered TLR4 (MPLA), TLR9 (CpG) associated adjuvants and S1 antigen of SARS-CoV-2 showed a potent humoral and cellular immune response.[101] Another study is using subunit proteins to support TLR1/TLR2 adjuvant, Diprovocim by pi-pi stacking, could stimulate strong antibody titers and CD4⁺ and CD8⁺ T cell immune responses in mice.[102] To achieve a precision vaccination, the adjuvants should be selected based on the characteristics of the targeted pathogens or disease to define the immune response for optimal protection. In general, the Th1-biased adjuvant should be chosen for tumor treatment, while it is necessary to stimulate both Th1 and Th2 effective responses for microbe infection.

Although aspherical particles have been applied extensively to interpret how their shape affects the physical properties in
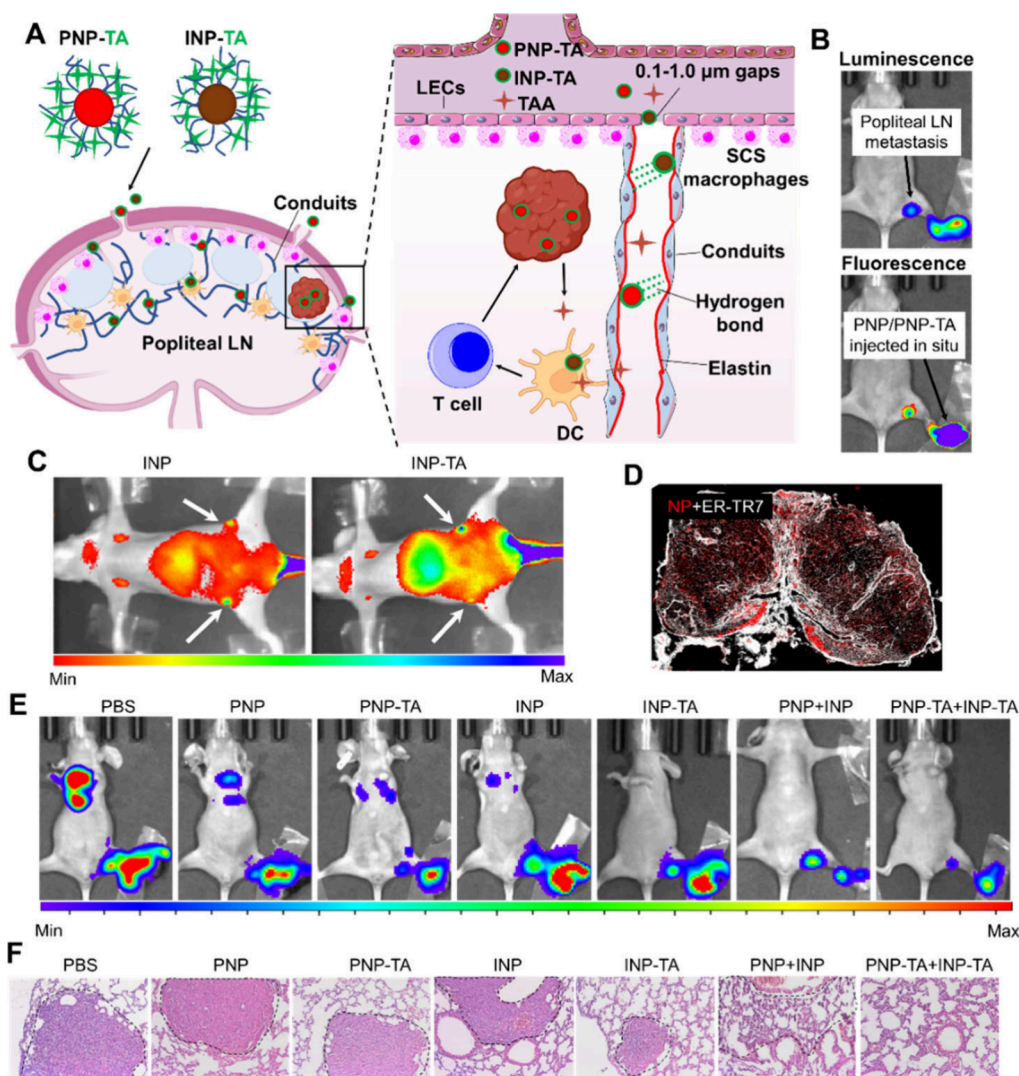
Figure 7. (A) Schematic of the tannic acid-modified nanoadjuvant and nanopaclitaxel targeting metastatic lymph node. (B) Images of building popliteal LN metastasis model and injection of PNP or PNP-TA *in situ*. (C) INP-TA distribution in inguinal lymph nodes at 24 h postinjection (color bar scale of radiant efficiency) (D) INP-TA showed enhanced enrichment in LNs via conduits targeting. (E) Representative images of tumor therapy with PNP, PNP-TA, INP and INP-TA, and monitoring the metastasis inhibition of LNs and lungs. (F) HE staining of lungs from the mice of different treated groups. Abbreviations, INP: IMQ nanoadjuvant; PNP: PTX nanoparticle; INP-TA: Tannic acid supported INP; PNP-TA: Tannic acid-supported PNP; TAA: Tumor-associated antigen; LECs: Lymphatic endothelial cells; SCS: Subcapsular sinus. Reproduced with permission from ref 104. Copyright 2022 ELSEVIER.

nanomedicine, most of them are based upon inorganic materials which are far from life requirements. In a recent study, molecular bottlebrushes (MBBs) with the same diameter and different lengths given by precision chemistry were applied to shape one-dimensional nanoadjuvants.[103] The MBBs had densely grafted cationic polymer branches and adopted wormlike morphology, whose contour lengths were controlled precisely by the degree of polymerization of polymeric backbones. Three MBBs of different aspect ratios (ARs) were loaded with the Toll-like receptor 9 (TLR9) agonist, CpG ODN, by electrostatic interaction. It was found that the nanoadjuvants with AR = 2 outperformed those with AR = 1 and AR = 4 in accumulation and retention at lymph nodes, and in colocalization in the late-endosomes of APCs, effectively activating TLR9. When vaccinated with HBsAg, the nanoadjuvants of AR = 2 also demonstrate a significant performance in the clearance of the virus in chronic HB mice

models, showing an obvious dependence upon the AR of one-dimensional nanovaccines (Figure 6).

Previous transport of the nanovaccines to the LNs based upon small size is passive targeting. It is known that the retention of nanovaccines in LNs is also important for immune responses. The reticular conduits are rich in the cortex of LNs. We reported new nanovaccines targeting the conduits that may promote payload infiltrating into the paracortex of LNs.[104] Tannic acid (TA) is a botanic polyphenol and likely forms hydrogen bonds with elastin, which is rich in conduits. In this case, the nanoparticles bearing TLR7/8 agonist, imiquimod (IMQ), were modified with TA to give INP-TA, which showed obvious longer retention in LNs and conduits than the unmodified one. This leads to high colocalization with residual DCs and thus high inflammation response. In a model of triple-negative breast cancer (TNBC), INP-TA covaccinated with the nanoparticle of paclitaxel that generated tumor-associated-antigens *in situ*. Synergized administration achieved
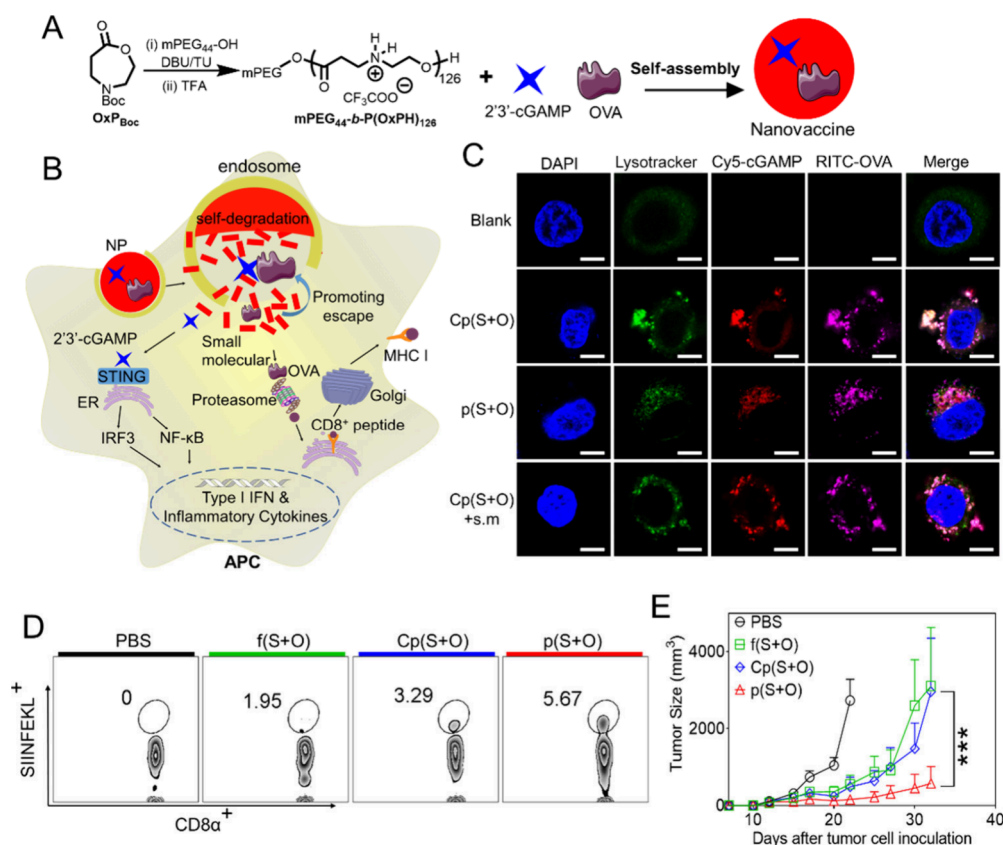
Figure 8. (A) Synthetic strategy diagram of the self-degradable poly(β-amino ester)s and the nanovaccine (p(S+O)) formulation. (B) Schematic of the nanovaccine promoting cytosolic delivery of antigen and agonist. (C) p(S+O) promoted OVA and 2′3′-cGAMP escaped from endosome. (D) Representative flow cytometry plots of SIINFEKL-tetramer$^+$ CD8$^+$ T cells on day 19 post the first vaccination. (E) Average tumor volume. Reproduced with permission from ref 105. Copyright 2022 ELSEVIER.

a robust tumor-specific T-cell response and successfully prevented tumor processing and lung metastasis (Figure 7).

Usually, the release of antigens and adjuvants in nanovaccines is primarily achieved through the degradation of materials or disintegration of complexes. However, for more precise control, a common strategy currently involves the use of stimulus-responsive methods, including acid-, redox-, temperature-, enzyme- and light response. This allows nanovaccines to achieve controlled release of antigens and adjuvants in specific tissues or organelles, or through external stimuli intervention. As shown in Figure 8, we found that a well-defined poly(β-amino ester)s by ring-opening polymerization (ROP) of N-Boc-1,4-oxazepan-7-one (OxPBoc), which could be self-degraded into linear oligo-amide. This polymer is cationic in PBS buffers and self-degraded induced by the amino groups along the polyester chain at pH > 6.5. After encapsulating cGAMP and OVA via electrostatic interaction, it was shown that the self-degradation products facilitated the release of 2′,3′-cGAMP and OVA from early endosome to the cytosol, where the two components strongly activated CD8$^+$ T lymphocytes and significantly enhanced IFN1, TNF, CXCL9, and CXCL10 expression. In a B16F10-OVA melanoma model, the vaccine could eradicate tumors efficiently.[105]

3.4. Nanovaccines Regulate $\gamma\delta$ T Cell Response

Different pathogens and diseases require the activation of specific immune cell subsets for an effective immune response. Tailoring nanovaccines to selectively activate or modulate innate immune pathways allow for precise control over the initiation and magnitude of immune responses. Similarly, designing nanovaccines to promote specific adaptive immune responses, such as Th1 or Th2 cell activation, enables the customization of immune responses to combat different pathogens or diseases effectively. Nowadays, novel vaccines for regulating both innate and adaptive immune responses have been thoroughly studied. For example, by adding different types of adjuvants to modulate antigen processing and presentation, thereby influencing T cell polarization. Or by altering the formulation and delivery methods to stimulate specific immune responses. However, more efforts should be focused on developing vaccines that can activate other significant specialized immune cells, such as $\gamma\delta$ T cells.

As another subtype of T cells, $\gamma\delta$ T cells recognize tumor cells through nonclassical mechanisms, such as stress-induced ligands and phosphoantigens, independent of major histocompatibility complex (MHC) presentation. This enables $\gamma\delta$ T cells to recognize a broader range of tumor antigens, including those not presented by conventional $\alpha\beta$ T cells. Moreover, $\gamma\delta$ T cells are enriched in epithelial and mucosal tissues, where they serve as frontline defenders against infections and tumors at barrier sites. Their tissue-resident nature enables $\gamma\delta$ T cells to exert localized antitumor effects and provides a first line of defense against tumor invasion. Given the above, Yang et al. prepared a microneedle (MN) delivered nanovaccine for activating $\gamma\delta$ T cells. The MNs contained the fusion vesicles composed of the tumor cell membrane, liposomes and CpG. After the MN vaccine inoculation, $\gamma\delta$ T cells were activated by antigens present on the tumor cell membrane and physical
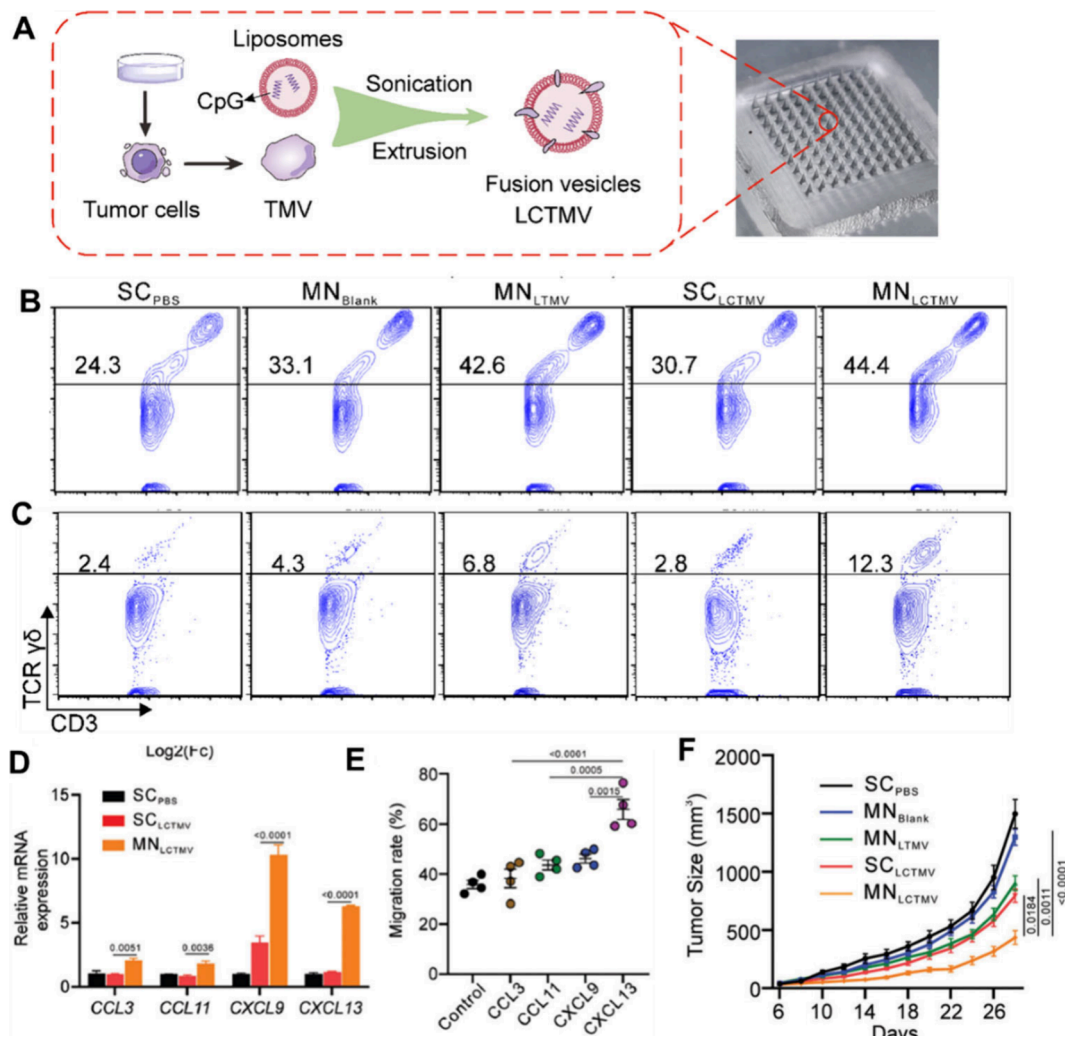
Figure 9. Microneedle assistant nanovaccine activated $\gamma\delta$ T cells to combat tumors. (A) Schematic of the MN nanovaccine preparation. $\gamma\delta$ T cells in (B) skin and (C) tumor postvaccination. (D) RNA levels of related chemokines genes in tumors and (E) spleen stimulated by MN nanovaccine. (F) MN$_{LCTMV}$ significantly inhibited Luc-4T1 tumor growth. Reproduced with permission from ref 106. Copyright 2023 Wiley Online Library.

stimulation of the dermis via microneedles. Then the CXCL13 induced by MN nanovaccine accumulated at the tumor site. Meanwhile, the MN nanovaccine promoted the DC maturation, prolonged the LN accumulation and showed a significant antitumor effect on various tumor models.[106] See Figure 9.

Herein, developing a variety of nanovaccines with different mechanisms of action and immune-modulating capabilities is essential for effectively regulating immune cell responses. These tailored approaches hold promise for improving vaccine efficacy, enabling personalized vaccination strategies, and addressing the diverse challenges posed by infectious diseases and tumors.

3.5. Nanovaccines Remodel the Suppressed Tumor Microenvironment

The tumor microenvironment (TME) shapes the immune landscape within the tumor, influencing immune cell infiltration, activation, and function. Immunosuppressive factors within the TME, including regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and immune checkpoint molecules, create an immunosuppressive milieu that facilitates tumor evasion from immune surveillance and promotes immune tolerance. Nanovaccine loaded with adjuvants and antigens can modulate the immune responses

within the TME by enhancing antigen presentation, promoting dendritic cell activation, and stimulating effector T cell infiltration. Furthermore, the immunogenic cell death within the TME induced by nanovaccines leads to the release of tumor-associated antigens and danger signals that stimulate antitumor immune responses. This promotes the recognition and elimination of tumor cells by the immune system, contributing to tumor regression and long-term immune memory. In addition, nanovaccines combined with other therapeutic modalities, such as chemotherapy, radiation therapy, or immune checkpoint blockade, achieve synergistic effects in tumor eradication.[107] By enhancing immune activation and overcoming therapy resistance, nanovaccine-based combination therapies offer new opportunities for improved patient outcomes in cancer treatment.

Epstein–Barr virus (EBV) is associated with Hodgkin's disease, Burkitt's lymphoma, post-transplant lymphoproliferative disorder, and nasopharyngeal carcinoma (NPC). EBV latent infection is the main cause of related tumor recurrence. The vaccines using EBV latency-associated antigens have great potential to clear latent infections. As shown in Figure 10, Liu et al. prepared an EBV-associated nanovaccine with a narrow particle size distribution and prolonged stability by mixing four
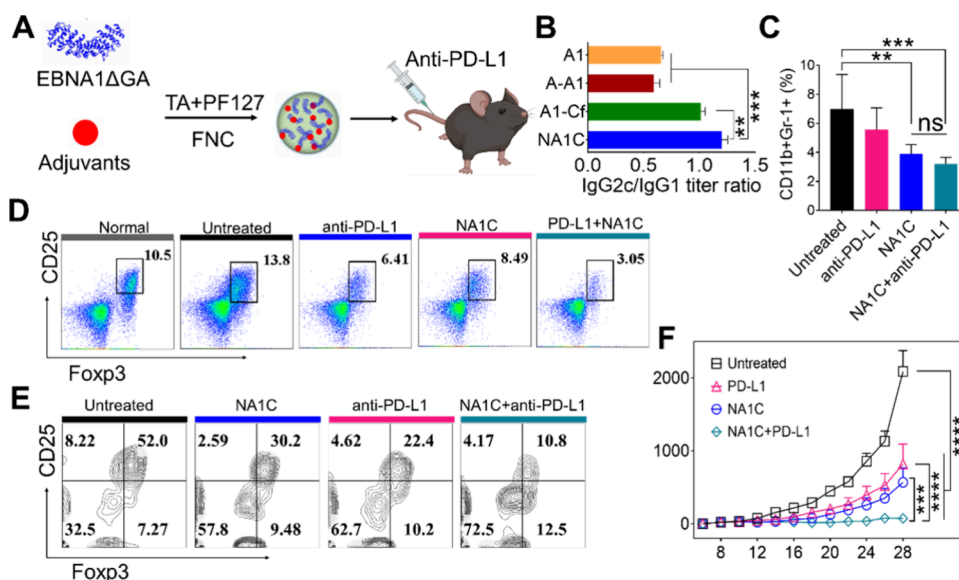
Figure 10. Nanovaccine combined with anti-PD-L1 remodeling the TME. (A) Schematic of the EBV-associated nanovaccine combined with anti-PD-L1 for tumor therapy. (B) Nanovaccine adjuvanted by CpG (NA1C) elicited a biased immune response. (C) NA1C synergized with anti-PD-L1 downregulated MDSC in TME. The Tregs in (D) PBMC and (E) TME were downregulated. (F) NA1C combined with anti-PD-L1 showed potent efficiency for tumor eradication. Reproduced with permission from ref 108. Copyright 2020 ELSEVIER.
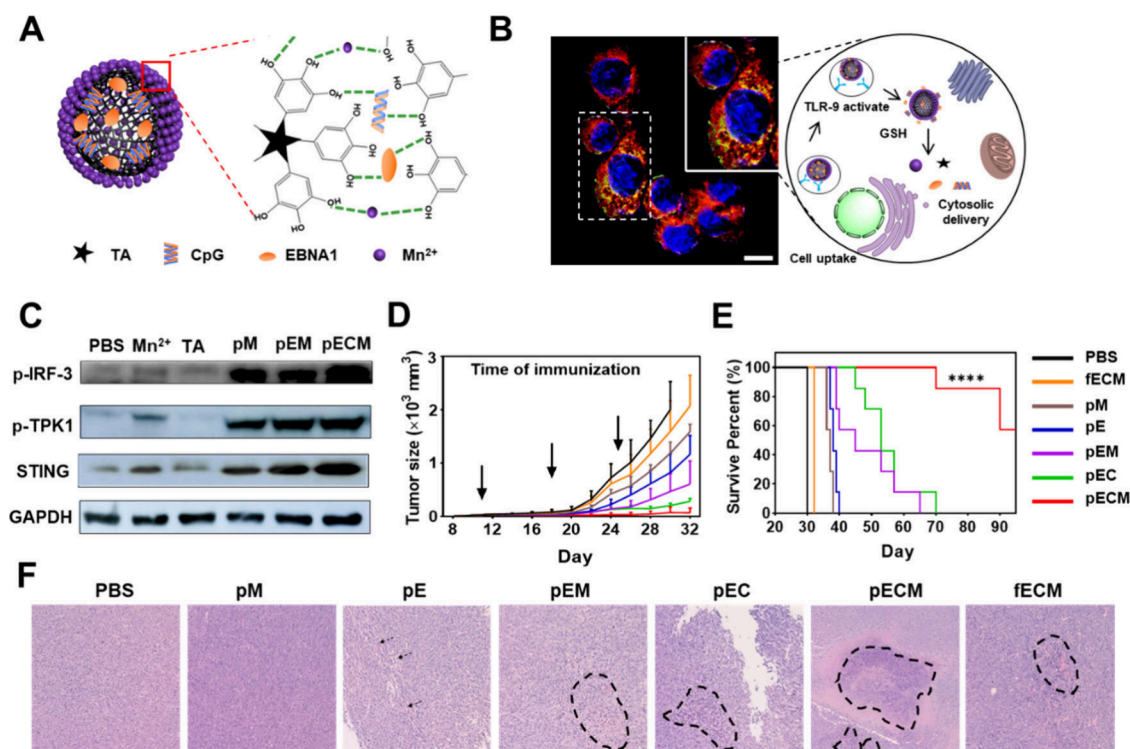

Figure 11. Nanovaccine promoted TLS formation to inhibit tumor progress. (A) Schematic of the Mn^{2+} assistant nanovaccine codelivered EBNA1 antigen and CpG for tumor therapy. (B) Nanovaccine promoted cell internalization and cytosolic delivery of CpG and Mn^{2+}. (C) Nanovaccine elicited a strong STING pathway response and (D, E) showed a potent tumor inhibition (F) HE staining showed the TLS formation using the Mn^{2+} assistant nanovaccine. Reproduced with permission from ref 109. Copyright 2023 ACS Publications.

solutions of tannic acid, Pluronic F-127 (PF127), subunit antigen (EBNA1), and adjuvants (CpG or IFN-α) with the FNC. The homogeneous and small nanovaccines exhibited strong targeted LN effects and induced potent Th1-biased immune response, demonstrating strong immunosurveillance of the tumorigenesis. Furthermore, the nanovaccine combined with anti-PD-L1 decreased the infiltration of Tregs and

MDSCs in the tumor and peripheral immune system, resulting in 70% of the tumor being eliminated in mice. high efficacy for activating CTLs.[108]

Additionally, tertiary lymphoid structures (TLS) are lymphoid structures found in nonlymphoid tissues and generally appear in chronic inflammatory tissues caused by autoimmune diseases, chronic infections, and cancer. For

tumors, the presence of TLS in tumors generally means a better prognosis and clinical outcomes after immunotherapy. Herein, strategies adopted to promote TLS formation to combat tumors are necessary. Inspired by the versatile functions of novel nanovaccines, Wen al et. used the TA as delivery material to load biadjuvants, Mn^{2+} and CpG, and EBNA1 antigen through hydrogen bond and coordination interaction (Figure 11A). The constructed nanovaccine could be internalized and delivered into the cytosol efficiently. Moreover, as Figure 11C,D indicated, in the mouse mimicry nasopharyngeal carcinoma model, Mn^{2+} as an adjuvant could activate the cGAS-STING pathway in Raw264.7 cells. Nanovaccine encapsulated with EBNA1, Mn^{2+} and CpG (pECM) showed significantly inhibited tumor growth compared with other vaccines. There were half of the mice in the pECM group survived during the 90-day monitoring. In addition, the biadjuvant nanovaccine pECM accelerated the formation of TLSs in tumors by increasing chemokines CCL19/CCL21, CXCL10, and CXCL13 to blood and lymph vessels, in turn, recruiting matured T cells, B cells, and DCs.[109]

4. PERSPECTIVE AND CONCLUSION

In comparison to traditional vaccines, novel nanovaccines, including those based on subunit antigens, peptides, nucleic acids, and innovative adjuvant combinations, exhibit clearer composition, enhanced immunogenicity, personalized design capabilities, and improved safety. The precise nanovaccines for the specific disease of the target population should be tailored by the selection and construction of potent antigens, safe delivery systems, the right size and surface properties, as well as the distribution of antigens and adjuvants in the nanoparticles. However, it may suffer in terms of complexity, reproducibility, cost and safety. Researchers need to balance the production and actual benefits brought by the precise vaccine design. Therefore, this Perspective outlined the elements and necessities that should be considered for the precise design and vaccination of nanovaccines.

(1) Developing a precise nanovaccine may not need to consider all factors. One needs to use experimentation and experience to determine the main factors affecting the precise vaccination while designing a nanovaccine. For subunit and peptide nanovaccines, the primary factors of formulation that impact the immunogenic performance are particle size and size distribution. For mRNA or DNA vaccines, the primary consideration is the delivery vehicle to ensure the expression of nucleic acid at the target location and expression efficiency.

(2) The adjuvants should be selected based on the desired type of immune response. Different adjuvants can stimulate different arms of the immune system, such as humoral-mediated adjuvants are important for infectious diseases, and cellular-mediated adjuvants are necessary for cancer therapy. Moreover, adjuvants should be compatible with the antigens being used in the vaccine formulation. Some adjuvants may enhance the immunogenicity of certain antigens more effectively than others. Compatibility between the adjuvant and antigen is essential for achieving optimal vaccine efficacy.

(3) Delivery materials should exhibit minimal toxicity and immunogenicity, allow for sustained release of vaccine components, shield antigens and adjuvants from premature clearance or degradation, promote efficient uptake by antigen-presenting cells, and comply with regulatory guidelines for clinical use. Meanwhile, the effects of delivery materials on the protein corona formation in vivo, as well as the subsequent impact of the protein corona on targeting and metabolism, also need to be studied.

(4) Depending on the administration routes, different formulations of nanovaccines need to be designed. The nanovaccine for intravenous administration was typically for tumor therapy and intramuscular, subcutaneous injection were mainly for infectious prevention.

(5) Mucosal immunity can evoke stronger immune protection in the body and is more suitable for preventing and controlling the spread of highly contagious pathogens. Breaking through mucosal immune barriers with precise targeting delivery techniques and developing new noninjectable vaccines with high protective efficacy and compliance against respiratory infectious diseases are the main research directions for mucosal vaccines. In the future, more efforts should be invested in the development of inhalable mRNA efficient delivery technology for respiratory mucosal immunity and novel delivery technology for intestinal mucosal immunity.

(6) For special populations, it is essential to tailor the nanovaccine to address the specific immunological characteristics and needs of the target population, such as infants, elderly individuals, pregnant women, or immunocompromised patients. The design should account for any potential age-related or immune-related factors that may impact vaccine efficacy or safety, such as immune senescence, immunodeficiency, or immune tolerance. In vulnerable populations, to minimize the risk of adverse reactions or complications. Additionally, vaccine acceptance, access, and delivery logistics should be taken into account to ensure equitable distribution and uptake among special populations.

In summary, for the precise design of nanovaccines, research should focus on the main factors influencing their pharmaceutical and immunogenic performance, rather than considering all factors. The precise design and precision vaccination of nanovaccines are interrelated, requiring scientists to deepen their research and exploration in fundamental immunology, materials science, pharmacy, and interdisciplinary fields.

■ AUTHOR INFORMATION

Corresponding Author

Yongming Chen — *College of Chemistry and Molecular Science and State Key Laboratory of Antiviral Drugs, Henan University, Zhengzhou 450046, China; School of Materials Science and Engineering, Key Laboratory for Polymeric Composite and Functional Materials of Ministry of Education, Sun Yat-sen University, Guangzhou 510275, China;* orcid.org/0000-0003-2843-5543; Email: chenym35@mail.sysu.edu.cn

Authors

Hong Liu — *College of Chemistry and Molecular Science, Henan University, Zhengzhou 450046, China; Translational*

Medical Center of Huaihe Hospital, Henan University, Kaifeng 475004, China

Haolin Chen − *School of Materials Science and Engineering, Key Laboratory for Polymeric Composite and Functional Materials of Ministry of Education, Sun Yat-sen University, Guangzhou 510275, China*

Zeyu Yang − *School of Materials Science and Engineering, Key Laboratory for Polymeric Composite and Functional Materials of Ministry of Education, Sun Yat-sen University, Guangzhou 510275, China*

Zhenfu Wen − *School of Materials Science and Engineering, Key Laboratory for Polymeric Composite and Functional Materials of Ministry of Education, Sun Yat-sen University, Guangzhou 510275, China*

Zhan Gao − *School of Materials Science and Engineering, Key Laboratory for Polymeric Composite and Functional Materials of Ministry of Education, Sun Yat-sen University, Guangzhou 510275, China*

Zhijia Liu − *School of Materials Science and Engineering, Key Laboratory for Polymeric Composite and Functional Materials of Ministry of Education, Sun Yat-sen University, Guangzhou 510275, China;* orcid.org/0000-0002-8438-6961

Lixin Liu − *School of Materials Science and Engineering, Key Laboratory for Polymeric Composite and Functional Materials of Ministry of Education, Sun Yat-sen University, Guangzhou 510275, China;* orcid.org/0000-0001-6133-4977

Complete contact information is available at:
https://pubs.acs.org/10.1021/jacsau.4c00568

Author Contributions

The manuscript was written through the contributions of all authors. CRediT: **Hong Liu** investigation, visualization, writing-original draft, writing-review & editing; **Haolin Chen** writing-original draft; **Zeyu Yang** writing-original draft; **Zhenfu Wen** writing-original draft; **Zhan Gao** writing-original draft; **Zhijia Liu** writing-original draft; **Lixin Liu** supervision, writing-review & editing; **Yongming Chen** conceptualization, investigation, supervision, writing-review & editing.

Notes

The authors declare no competing financial interest.

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