Nanovaccines: Merits, and diverse roles in boosting antitumor immune responses

Qiliang Yin<b><sup>1,2</sup></b>, Ying Wang<b><sup>1,2</sup></b>, Yipeng Xiang<b><sup>1,2</sup></b>, and Feng Xu<b><sup>1</sup></b>

**ABSTRACT**
An attractive type of cancer immunotherapy is cancer therapeutic vaccines that induce antitumor immunity effectively. Although supportive results in the recent vaccine studies, there are still numerous drawbacks, such as poor stability, weak immunogenicity and strong toxicity, to be tackled for promoting the potency and durability of antitumor efficacy. NPs (Nanoparticles)-based vaccines offer unique opportunities to breakthrough the current bottleneck. As a rule, nanovaccines are new the generations of vaccines that use NPs as carriers and/or adjuvants. Several advantages of nanovaccines are constantly explored, including optimal nanometer size, high stability, plenty of antigen loading, enhanced immunogenicity, tunable antigen presentation, more retention in lymph nodes and promote patient compliance by a lower frequency of dosing. Here, we summarized the merits and highlight the diverse role nanovaccines play in improving antitumor responses.

**Introduction**
Tumor vaccine has been recognized as a novel and attractive immunotherapy in preclinical and clinical trials by boosting host antitumor immune responses. However, the clinical translation of this treatment strategy has been challenging. Previous studies of cancer vaccines used vaccine designs that lacked sufficient immunogenicity to induce robust antitumor immunity. Moreover, it has proved that traditional approaches from the field of infectious disease vaccines cannot be applied to cancer treatment. Specifically, there are numerous limitations to conventional subunit vaccines, they are usually poor serum stability and biocompatibility, short half-life, strong toxicity, have weak immunogenicity and inefficient vaccine delivery, and only induce short-term immune responses. Although some subunit vaccines have been tested for the research of antitumor immunotherapy, most of them have failed to produce adequate preclinical/clinical outcomes for cancer therapy. Thereby, designing a cancer vaccine using nanotechnology could be an effective new strategy. NPs are widely applied nanostructures with diameters ranging from 1 to 500 nm, and they are often used as nanovaccine carriers. Efficacy of these NPs-based vaccines are often determined by numerous parameters, including but not limited to: nanometer size, surface properties (e.g., charge, hydrophilic property), geometry, kinetics, stability, biocompatibility, and safety. There are many merits to nanovaccines prepared with nanotechnology over traditional naked formulations (free peptide, DNA, and mRNA) and bioplatforms (DCs, viral, or bacterial vectors), such as adjustable size, high loading capacity, customized surface chemistry, controlled release, and serum stability. Accordingly, nanovaccines encapsulating tumor antigens possess improved bioavailability and pharmacokinetic properties, which is the essence elements to elicit sufficiently strong and long-lasting antitumor immune response.

In general, various kinds of NPs can be used as delivery systems or adjuvants, such as polymeric NPs, liposomes, micelles, carbon nanotubes, gold NPs, virus NPs, they have been used to deliver antigens to essential cell types and to enhance innate and adaptive immune responses. The nanovaccines are able to intrinsically improve the cross-presentation by enhancing antigen uptake and internalization of antigen presenting cells (APCs) and facilitating maturation of DCs. In addition, they offer unique opportunities to boost antitumor immune responses since NPs can be designed for targeted delivery of antigens to specific organs, tissues, and cells, in this way, the nanovaccines have a capacity to induce immunological reaction in situ. Further, the formation of nanovaccine depot at the injection site through vaccination, which induces antitumor immune responses through a time-dependent APCs recruitment and antigen delivery process (defined as depot effect). Importantly, the stability of nanovaccine is greatly improved to protect antigen from degradation by intracellular proteases and sustainedly release the loaded antigen. Consequently, in this review, we discuss diverse roles nanovaccines play in enhancing antitumor immune responses based on their exclusive superiority.

**The properties of nanovaccines related to antitumor immune response**
Since most of antigens are negatively charged, the cationic nanoadjuvants properly bond antigens by electrostatic adsorption to yield the adjuvant/antigen assemblies with nanometric sizes, they are steady, inadequate biodegradability, and rapidly
endocytosed by APCs in draining lymph node. The NPs’ size is one of the most important properties in nanovaccines, researchers have systematically explored the interaction between the nanovaccines’ diameter and the immunogenicity. Typically, when the size of nanovaccine is less than 100 nm, the internalization of nanovaccine by DCs is greater. Nevertheless, when the size surpasses 100 nm, the internalization of nanovaccine by DCs conversely reduces, and the nanovaccine is more likely taken up by macrophages via phagocytosis at the injection site. Namely, the nanovaccines with smaller size makes them easily transferred through the epithelia and other biological barriers, the particle diameters ranging from 20 to 50 nm is more prone to drain to the lymphatic vessels and accumulate in the lymph nodes. The interplay between various NPs diameters and the immunogenicity are complicated dynamic processes that can also be influenced by the administration routes, types of NPs, and injection sites.

The shape is also an important property of the nanovaccines. The nanovaccines with a different shape can not only modulate uptake and internalization of immune cells but also influence the biodistribution in vivo, which can further affect the immunogenicity of the nanovaccines. A study was conducted to determine whether the shape of gold nanoparticles coated with protein affected their cellular uptake, the results revealed that spherical NPs showed the fastest endocytosis rate, followed by cubic NPs, then rod- and disk-like NPs. Other studies have also clarified that the cellular uptake level of elliptical NPs is relatively much lower than spherical NPs, moreover, star-shaped NPs showed similar performance with the spherical ones in antigens delivery, which can be interesting to use as guidance for the shape of nanovaccine. Furthermore, it is proved that particle shape directly influences the type of immune response. The smaller-sized NPs are more effective than larger particles at inducing Th1 and CD8+ T cells, which is most pronounced when these small particles are spherical. In contrast, the larger sized NPs are more potent at inducing Th2 responses, which are most effective when utilizing rod-shaped particles.

Surface charge of nanovaccines is another determining factor in delivering antigens to dendritic cells and stimulating immune responses. In contrast to anionic NPs, the immunostimulatory properties of cationic nanoparticle were demonstrated to be superior, such as enhancing internalization of DCs, inducing T cell proliferation and improving transport efficiency of antigens. Cationic biomimetic NPs provided a suitable interface environment for adsorption, presentation, and targeting of antigens in vivo. Thereby, antigens can efficiently be presented by tailored biomimetic particles for the design of nanovaccines. Classically, cationic liposomes possess the potential ability to magnify the immunogenicity of the loaded antigen and have attracted enormous attention as a promising vaccine delivery platform for cancer immunotherapy. Importantly, positively charged liposomes arouse a higher degree of immunogenic response than neutral or negatively charged ones. Furthermore, cationic liposome has been reported to be more effective in cellular uptake and DCs maturation. Similarly, a direct comparison of the cationic NPs (chitosan-coated PLGA- and maltodextrin-based particles) with their anionic equivalents revealed superior intracellular protein delivery with cationic NPs. As a result, NPs with positive charges are an excellent choice for the preparation of nanovaccines.

Furthermore, apart from the size, shape, and surface charge of NPs, other factors such as hydrophobicity and loading density of antigens can also influence the therapeutic efficacy of nanovaccines. Obviously, the cationic NPs with optimal sizes and shapes are the best candidate for the preparation of vaccines, and the resulting nanovaccine is able to enhance immunogenicity, heighten antigen delivery and exert a sustained immune effect. Accordingly, by exploiting these merits (Table 1), nanovaccines can boost antitumor immune responses and overcome the current barrier to cancer vaccination.

### Effect of the nanovaccines on DCs

Dendritic cells (DCs) are paramount in inducing a proper antitumor immune. DCs, as crucial APCs, have the ability to initiate an antitumor cascade by the uptake of particles derived from tumor cells and cross-presenting the tumor antigens on major histocompatibility complex I (MHC I) for effective activation of CD8+ T cell responses. Hence, nanovaccines are presented to DCs is the prime step to induce an antitumor immune response. Once recognized as antigens, DCs shift to secondary lymphoid organs and uptake, process and present antigens to T cells, thus, the CD8+ T cells are activated to boost adaptive antitumor immunity. Meantime, γδT cells are stimulated by the nanovaccines to induce innate antitumor immunity (Figure 1). The targeting of antigen toward DCs using a NP carrier is a broad topic of current interest. The challenge of delivering antigen to DCs involves its transport to DCs-rich areas such as the draining lymph node, its binding to DCs and its internalization by DCs for antigen processing and presentation. Nanovaccines for antigen delivery were developed in order to

| Table 1. Characteristics and functions of nanoparticles. |
|------------------|------------------|------------------|
| **Properties of** | **Types** | **Effect** |
| **nanovaccines** |       |       |
| Size               | 20–50 nm         | Tend to drain to the lymphatic vessels and accumulate in the lymph nodes |
|                   | 50–100 nm        | Internalization of NPs by DCs is greater |
|                   | >100 nm          | More likely taken up by macrophages via phagocytosis at the injection site |
| Shape              | Sphericity       | The fastest endocytosis rate, the minimal membrane-bending energy change and high efficacy for vaccine delivery |
|                   | Star             | Similar behavior with the spherical ones in wrapping time and high efficacy for antigens delivery |
|                   | Cube             | - |
|                   | Rod-like         | - |
|                   | Disk-like        | Maximal membrane-bending energy change and low endocytosis rate |
| Surface charge     | Cation           | Efficient antigen uptake, molecular activation of APCs and distinct biodistribution profiles |
|                   | Anion            | - |
| Polarity           | Hydrophilicity   | Tend to form small NPs, easier to accumulate in B cells and increase immune cellular internalization |
|                   | Hydrophobicity   | Trigger the formation of larger particles |
protect the delivered specific antigen from being degraded post-administration. Importantly, the primary advantage of nanovaccines lies in its innate ability to protect the antigen they deliver. For instance, PLGA NPs showed strong antitumor efficacy through CD40-targeting in DCs when co-delivered with ovalbumin (OVA) antigen, Pam3CSK4 and poly adjuvants. Targeted OVA adjuvants coloaded PLGA nanovaccines greatly promoted maturation and activation of DCs compared with non-targeted nanovaccines. Similarly, researchers constructed the PEG-b-poly(L-lysine)-b-poly(L-leucine) micelles (NPs) to co-deliver PMP (polypeptide-poly(L-C))/OVA/siRNA vaccine, it is proved that the nanovaccines can improve in vitro DCs maturation and mobilization, and elicit in vivo cytotoxic CD8+ T cell proliferation and Th1 immune response compared with PMP/OVA and OVA controls. Consequently, the nanovaccines are able to deliver the entire antigen to DCs.

Immature DCs have the inherent ability to internalize and endocytose particles and molecules through several mechanisms, including constitutive macropinocytosis, receptor-mediated endocytosis and phagocytosis. Once DCs phagocytose nanovaccine, they undergo maturation and transfer to secondary lymphoid organs, and the mature DCs present antigens to helper or effector T cells by T cell receptor (TCR) to trigger specific cytotoxic T lymphocytes (CTLs) responses. In the meantime, DCs that uptake antigens were also proved to have an increased expression of co-stimulatory molecules such as CD40, CD80, as well as CD86 and secretion of proinflammatory cytokines and chemokines (Figure 2). Subsequently, mature DCs completely close down macropinocytosis, but they can still internalize antigens with high effectiveness using receptor-mediated endocytosis and even phagocytosis. Therefore, mature DCs continuously activate immune responses as long as they encounter antigens for an extended period following vaccination. Thus, the activated cytotoxic T lymphocytes (CTLs) are able to constantly elicit the tumor-killing effect (Figure 2). For this reason, nanovaccines that continuously release antigens may induce a sustained immune response against tumors. It is reported that diverse delivery platforms of nanovaccine have exhibited potential superiority in priming DCs, for instance, the self-assembly nanovaccine containing TLR7/8 agonist and STAT3 inhibitor, a simple self-adjuvant biomimetic nanovaccine self-assembled with the conjugate of phospholipids and nucleotides, and the nanovaccines loaded with whole-cell components of tumor tissues or cells. In addition, liposomes are spherical, single or multilayered membranous vesicles ranging from 50 to 500 nm in size, and they are usually used as vectors to co-encapsulate antigens and adjuvants to improve DC maturation and cytokine production. Further, as a carrier of nanovaccine, cationic liposomes have been notably acknowledged for better cellular uptake, antigen delivery and DCs initiation. They are well-known DCs inducers and enhance expression of CD40, CD80, and CD86, resulting in the presentation of class I antigen and priming of antigen-specific CD8+ T cells.
**Effect of the nanovaccines on NKS**

NK cells are the crucial component of the innate immune system, which play an important role in antitumor immunity. Nanovaccine can be recognized by NK cells after vaccination.\(^{51,67}\) However, it is uncommon to research the effect of the nanovaccines on NK cells. Yisi Tang et al developed a liposomal nanovaccine containing a recombinant protein to target tumor-associated macrophages (TAM).\(^{67}\) The nanovaccine was spherical and approximately 95 nm in diameter, and it showed high stability in PBS buffer and water. Subcutaneous immunization with the nanovaccine resulted in potent antitumor activity, including an increase in intratumoral IFN-γ+ and GramzB+ NK cells.\(^{67}\) Furthermore, some components of nanovaccine such as bisphosphonates (BPs) and calcium could serve as the biodegradable nanocarrier, which triggered the proliferation and activation of DCs and innate-like $\gamma \delta T$ cells.\(^{51,68}\) With innate and adaptive dual-immunity activation, the nanovaccine can present evident and long-acting tumor therapeutic and prophylactic efficacy, as well as good biosafety in vivo.\(^{51}\)

**Antigen depot effect of the nanovaccines**

Most commonly, subcutaneous injection is the main way of vaccination. There is increasing evidence that both the antigen depot at the injection site and migration to the draining lymph nodes are essential for the development of vaccines\(^{69,70}\) (Figure 3). Thus, the antigen depot effect of nanovaccines in injection site is the decisive factor for boosting antitumor immune responses.\(^{22}\) Research indicates that even larger, micron-scale injectable or implantable macromolecular transfer strategies, normally hydrogels, stents, or microneedle, can form a depot in the surrounding tissue, improving peripheral tissue APCs infiltration, followed by their migration to lymph nodes.\(^{71,72}\) It is possible for sustained-release systems to build a depot in the tissue that gradually releases antigen, thereby avoiding booster doses.\(^{73}\) Interestingly, the depot effect can be achieved by NPs encapsulating antigen as nanovaccines. Put differently, they retain the antigen at the surface of injection site, release antigen gradually and thus prolong the time of antigen exposure with immune cells.\(^{74,75}\) Notably, NPs showed a higher depot effect than microparticles, which has great scientific value for developing nanovaccines.\(^{76}\)
Intriguingly, the antigen depot effect of the nanovaccines plays a key role in enhancing antitumor immune responses (Figure 3). The erythrocyte membrane-enveloped poly (D, L-lactide-co-glycolide) (PLGA) NPs loaded with antigenic peptide and toll-like receptor 4 agonist, monophosphoryl lipid (MPLA) was developed by researchers, the resulting nanovaccine showed the retained protein content in erythrocyte and enhanced in vitro cell uptake. The antigen depot effect was observed in administration site with promoted antigen retention in draining lymph nodes. Similarly, the antigen was formulated into NPs consisting of nanomultilamellar vesicles combined with the adjuvant MPLA, which demonstrated a noticeable depot effect, meanwhile, it led to controlled antigen release and induced stronger antibody responses than in mice immunized with the purified protein combined with the adjuvants. Additionally, the Nano-B5 platform is demonstrated to be capable of producing in vivo stable, self-assembling nanovaccines bearing diverse antigens, including peptides and polysaccharides, a dramatic increase in injection site retention time and lymph node accumulation was observed after nanovaccines were injected into the tail base of mice. Accordingly, the antigen depot effect of nanovaccine enhances lymph node targeting and elicits potent immune activation. Last but not least, the depot effect of the nanovaccine based on cationic liposomes is also promoted after s.c. injection, as a result of the administration of Ag85B-ESAT-6 with liposomes, detectable levels of the antigen remain at the injection site 14 days after injection.

**Stability of the nanovaccine**

The stability of vaccines has always been a major challenge for vaccines development, mainly involving protecting vaccine from degradation and inactivation. Ordinarily, peptide vaccines as one of the main types of personalized cancer immunotherapy contain only key amino acid sequences of tumor neoantigens, hence they can offer precise activation of immune responses. However, the peptide vaccine is prone to be degraded in vivo, thus it is insufficient to activate effective CD8+ T cell responses for tumor elimination. Stability is the prominent virtue of nanovaccine. The stability and integrality of nanovaccines can be maintained by a gentle method of loading antigens, such as physical adsorption based on charge or hydrophobic interactions encapsulation, and biobinding. For this reason, the stability of nanovaccines is crucial for boosting antitumor immune responses.

In NP-based vaccine design, the stability of NPs should be taken into consideration, as it is closely related to the immune efficacy of NPs. It is generally known that ferritin is regarded as an attractive vaccine platform and has considerable potential as a nanovaccine carrier on the grounds of its uniform structure, good plasticity, and desirable stabilities, ferritin is extremely thermally and chemically stable and can be engineered to carry antigens and expose immunogens in a repetitive and well-organized manner. Besides, CpG ODNs with negatively charged hardly penetrate the cell membrane and are also easily degraded by nucleases in physiological conditions. thus, CpG-ODNs were coated onto mesoporous silica NPs to facilitate the induction of cytokines by TLR9. As a result of NPs protection, CpG/MSN-NH2 NPs showed enhanced serum stability, significant uptake by cells, and elevated cytokine levels. In addition, the bisphosphonate (BP, alendronate)-stabilized calcium phosphate NPs (BCP NPs) were designed and developed to allow the encapsulation of plasmid DNA and surface coating of bovine serum albumin (BSA), and these stable and well-dispersed BCP NPs demonstrated excellent uptake efficiency, endo-lysosome escape property, and macrophage transfection with plasmid DNA.

**Effect of the nanovaccines on cancer immunotherapy**

NPs are the specific and efficient delivery platform for antigens to enhance immunogenicity and stability. Physical parameters of nanoparticles, such as size, shape, and surface properties, can readily be modified to influence the immune responses against cancer. Indeed, a significant factor controlling antigen retention and vaccine immunogenicity is the nanostructure construction approach in nanovaccines. Generally, a valid nanovaccine should meet the requirement that can effectively co-deliver the immunologic adjuvants and antitumor antigens to lymphoid tissues and APCs for a successful promotion of antitumor activation.

In preclinical studies, many types of antigens and adjuvants have been developed for applications in cancer nanovaccines. The different types of nanovaccines are able to stimulate an effective antitumor immune response in vivo and in vitro (Table 2). Lin Ma et al loaded both water-insoluble and water-soluble components of tumor tissues/cells to both inside and on the surface of poly (lactic-co-glycolic) (PLGA) NPs, the resulting nanovaccines could efficiently deliver cancer vaccines into APCs and exhibited excellent efficacy in preventing and treating melanoma, lung cancer and triple-negative breast cancer in mouse models. Furthermore, an orchestrated nanovaccine was developed by researchers to remodel the immunosuppressive tumor microenvironment (TME) and activate tumor-infiltrating lymphocytes (TILs) using hybrid micelles (HM), the nanovaccine was encapsulated with colony-stimulating factor 1 receptor inhibitor BLZ-945 and indoleamine 2,3-dioxygenase (IDO) inhibitor NLG-919 in its core and displayed ovalbumin (OVA) as the model antigen (denoted as BN@HM-OVA). Notably, the nanovaccines were able to induce a “depot effect” at injection sites, causing DCs to be exposed to antigens for a prolonged period of time. Similarly, the novel all-in-one biomimetic NPs (i.e. CS-I@CM) were constructed for the immunotherapy of glioblastoma (GBM). The resultant nanovaccines consist of ultrasmall Cu2-xSe NPs, indoximod (IND, an inhibitor of indoleamine-2,3-dioxygenase in tumor), JQ1 (an inhibitor for reducing the expression of PD-L1 by tumor cells), and tumor cell membrane, which could drastically activate the immune responses through remodeling immunosuppressive TME of GBM. In addition, peptide-based antigens are being extensively assessed in cancer nanovaccine designs. For example, a variety of cell-penetrating peptide (CPP)-mediated vaccine delivery systems based on nanotechnology have been proposed, most of which
are designed to promote the stability of antigens in vivo and their delivery into immune cells.\textsuperscript{114} in particular, the CPP-mediated nanovaccines can enhance antigen uptake, processing, and presentation by APCs, which are the essential steps in activating an antitumor immune response.\textsuperscript{114} Furthermore, Daniel Shae et al developed a synthetic cancer nanovaccine platform (nanoSTING-vax) to mimic immunogenic cancer cells in its capacity to efficiently improve co-delivery of peptide antigens and the STING agonist,\textsuperscript{103} the nanoSTING-vax significantly enhanced CD8+ T cells’ responses to peptide antigens, resulting in a dramatic improvement in response to immune checkpoint blockade in murine colon cancer and melanoma models.\textsuperscript{103} Additionally, a Nano-B5 platform is established for formulating in vivo fully protein-based, self-assembling, stable nanovaccines with diverse antigens, such as peptides and polysaccharides, it is found that the nanovaccines can efficiently enhance antigen immunogenicity and activate CD8+ T cells in blood circulation.\textsuperscript{100}

Currently, clinical studies of antitumor nanovaccines are less common. Patients with biochemically relapsed prostate cancer were enrolled in a phase I study, an open-label, parallel design study, a HAAH directed NP vaccine (PAN-301-I) was administered intradermally using a fixed dose escalation scheme every 21 days. This study evaluates the safety and immunogenicity of the PAN-301-I vaccine in patients with biochemically relapsed prostate cancer. In another phase I clinical trial, a novel RNA-nanoparticle vaccine is formulated to treat early melanoma recurrence following adjuvant anti-PD-1 antibody treatment, the goal of this phase I trial is to evaluate the toxicity and feasibility of a tumor-specific RNA-NP vaccine in patients with stage IIB-IV melanoma who have progressed on anti-PD1 (a-PD1) adjuvant therapy. Furthermore, PRECIOUS-01 is an immunomodulating agent composed of the invariant natural killer T cell (iNKT) activator threitolceramide-6 (ThrCer6, IMM60) and the New York Esophageal Squamous Cell Carcinoma-1 (NY-ESO-1) cancer-testis antigen peptides encapsulated in a poly (lactic-co-glycolic acid) (PLGA) NP, PRECIOUS-01 is being developed for the treatment of cancer patients who are NY-ESO-1 positive. In phase I clinical trial, a dose escalation study of immunomodulatory NPs (PRECIOUS-01) is being conducted on patients with advanced solid tumors. In addition, an open-label, Phase I clinical trial is conducted to evaluate the safety, tolerability and immunogenicity of EGFR(V)-EDV-Dox in subjects with recurrent glioblastoma multiforme, in this study, dose exploration and immunogenicity testing is conducted in subjects with recurrent glioblastoma (GBM) to determine the efficacy of EGFR(V)-EDV-Dox as a single agent. Despite the fact that most studies are in phase I clinical trials and most of the results have not yet been published, as antitumor immunotherapy develops rapidly, more and more nanovaccines will be developed and tested in clinical trials.

\textbf{Conclusion}

With the discovery of obstacles that cannot be addressed by traditional vaccination, it has become essential to explore new areas regarding vaccination. The advantages provided by NPs’ proprieties have been extensively exploited in the formulation
of nanovaccines. This review focuses on the merits of nano-vaccine based on NPs for cancer immunotherapy. The size, shape, surface charge, and hydrophobicity level of NPs have a profound impact on the interaction between nanovaccines and immune cells. These properties empower nanovaccines to overcome some of the challenges, such as weak immunogenicity, poor stability, low response rates and immune-related adverse events, faced by current cancer therapeutic vaccines. Accordingly, the nanovaccines are developed to significantly facilitate the activation of DCs, formation of antigen depot, and maintenance of stability, which is promising to maximize the potential of cancer therapeutic vaccines. Notably, these attributes of NPs can independently influence the effectiveness of vaccines, the best vaccine candidates are determined by the comprehensive regulation of NPs. Thus, future studies should weigh the benefits of NPs with adjustable properties in nanovaccine design. Collectively, as new nanotechnology-based vaccine candidates emerge, they could revolutionize or supplement the current landscape of cancer immunotherapy. It is expected that future vaccination will depend on the development of stable, safe, and individualized nanovaccines that guarantee strong and lasting antigum immune responses.

Disclosure statement
No potential conflict of interest was reported by the author(s).

Funding
The compilation of this review was financially supported by the Project Agreement for Science & Technology Development, Jilin Province [grant no. 20200404135Y].

ORCID
Qiliang Yin http://orcid.org/0000-0001-6863-6900

References
1. Yousefi H, Yuan J, Keshavarz-Fathi M, Murphy JF, Rezaei N. Immunotherapy of cancers comes of age[J]. Expert Rev Clin Immunol. 2017;13(10):1001–1015. doi:10.1080/1744668X.2017. 1366315.
2. Platten M, Bunse L, Wick A, Bunse T, Le Cornet L, Harting I, Sahm F, Sangbvi K, Tan CL, Poschke I. A vaccine targeting mutant IDH1 in newly diagnosed glioma[J]. Nature. 2021;592 (7854):463–468. doi:10.1038/s41586-021-03363-z.
3. Bowen WS, Srivastava AK, Batra L, Barsoumian H, Shirwan H. Current challenges for cancer vaccine adjuvant development[J]. Expert Rev Vaccines. 2018;17(3):207–215. doi:10.1080/14760584. 2018.1434000.
4. Saxena M, Van Der Burg SH, Melief CJM, Bhardwaj N. Therapeutic cancer vaccines[J]. Nat Rev Cancer. 2021;21 (6):360–378. doi:10.1038/s41568-021-00346-0.
5. Vermaelen K. Vaccine strategies to improve anti-cancer cellular immune responses[J]. Front Immunol. 2019;10:8. doi:10.3389/ fimmu.2019.00008.
6. Qin L, Zhang H, Zhou Y, Umeshappa CS, Gao H. Nanovaccine-based strategies to overcome challenges in the whole vaccination cascade for tumor immunotherapy[J]. Small. 2021;17(28):2006000. doi:10.1002/smll.202006000.
7. Das A, Ali N. Nanovaccine: an emerging strategy[J]. Expert Rev Vaccines. 2021;20(10):1273–1290. doi:10.1080/14760584.2021. 1984980.
8. Wen R, Umeano AC, Kou Y, Xu J, Farooqi AA. Nanoparticle systems for cancer vaccine[J]. Nanomedicine (Lond). 2019;14 (5):627–648. doi:10.2217/nmm-2018-0147.
9. Koirala P, Bashiri S, Toth I, Skwarczynski M. Current prospects in peptide-based subunit nanovaccines[J]. Methods Mol Biol. 2022;2412:309–338.
10. Lu L, Duong VT, Shalash AO, Skwarczynski M, Toth I. Chemical conjugation strategies for the development of protein-based sub-unit nanovaccines[J]. Vaccines (Basel). 2021;9(6):563–588.
11. He X, Zhou S, Quinn B, Jahagirdar D, Ortega J, Abrams SI, Lovell JF. HPV-associated tumor eradication by vaccination with synthetic short peptides and particle-forming liposomes[J]. Small. 2021;17(11):e2007165. doi:10.1002/smll.202007165.
12. Cordeiro AS, Patil-Sen Y, Shivkumar M, Patel R, Khedr A, Elsayw MA. Nanovaccine delivery approaches and advanced delivery systems for the prevention of viral infections: from development to clinical application[J]. Pharmaceutics. 2021;13(12):2091. doi:10.3390/pharmaceutics13122091.
13. Yin WM, Li YW, Gu YQ, Luo M. Nanoengineered targeting strategy for cancer immunotherapy[J]. Acta Pharmacol Sin. 2020;41(7):902–910. doi:10.1038/s41401-020-0417-3.
14. Bachmann MF, Jennings GT. Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns[J]. Nat Rev Immunol. 2010;10(11):787–796. doi:10.1038/nri2868.
15. Zhao L, Seth A, Wibowo N, Zhao C-X, Mitter N, Yu C, Middelberg APJ. Nanoparticle vaccines[J]. Vaccine. 2014;32 (3):327–337. doi:10.1016/j.vaccine.2013.11.069.
16. El-Sayed N, Korotchenko E, Scheibhöfer S, Weiss R, Schneider M. Functionalized multifunctional nanovaccine for targeting dendritic cells and modulation of immune response[J]. Int J Pharm. 2021;593:120123. doi:10.1016/j.ijpharm.2020.120123.
17. Wen R, Banik B, Pathak RK, Kumar A, Kolishetti N, Dhar S. Nanotechnology inspired tools for mitochondrial dysfunction related diseases[J]. Adv Drug Deliv Rev. 2016;99( Pt A):52–69. doi:10.1016/j.addr.2015.12.024.
18. Hu X, Wu T, Bao Y, Zhang Z. Nanotechnology based therapeutic modality to boost anti-tumor immunity and collapse tumor defense[J]. J Control Release. 2017;256:26–45. doi:10.1016/j.jconrel. 2017.04.026.
19. Kim CG, Kye YC, Yun CH. The role of nanovaccine in cross-presentation of antigen-presenting cells for the activation of CD8(+)/ T cell responses[J]. Pharmaceutics. 2019;11(11):612. doi:10.3390/pharmaceutics11110612.
20. Du G, Sun X. Engineering nanoparticulate vaccines for enhancing antigen cross-presentation[J]. Curr Opin Biotechnol. 2020;66:113–122. doi:10.1016/j.copbiotech.2020.06.015.
21. Cai T, Liu H, Zhang S, Hu J, Zhang L. Delivery of nanovaccine towards lymphoid organs: recent strategies in enhancing cancer immunotherapy[J]. J Nanobiotechnology. 2021;19(1):389. doi:10. 1186/s12951-021-01146-2.
22. Zhang L-X, Sun X-M, Jia Y-B, Liu X-G, Dong M, Xu ZP, Liu R-T. Nanovaccine’s rapid induction of anti-tumor immunity significantly improves malignant cancer immunotherapy. Nano Today. 2020;35:100923. doi:10.1016/j.nantod.2020.100923.
23. Chen W, Zuo H, Li B, Duan C, Rolfe B, Zhang B, Mahony T, Xu ZP. Clay nanoparticles elicit long-term immune responses by forming biodegradable depts for sustained antigen stimulation[J]. Small. 2018;14(19):e1704465. doi:10.1002/smll.201704465.
24. Bhardwaj P, Bhatia E, Sharma S, Ahamad N, Banerjee R. Advancements in prophylactic and therapeutic nanovaccines[J]. Acta Biomater. 2020;108:1–21. doi:10.1016/j.actbio.2020.03.020.
25. Gheibi Hayat SM, Darroodi M. Nanovaccine: a novel approach in immunization[J]. J Cell Physiol. 2019;234(8):12530–12536. doi:10.1002/jcp.28120.
26. Carmona-Ribeiro AM, Perez-Betancourt Y. Cationic nanostructures for vaccines design[J]. Biomimetics (Basel). 2020;5(3). doi:10.3390/biomimetics5030032.
76. Guo Y, Wang D, Song Q, Wu T, Zhuang X, Bao Y, Kong M, Qi Y, Tan S, Zhang Z. Erythocyte membrane-enveloped polymeric nanoparticles as nanovaccine for induction of antitumor immunity against melanoma[J]. ACS Nano. 2015;9(7):6918–6933. doi:10.1021/acsnano.5b01042.

77. Venceslau-Carvalho AA, Teixeira De Pinho Favaro M, Ramos Pereira L, Rodrigues-Jesus MJ, Santos Pereira S, Andreata-Santos R, dos Santos Alves RP, Castro-Amarante MF, Bitencourt Rodrigues K, Ramos da Silva J, et al. Nano-Multimamellar lipid vesicles loaded with a recombinant form of the chikungunya virus E2 protein improve the induction of virus-neutralizing antibodies[J]. Nanomedicine. 2021;27:102445. doi:10.1016/j.nano.2021.102445.

78. Zhu D, Hu C, Fan F, Qin Y, Huang C, Zhang Z, Lu L, Wang H, Sun H, Leng X, et al. Co-delivery of antigen and dual agonists by programmed mannose-targeted cationic lipid–hybrid polymersomes for enhanced vaccination[J]. Biomaterials. 2019;206:25–40. doi:10.1016/j.biomaterials.2019.03.012.

79. Pan C, Wu J, Qin S, Zhang X, Zhang L, Yue H, Zeng M, Wang B, Yuan Z, Qiu Y, et al. Biosynthesis of self-assembled proteinaceous nanoparticles for vaccination[J]. Adv Mater. 2020;32(42): e2002940. doi:10.1002/adma.202002940.

80. Henriksson-Lacey M, Bramwell VW, Christensen D, Agger E-M, Andersen P, Perrie Y. Liposomes based on dimethyldioctadecylammonium promote a depot effect and enhance immunogenicity of soluble antigen[J]. J Control Release. 2010;142(2):180–186. doi:10.1016/j.jconrel.2009.10.002.

81. Qu Y, Wang L, Yin S, Zhang B, Jiao Y, Sun Y, Middelberg A, Bi J. Stability of engineered ferritin nanovaccines investigated by combined molecular simulation and experiments[J]. J Phys Chem B. 2021;125(15):3830–3842. doi:10.1021/acs.jpcb.0c02767.

82. Bai S, Jiang H, Song Y, Zhu Y, Qin M, He C, Du G, Sun X. Aluminum nanoparticles deliver a dual-epitope peptide for enhanced anti-tumor immunotherapy[J]. J Control Release. 2022;344:134–146. doi:10.1016/j.jconrel.2022.02.027.

83. Li AW, Sobral MC, Badrinath S, Choi Y, Graveline A, Stafford AG, Weaver JC, Dellacherie MO, Shih T-Y, Ali OA, et al. A facile approach to enhance antigen response for personalized cancer vaccination[J]. Nat Mater. 2018;17(6):528–534. doi:10.1038/s41563-018-0028-2.

84. Li C, Wang J, Wang Y, Gao H, Wei G, Huang Y, Hu Y, Gan Y, Wang Y, Mei L, et al. Recent progress in drug delivery[J]. Acta Pharm Sin B. 2019;9(6):1145–1162. doi:10.1016/j.apsb.2019.08.003.

85. Zhu G, Mei L, Vishwasrao HD, Jacobson O, Wang Z, Liu Y, Yung BC, Fu X, Jin A, Niu G, et al. Intertwining DNA–RNA nanocapsules loaded with tumor neoantigens as synergistic nanovaccines for cancer immunotherapy[J]. Nat Commun. 2017;8(1):1482. doi:10.1038/s41467-017-01386-7.

86. Hu C, Yang X, Liu R, Ruan S, Zhou Y, Xiao W, Yu W, Yang C, Gao H. Co-administration of iRGD with multistage responsive nanoparticles enhanced tumor targeting and penetration abilities for breast cancer therapy[J]. ACS Appl Mater Interfaces. 2018;10(26):22571–22579. doi:10.1021/acsami.8b04847.

87. Liu ZH, Xu HL, Han GW, Tao L-N, Lu Y, Zheng S-Y, Fang W-H, He F. A self-assembling nanoparticle: implications for the development of thermostable vaccine candidates[J]. Int J Biol Macromol. 2021;183:2162–2173. doi:10.1016/j.ijbiomac.2021.06.024.

88. Deshpande S, Masurkar ND, Girish VM, Desai M, Chakraborty G, Chau JM, Drum CL. Thermostable exoshells fold and stabilize recombinant proteins[J]. Nat Commun. 2017;8(1):1442. doi:10.1038/s41467-017-01585-2.

89. Chen W, Jiang M, Yu W, Xu Z, Liu X, Jia Q, Guan X, Zhang W. CpG-based nanovaccines for cancer immunotherapy[J]. Int J Nanomedicine. 2021;16:5281–5299. doi:10.2147/IJNN.S337626.

90. Liang Z, Cui X, Yang L, Hu Q, Li D, Zhang X, Han L, Shi S, Shen Y, Zhao W, et al. Co-Assembled nanocomplexes of peptide neoantigen Adpgk and Toll-like receptor 9 agonist CpG ODN for efficient colorectal cancer immunotherapy[J]. J Pharm. 2021;608:121091. doi:10.1016/j.jpharm.2021.121091.
