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Nanovaccines for cancer immunotherapy: Current knowledge and future perspectives

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ABSTRACT

Cancer immunotherapy harnesses the immune system to attack tumors and has received extensive attention in recent years. Cancer vaccines as an important branch of immunotherapy are designed for delivering tumor antigens to antigen-presenting cells (APCs) to stimulate a strong immune response to against tumors, representing a potentially therapeutic and prophylactic effect with the long-term anticancer benefits. Nevertheless, the disappointing outcomes of their clinical use might be attributed to dilemma in antigen selection, immunogenicity, lymph nodes (LNs) targeting ability, lysosomal escape ability, immune evasion, *etc.* Nanotechnology, aiming to overcome these barriers, has been utilized in cancer vaccine development for decades. Numerous preclinical and clinical studies demonstrate positive results in nanomaterials-based cancer vaccines with considerable improvement in the vaccine efficacy. In this review, we systematically introduced the characteristics of nanovaccines and highlighted the different types of nanomaterials used for cancer vaccine design. In addition, the opportunities and challenges of the emerging nanotechnology-based cancer vaccines were discussed.

1. Introduction

Cancer immunotherapy, an effective treatment that utilizes the patient's own immune system to recognize and destroy tumor cells [1], is regarded as the fourth major therapy after surgery, radiotherapy and chemotherapy [2]. Cancer immunotherapy includes cytokine therapeutics [3,4], immune checkpoint blockades (ICB) [5,6], chimeric antigen receptor T cells (CAR-T) [7–9], cancer vaccines [10–13] and so on. Although these immunotherapies have made some progress in their fields, respective deficiencies limited their clinical applications. For example, cytokine therapeutics have issues of high toxicity and low efficacy [14], ICB therapeutics have low tumor targeting [15], CAR-T therapeutics show the risk of cytokine release syndrome and neurotoxicity [15], and cancer vaccines have problems of low immunogenicity and immunosuppression [16]. To cover the shortcomings of single immunotherapies, combinations between different immunotherapies have been tried as a promising cancer treatment [17,18]. Today many studies on cancer vaccines are combined with immune checkpoint inhibitors (ICI), which address to some extent the problem of immunosuppression of cancer vaccines. For example, in research on human papillomavirus (HPV), Wieking *et al.* have previously developed a cancer vaccine (Ad5 [E1-,E2b-]-E6/E7) [19]. Vaccine therapy alone induced great antitumor immune response in early tumor models; however its efficacy was significantly limited in more established tumor models. Thus, in subsequent studies, they combined cancer vaccine with PD-1 ICB therapy, which is demonstrated to produce a powerful antitumor response that is superior to monotherapy [20].

As a vital branch of immunotherapy, cancer vaccines aim to treat cancer without damaging healthy cells by co-delivering antigens and adjuvants to antigen-presenting cells (APCs), especially dendritic cells (DCs), which is demonstrated to present antigens to T cells and activate them, thereby triggering the body's antitumor immune response [10–12]. Due to a series of shortcomings such as low immunogenicity, low antigen loading efficiency, poor LNs targeting ability, weak lysosomal escape ability, and immune evasion, vaccines using conventional delivery platform without the application of nanotechnology have poor clinical efficacy evaluation for against tumors [12,21–23].

With the fast advancement of nanotechnology and biomedicine, nanobiomaterials have attracted increasing attention and proved to effectively improve the performance of cancer vaccines [24,25]. Nanoparticles are usually vehicles with a diameter of less than 200 nm, which have been widely used as carriers in cancer

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vaccines. Such particle size characteristics also give nanomaterials a natural advantage in LNs delivery [26]. To a certain extent, this can solve the problem of poor LNs targeting ability of conventional cancer vaccines. A previous study revealed that the optimal size range of the carrier particles was narrowly limited to between 40 nm and 50 nm [27]. As a carrier, nanomaterials have a good performance in stimulating the body's immune response by simulating some key characteristics of pathogens, such as size, shape, and surface coating [28]. By the way of their manufacture, nanobiomaterials that utilized for nanovaccine design can be categorized into three classes, namely, synthetic nanomaterials, semi-biological nanomaterials and biogenic nanomaterials [12]. In general, nanovaccines consist of three parts: antigens (including nucleic acids encoding antigens), adjuvants and carriers. Antigens, including tumor-associated antigens (TAAs) and neoantigens [10,12], can trigger the body's immune response to achieve the purpose of antitumor. It is worth mentioning that neoantigens are antigens expressed only in tumor cells, and their immunogenicity is usually greater than TAAs [29]. The addition of adjuvants for cancer vaccines can strengthen the immune response caused by the antigen or change the type of it [30]. The choice of adjuvant is one of the keys to improve the low immunogenicity and immune evasion of conventional vaccines. While protecting antigens and adjuvants, nanocarriers can efficiently target them to immune system [12,31], thereby enhancing the body's antitumor immune response. The construction and modification of nanocarriers is a key link for cancer vaccines to exert their efficacy.

In our review, we summarize the characteristics of nanocarriers in cancer vaccines. The various cancer nanovaccines with different types of carriers are overviewed in detail. Meanwhile, Table 1 summarizes the various nanovaccines mentioned in this review. Furthermore, we discussed some clinical trials related to

Table 1
An overview of nanovaccines for cancer immunotherapy.

Antigen	Delivery platform	Adjuvant	Immunological effects	Refs.
OVA and HBs Ag	Supramolecular protein chaperone compd. **1**	-	Enhancing the secretion of specific antibodies and cytokines, promoting DCs maturation.	[38]
OVA	DOPE	CpG-ODN	Activating T-cell maturation and increasing secretion of TNF-α and IL-10.	[39]
OVA	MSNs with extra-large pores	CpG-ODN	Targeting lymph nodes, enhancing cross-presentation and inducing specific CTLs.	[47]
TRP-2	Mesoporous silicon vector	CpG-ODN and MPLA	Significantly prolonged survival in the mouse model of melanoma lung metastasis.	[48]
mRNA encoding MUC1	Lipid/calcium/phosphate NPs	-	Producing a robust CTL response and prolonging survival in the triple negative breast cancer mouse model.	[54]
TRP-2	Poly(β-amino esters)	MPLA	Targeting and maturing DCs, inducing the production of antigen specific CTLs.	[55]
OVA	DOPE/EYPC liposomes	-	Enhanced cross-presentation led to the production of OVA-specific CTL.	[60]
Tumor cell-derived mRNA complexes	DOTAP	-	Increasing the percentage of PD-L1$^+$ CD86$^+$ myeloid cells in organs and tumor microenvironment.	[70]
mRNA encoding neoantigen	DOTAP/DP7-C	-	Inhibiting *in situ* growth and subcutaneous growth of lung carcinoma in mice.	[71]
Antigenic peptides	Cobalt–porphyrin (CoPoP) liposome	QS-21 and PHAD	Showing good tumor suppressive properties in a variety of tumor models (CT26, CMS4, 4T07).	[74]
OVA	PLGA	Riboxxim	Stimulating the maturation of DCs and the activation of CD8$^+$ T cells.	[79]
OVA peptides	Poly(propylacrylic acid)	α-GalCer	Enhancing the antigen cross-presentation.	[86]
OVA	PLGA and OEGMA hydrogel	R837	Significant increase in CD8$^+$ CTL and NK cells.	[91]
OVA	Magnetic nanoparticle-filled micellar system	polyIC-R837 complex	It showed sustained resistance to melanoma cells.	[94]
OVA	PEG-phospholipid micelles	Magnetic iron oxide nanoparticles and lipooligosaccharide	Protection against melanoma cells reached 100% when PD-L1 expression was abrogated in tumor cells.	[95]
OVA	Polymer micelle PEOz-PDLLA	CL264	Promoting antigen cross-presentation and enhancing humoral and cellular immune responses.	[101]
OVA	MSN and MSR	CpG-ODN and GM-CSF	DCs recruitment and IFN-γ release from CD8$^+$ T cells increased.	[107]
OVA	Multi-walled carbon nanotubes	CpG and anti-CD40 antibody	Inhibiting the growth of solid tumors and pseudometastatic lung tumors.	[111]
OVA peptides	P22 VLP	-	Increasing CD4$^+$ T cells and CD8$^+$ T cells and decreasing bone marrow-derived suppressor cells.	[119]
OVA and gp100 double antigens	HBc VLP	-	Enhancing T cell response, powerfully inhibiting tumor growth and metastasis formation.	[121]
iPSCs proteins	Red blood cell membrane-encapsulated mannose-modified lipid	-	Accumulateing sufficiently in the spleen to activate APCs and trigger an anti-tumor response.	[129]
Melanoma cell membranes	PLGA	R837	Targeting DCS and lymph node retention.	[133]
OVA	DCM/HCtSA/OVA micelles	-	Targeting DCs, enhancing antigen cross-presentation and driving T cell activation and secretion of relevant anti-tumor cytokines.	[135]
Ag from TEX	TEX	High-mobility group nucleosome-binding protein 1	Improving the immune microenvironment with an increase of CD8$^+$ T cells and anti-tumor cytokines and a decrease of Treg cells.	[141]
Alpha-fetoprotein	DEX	-	The increase of immunostimulatory cytokines and infiltrating CD8$^+$ CTLs, and the decrease of immunosuppressive cytokines and Treg cells.	[145]
OVA	OMV	-	Inducing DCs maturation and antigen presentation, and triggering antigen-specific immune responses.	[156]

cancer nanovaccines. Finally, we put forward the opportunities and challenges facing the development of current cancer nanovaccines.

2. The characteristics of nanocarriers in cancer vaccination

2.1. Controlled-release and improved antigen stability

Some antigens alone are weak immunogens, partly because they are susceptible to degraded by enzymes in the blood microenvironment before they take effect [25,32]. The use of nanomaterials as delivery systems for the encapsulation of antigens is an emerging methodology to address this drawback. It can achieve directed and/or controlled-release of associated antigens while protecting them from degradation [32,33]. Generally, the antigen in the sustained-release platform can maintain a complete conformation and be released in a continuous or intermittent manner to prolong the time for the antigen to stimulate immunity [34,35]. Nanomaterials designed for controlled-release platforms can usually change their properties in response to internal and external stimuli, in order to achieve controlled drug release [36,37]. In the study of Wang *et al.* [38], coassembled nanofiber/hydrogel as an antigen delivery system has shown great potential in controlled-release drug delivery. The supramolecular protein chaperone in this system can facilitate both the cellular uptake of protein antigens and the release of the proteins after uptake. Moreover, Liu *et al.* [39] developed a self-adjuvanting biomimetic antitumor nanovaccine, which could achieve long-term controlled-release of antigens, and at the same time, antigens could stimulate $CD8^+$ T cell responses by the entry to MHC-I pathway through cross-presentation. In another research, Li and his colleagues [40] introduced the synthesis of a new type of pro-nanostimulant, which is formed by coupling the semiconducting polymer nanoparticles core with immunostimulants. Upon near-infrared light irradiation, it can release TAAs and ICI NLG919 on demand, which can induce powerful antitumor immune response. The above examples prove the feasibility of nanomaterials in antigens protection and controlled-release.

2.2. Co-delivery of antigen and adjuvant

In addition to the degradation of antigens alone *in vivo*, they show low immunogenicity due to the immunotolerance in the tumor microenvironment [41]. Therefore, a potent adjuvant, including various immunostimulatory molecules such as stimulator of interferon genes (STING) agonists, Toll-like receptor (TLR) agonists, cytokines and costimulatory ligands [42,43], is a crucial component of cancer vaccines. They can stimulate the immune system and enhance the antitumor immune response caused by antigens [41]. Nano drug delivery system can fulfill the co-delivery of antigens and adjuvants. The silicon material is demonstrated to be a biodegradable and biocompatible material [44,45]. In recent years, mesoporous silica has become a promising carrier material for immunotherapy drugs [46]. In the research of Cha *et al.* [47], a kind of mesoporous silica nanoparticles (MSNs) with extra-large pores was synthesized, which greatly increased the loading capacity of large biomolecules. General mesoporous silica particles have a small pore size of about 3 nm, which has limitations for loading large biomolecules. Because of the structural characteristics of extra-large pore size of approximately 25 nm, XL-MSNs had the ability to simultaneously load antigen proteins and Toll-like receptor agonists and deliver them to DCs. In another study on mesoporous silica, Zhu *et al.* [48] used a mesoporous silicon vector to co-deliver tyrosinase-related protein 2, cytosine-phosphate-guanine (CpG) and monophosphoryl-lipid A (MPLA) into the same DC. *In vivo* and *in vitro* studies have proved that this mesoporous silicon vector-based vaccine can induce a more effective and durable immune response.

2.3. Targeting immune cells

After the antigen into the immune system, to induce an antitumor immune response, it must be captured, processed, and presented by APCs. The antigen presentation ability of DCs is regarded as the most powerful and they are known as “professional APCs” [49,50]. Specific receptors expressed on the surface of DCs include mannose receptor, DEC-205, DC-SIGN, and so on [51–53], which makes it promising to improve the immune responses mediated by DCs by targeting antigens to specific DCs surface receptors [50]. Liu *et al.* [54] developed a new kind of intracellular delivery system lipid/calcium/phosphate NPs. After the surface modification with mannose, mRNA encoding MUC1 will be effectively delivered to LNs DCs. Also using mannose receptor to target DCs, Zou *et al.* [55] developed a nanovaccine based on cationic polymer poly(β-amino esters) (PBAE), loaded with antigen peptide Trp-2 and TLR-4 agonist. Compared with unmodified cationic particles, the modification with mannose improved the ability of the vaccine to actively target DC, thereby better stimulating the immune response. In addition to targeting the mannose receptor, targeting DC-SIGN also received attention in antitumor aspects. Alam’s team [56] developed a glycan-modified virus-like particle (VLP) which can achieve antigen delivery into DCs by targeting DC-SIGN. The previous reports showed that VLPs as vaccine carriers usually stimulated humoral immunity rather than cellular immunity required to fight tumors [57,58]. While in Alam’s study, the addition of DC-SIGN ligand reversed this situation. The modified VLP vaccine has been shown to elicit antitumor cell immunity.

2.4. Facilitating cross-presentation of antigen

Vaccine antigens are usually exogenous antigens to the body, theoretically activate $CD4^+$ T cells through MHC-II molecules. However, for the purpose of activating the $CD8^+$ cytotoxic T cell responses which are considered to the major antitumor responses [59,60], after entering the DC cytoplasm, vaccine exogenous antigens need to be presented to the cell surface by MHC-I molecules, which mainly present endogenous antigens. This process is called cross-presentation of exogenous antigens [61]. In short, cross-presentation provides a pathway for MHC-I molecules to recognize extracellular antigens. Extracellular antigens can thus stimulate $CD8^+$ cytotoxic T cell responses normally induced by intracellular antigens. Fig. 1 showed the intracellular presentation pathways of MHC-I and MHC-II. Nanomaterials designed to facilitate cross-presentation focus on increasing the rate of antigen uptake by DCs and improving the endosomal escape ability of the antigen after being taken up [62]. In common, there are basically two pathways included in cross-presentation: cytosolic and vacuolar pathway [63], which are also briefly illustrated in Fig. 1. Some cationic nanoparticles including cationic polymers or lipids can induce endosome destruction through the proton sponge effect to increase antigen escape. Besides, the fusion of some pH-sensitive nanoparticles with the endosomal membrane can also achieve antigen escape to enhance cross-presentation. For instance, Yuba *et al.* [60] modified DOPE/EYPC liposomes with pH-sensitive polymers such as MGlu-HPG and MGlu-LPG. Studies have shown that compared with unmodified liposomes, these polymer-modified liposomes can significantly increase endosomal escape of antigen through membrane fusion, thereby inducing strong specific cellular immunity in mice [60]. In the previous description, it is mentioned that DCs expressed the specific receptor DEC-205 on surface. Based on this characteristic, a kind of nanoparticle prepared from biodegradable polymer poly(lactic-*co*-glycolic acid)

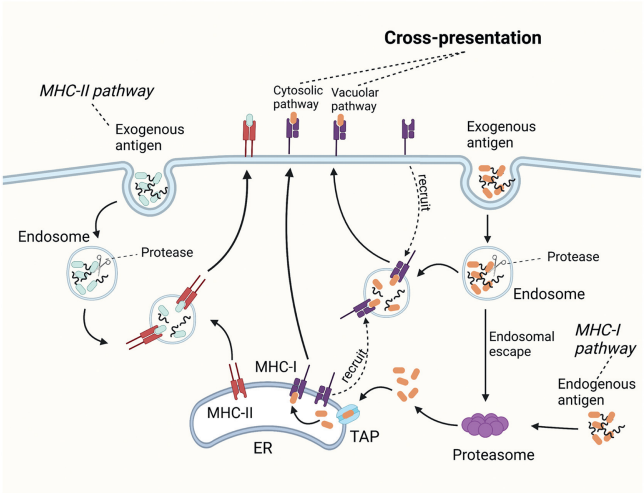

Fig. 1. Schematic illustration of the intracellular presentation pathways of MHC-I and MHC-II. MHC-I molecules mainly present endogenous antigens, and MHC-II molecules present exogenous antigens. Exogenous antigens can gain access to the MHC-I pathway through cross-presentation, which consists of two pathways, the cytosolic pathway and the vacuolar pathway. ER: endoplasmic network; TAP: transporter associated with antigen processing. This figure was created using BioRender.

(PLGA) that can target DCs was developed by Hanlon *et al.* [50]. The study found that when loaded with protein antigens, the nano-delivery system enhanced the levels of cross-presentation of a clinically relevant melanoma epitope.

3. Cancer vaccines with different nanomaterials as carriers

3.1. Synthetic nanocarriers as drug delivery systems

3.1.1. Lipid-based nanoparticles

Liposomes are specialized lipid vesicles which comprise of a watery center encompassed by a lipid bilayer shell [64]. With such structural characteristics, liposomes show great potential as a drug-loading platform because they can simultaneously load hydrophilic drugs (dissolved in the aqueous core) and lipophilic drugs (embedded in lipid bilayer) [25,65]. In addition, due to its good biodegradability and surface modification, it has been regarded as a good carrier platform for cancer vaccines in recent years [25,64,66]. The surface charge of liposomes plays a critical part in stimulating the immune response. Positively charged liposomes have been demonstrated to induce a more powerful immune response than negatively charged or neutralized liposomes [67]. DOTAP is one of the first cationic liposomes to be developed and has been under investigation for decades [68]. Past studies have proved the safety of DOTAP as a cationic liposome in first-in-human trial application [69]. Sayour *et al.* [70] combined tumor cell (B16F0, B16-F10, LLC, KR158-luc)-derived mRNA complexes with positively charged DOTAP through electrostatic force to develop an RNA nanovaccine based on cationic liposomes. These RNA-NPs can significantly increase the percentage of PD-L1^{+} CD86^{+} myeloid cells in organs and tumor microenvironment. In addition, they can synergize antitumor efficacy with ICI and demonstrate safety in canine and mice models. In another work on DOTAP reported by Zhang's lab, they created a common mRNA delivery system based on DOTAP [71]. In the previous research, they developed a cationic peptide DP7-C that can serve as both carrier and immune adjuvant [72,73]. DP7-C is cholesterol-modified cationic peptide AMP DP7 (VQWRIRVAVIRK), which can be used as a peptide-based vaccine carrier to increase the efficiency of antigen delivery to DCs. Although DP7-C showed great anti-cancer potential, it failed to deliver mRNA to cells, which may be that the length of mRNA exceeded the carrying capacity of DP7-C. The high plasticity of DOTAP allows it to be functionalized by modifications. Therefore, they modified the cationic liposome DOTAP with DP7-C. The resulting DOTAP/DP7-C liposome inherited the advantages of the two. While improving the efficiency of mRNA loading, it can also stimulate DCs to produce a stronger antitumor immune response. In addition to being used in gene vaccines, liposomes also perform well in protein/peptide vaccines. This is exemplified in the work undertaken by He and colleagues. They developed a cobalt–porphyrin (CoPoP) liposome as short peptide vaccines delivery system [74]. CoPoP and antitumor immune adjuvants (QS-21, a saponin, and PHAD, a synthetic monophosphoryl lipid A) are contained in the lipid bilayer together. The liposome-based adjuvant system can be mixed with MHC-I epitope peptide A5 (antigenic peptide derived from the AH1 epitope of gp70 protein in murine leukemia virus) to form stable nanoparticles which had shown good efficacy in mouse CT26 tumor models as preventive or therapeutic vaccine. Although liposomes have received a lot of preclinical attention as a promising vaccine delivery vehicle, there are many problems to overcome in clinical transformation such as the stability of liposomes, entrapment efficiency, pilot plant-scale production [66,75]. Future research on next-generation liposomes should be devoted to solving the above-mentioned problems, and a possible method is to modify the properties of liposomes by incorporating new ingredients.

3.1.2. Polymeric nanoparticles

Polymeric nanoparticles have some exceptional characteristics which are attracting more and more attention for vaccine delivery, such as good biocompatibility, biodegradability, side chain modification and nonimmunogenicity [25,76]. Common polymer nanomaterials used in nanocarrier system include polyethyleneimine (PEI), poly(lactic-*co*-glycolic acid), polylactic acid (PLA), polyethylene glycol (PEG), polycaprolactone (PCL), *etc.* Several polymer materials approved by the US Food and Drug Administration (FDA), such as PLGA, PEG and PLA, are one of the most extensively studied materials as vaccine carriers [63]. The biocompatibility of PLGA is embodied in that it can be hydrolyzed into lactic acid and glycolic acid upon contact with aqueous solutions, and the latter can be further metabolized into carbon dioxide and water [77]. Furthermore, PLGA has been shown to stimulate the immune response [78]. In a recent study, Koerner's team developed a new cancer vaccine based on PLGA that can synergistically promote the efficacy of ICI [79]. They used PLGA to co-encapsulate the protein antigen and Riboxxim, that is a double-stranded RNA of the TLR3/RIG-I ligand approved for use as an immune stimulant in humans, and found that both nanoparticles and microparticles can cause similar cytotoxic lymphocyte (CTL) responses in mice. Excitingly, it is reported that this vaccine combined with ICI is undergoing the first small phase I human clinical trial. Similar to PLGA, PLA has also received attention in vaccine carrier materials. In general, the cationic liposomes mentioned above are often preferred as carriers for RNA-based vaccines because of their surface charge [80–84]. PLA-NPs are negatively charged on the surface like mRNA, so it is difficult to directly use as a carrier for mRNA. However, Coolen *et al.* [85] formed complexes between the cationic cell-penetrating peptides and mRNAs, and then absorbed the complex to PLA-NPs, thus realizing the vectorization of mRNAs using PLA-NPs. This shows that other polymeric materials like PLA are expected to use similar methods to deliver negatively charged nucleic acid antigens. Some polymer nanomaterials are also used as antigen carriers that can promote endosomal escape because of their pH-dependent membrane destabilizing activity. Poly(propylacrylic acid) (pPAA) is one of them. Qiu's team had developed a peptide antigen platform utilizing such characteristics of pPAA [86]. The nanocomposite can be obtained mo-

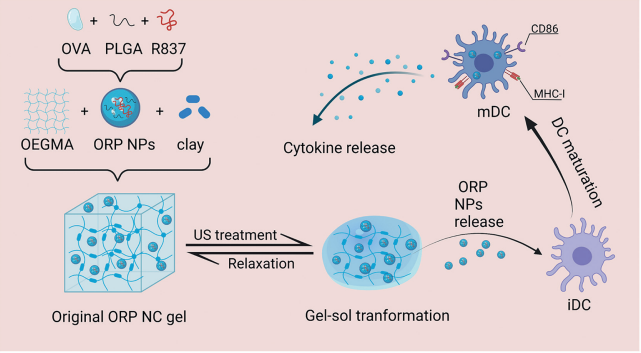

Fig. 2. Schematic illustration of an ultrasonic response hydrogel controlled-release system. The nanovaccines are encapsulated in the gel system with ultrasound response, and the gel collapses into viscous flow sol when stimulated by ultrasound leading to the release of the nanovaccines. The system can achieve sustained and controllable release of cancer vaccines. ORP NPs: OVA@R837-PLGA nanoparticles; OEGMA: oligo (ethylene glycol) methacrylate; NC gel: nanocomposite hydrogel. This figure was created using BioRender.

mentarily by mixing the decalysine-modified peptides and pPAA, and the synthesis steps are simple and fast. This delivery system enhanced antigen cross-presentation, effectively improved the immunogenicity of antigenic peptides, and was proven to be successful in preventing tumor growth in the B16 mouse tumor model. In normal circumstances, nanovaccines need to be administered repeatedly to achieve the best effect, but this will affect the patient's compliance to a certain extent [87–89]. Therefore, polymeric hydrogel systems that can release substances slowly over a period of time have been studied as a drug delivery platform [90]. For example, an ultrasound-responsive self-healing hydrogel system is developed for the sustained and controlled release of cancer vaccines (Fig. 2) [91]. They used OVA as antigens and R837 as immune adjuvants to prepare nanoparticles by the double-emulsion method. The obtained OVA@R837-PLGA nanoparticles (ORP NPs) were encapsulated in a hydrogel system composed of oligo (ethylene glycol) methacrylate and inorganic clay. Upon ultrasonic treatment, the antigen together with adjuvant would be released due to the collapse of the hydrogel. After the ultrasound removed, the hydrogel would be self-healed. Based on this property, the system can achieve improved patient compliance through a single injection and multiple ultrasound treatments. Notably, the immune response elicited by a single injection of the hydrogel was more potent than that elicited by the same dose of the nanovaccines by multiple direct injections. In addition, to utilize this ultrasound-responsive hydrogel system for personalized vaccine delivery, membrane-coated R837-PLGA nanoparticles (RPM NPs) were further prepared using cancer cell membranes collected from excised tumors. The combination of RPM NPs with α-PD-1 showed excellent tumor suppression with a survival rate of 83.3% in mice at 80 days. Indeed, such a drug delivery system can be used for the delivery of other types of vaccines as well.

3.1.3. Micelles

Micelles are usually spherical aggregates formed spontaneously in water by amphiphilic lipids [92]. Unlike liposomes with a double-layered hydrophilic core, the core of micelles is generally hydrophobic in a single layer, which makes it possible to carry hydrophobic substances that are beneficial to immunotherapy through micelles [93]. Some scholars previously filled magnetic nanoparticles in phospholipid micelles to track the migration of vaccines from the site of injection to LNs and tumors through magnetic resonance and nuclear imaging [94]. The magnetic nanoparticle-filled micellar system was biofunctionalized by the polyIC-R837 complex (a powerful adjuvant capable of stimulating TLR3 and TLR7) and the model tumor antigen OVA, showing strong antitumor immunity against highly aggressive B16-F10 melanoma cells. Similarly, in Traini's study [95], they also encapsulated magnetic iron oxide nanoparticles (IONPs) in PEG-phospholipid micelles, because the intrinsic redox and magnetic properties of IONP have been shown to be applied to potentiate the efficacy of DC-based vaccines [96]. At the same time, they demonstrated for the first time that lipooligosaccharide from the plant pathogen Xanthomonas campestris pv. campestris as adjuvants can stimulate TLR4 and cause an effective immune response. The hydrodynamic diameter of the IONPs functionalized using this feature was approximately 20 nm, with a size distribution in the ideal range for lymphatic delivery (20–100) nm. In addition, the flexibility of the composition of the polymer micelles and the modifiability of their surfaces allow them to acquire additional functions to aid delivery [97–99]. The surface of micelles could be modified with corresponding ligands to achieve targeted delivery, or combined with stimulus-sensitive functional groups to control drug release [92,100]. Based on this characteristic of micelles, Li *et al.* [101] reported a LNs-targeting polymer micelle constructed by amphiphilic diblock copolymer poly(2-ethyl-2-oxazoline)-poly(D,L-lactide) (PEOz-PDLLA) combined with carboxyterminated-Pluronic F127. The surface modification with carboxylic groups imparted the micellar-based system with endocytic receptor-targeting capabilities. Moreover, the incorporation of PEOz promoted endosome escape and cytoplasmic release of antigens. Loading the OVA antigen and TLR7 agonist CL264, this system was proven to significantly prevent the tumor growth in C57BL/6 mice.

3.1.4. Inorganic nanoparticles

Although most inorganic nanoparticles are not biodegradable, they are widely used for vaccine delivery because of their rigid structure and controllable synthesis and modification [102,103]. In addition, the properties of some inorganic nanomaterials can change according to pH, light, heat, magnetism and other factors, so they can be used as controlled-release carriers [25]. AuNPs can be fabricated into different forms (spherical, rod, cubic, *etc.*) and sizes according to requirements [104,105]. Coupled with its chemical inertness, biocompatibility and modifiability, AuNPs have been widely used in cancer vaccine delivery [32,65,103]. Previous studies have shown that AuNPs coated with a mixture of 1-octanethiol and 11-mercaptoundecanesulfonic acid could drain immunostimulatory TLR7 ligands into LNs, which prevented the growth of tumors in tumor-bearing mice and prolonged their survival [106]. Mesoporous silica is another promising inorganic material for improving cancer immunotherapy. Its various properties like high porosity, high biocompatibility, facile surface modification, and self-adjuvanticity aroused scientists' interest in its application in cancer immunology [46]. At present, the majority of research is on nano-sized mesoporous silica nanoparticles and micro-sized mesoporous silica rods (MSRs). The former can be absorbed by APCs, and the latter can form a three-dimensional scaffold to recruit APCs, both of which can stimulate the immune response [46]. In a recent research, Nguyen *et al.* [107] combined the properties of these two mesoporous silica materials to develop an MSR-MSN injectable dual-scale mesoporous silica cancer vaccine (Fig. 3). After injection, MSRs would form a three-dimensional macroporous scaffold and recruit a large number of DCs by releasing chemokines. Subsequently, MSNs, together with antigens and adjuvants, were absorbed by the recruited DCs, resulting in activating DCs. Mature DCs in conjunction with checkpoint blockade a-CLTA-4 activated cytotoxic T cells so that induced antitumor immune responses and improved the survival of melanoma bearing mice. Compared with a single MSR or MSN vaccine, this dual-scale vaccine could generate more antigen-specific T cells. Carbon nanotubes (CNTs), derived from carbon allotropes, can be used as plat-

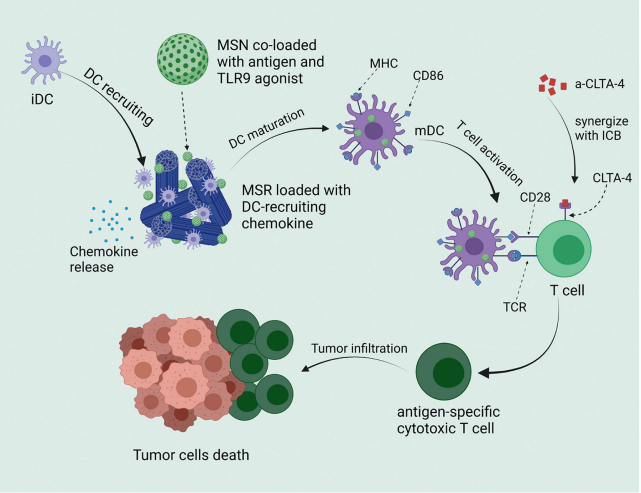

Fig. 3. Schematic diagram of the dual-scale mesoporous silica vaccine eliciting antitumor immune responses. MSN is loaded with antigens together with adjuvants. MSR recruits plenty of DCs. The antitumor response is triggered by recruited DCs, which phagocytosed the antigens and adjuvants. The combination of MSN and MSR can enhance the antitumor immune response. TCR: T cell receptor; CLTA-4: cytotoxic T lymphocyte-associated antigen-4. This figure was created using BioRender.

forms for vaccine delivery systems [108,109]. Large specific surface area, flexible surface chemistry and enhanced cell internalization are their attractive features for immunotherapy [108,110]. In previous research by Hassan *et al.*, they used multi-walled carbon nanotubes (MWNTs) to co-deliver OVA antigen together with the adjuvant CpG and anti-CD40 antibody (αCD40) to APCs [111]. MWNT significantly improves the ability of the active components to inhibit the growth of OVA-expressing B16F10 melanoma cells in subcutaneous or lung pseudo-metastatic tumor models.

3.2. Semi-biogenic nanocarriers as drug delivery systems

Semi-biogenic nanomaterials combine bio-derived materials with synthetic materials, and under ideal circumstances may inherit the advantages of both. Cancer vaccines based on semi-biological nanomaterials aim to reproduce the characteristics of natural organisms that are conducive to stimulating antitumor immune responses [112,113].

VLP, one of the most widely studied semi-biogenic nanocarriers for cancer vaccines, is a biomolecular nanoparticle similar to natural virion formed by self-assembly of viral structural proteins [114]. However, the difference from virions is that they only possess the envelope or capsid of the virions without any genetic material, so they cannot replicate and lack infectivity. Like synthetic vaccines, antigen-loaded VLPs can be effectively delivered to APCs, and because VLPs themselves have viral epitopes that can be recognized by the immune system, they are capable of triggering a strong humoral and/or cellular immune response [90,115,116]. VLPs have been proven as promising vaccine vectors [12], as exemplified by the fact that three VLPs-based human papillomavirus vaccines have been approved for clinical use [117,118]. In addition, there are still many VLP vaccines showing great potential in preclinical studies. A recent study showed that P22 VLPs have great prospects in peptide antigen delivery, and hopefully provide a simple and universal platform for personalized cancer vaccines [119]. P22 as a Salmonella typhimurium bacteriophage can be used as VLP, which is self-assembled by the scaffold protein SP and the coat protein CP. The C-terminus of the CP protein located outside the VLP can be used for fusion with peptide antigens [120]. VLP-OVAB and VLP-OVAT developed based on this feature have been demonstrated to produce strong humoral and cellular immunity, respectively. Furthermore, CP protein and SP protein can be expressed in the same vector and self-assemble into nanoparticles with good stability, which is of great significance for the clinical transformation and large-scale production of the vaccine. In another study, based on the hepatitis B virus core protein, Cheng's group developed a dual-antigen-loaded VLP for immunotherapy [121]. Through genetic engineering, antigen peptides can be inserted into the main immune region of hepatitis B core protein which can be expressed in large quantities by *E. coli* [122,123]. Based on these characteristics, they developed two hepatitis B core (HBc) VLPs: HBc-gp100 VLP and HBc-OVA VLP. By breaking the disulfide bond, the two VLP monomers reassemble into a dual antigen-loaded hybrid HBc VLPs under specific conditions. Subsequent experiments proved that hybrid HBc VLPs can accelerate the maturation of DCs *in vitro* and exhibit good therapeutic effects on mice subcutaneous melanoma model and lung metastasis tumor model. In summary, the potential of VLPs as cancer vaccines is demonstrated by the above examples.

Cell membrane-based nanoparticles, as an emerging class of semi-biological delivery platform, such biomimetic nanoparticles inherit various features of the cell membrane of the source cell, such as antigen diversity, targeting ability or extended circulation time [124,125]. Red blood cell (RBC) membrane-based nanoparticles can avoid the elimination of the mononuclear phagocytic system *in vivo* to prolong the circulation time and target specific tissues according to the original physiological characteristics of RBCs [126,127]. Previous studies have shown that aged or damaged red blood cells will be intercepted in the spleen, as an important lymphoid organ, which contains a large number of APCs [128,129]. Therefore, Zhai *et al.* [130] fused the RBC membrane with mannose liposomes to further target APCs on the basis of spleen retention. Moreover, the addition of liposomes solved the problem of drug loading defects while retaining the excellent biological properties of RBCs, which made the delivery system capable of loading iPSCs proteins. Subsequent experiments verified that the iPSC@RBC-Mlipo nanovaccine they developed was effective in suppressing B16F10 tumors and 4T1 lung metastases. Cancer cell membranes for vaccine delivery are advantageous in their abundant membrane antigens and the ability to self-target homologous cells [124,131–133]. Yang *et al.* [134] used melanoma cell membranes as tumor-specific antigens to encapsulate PLGA nanoparticles carrying the TLR7 agonist R837, and the resulting particles were further modified with mannose to obtain APCs targeting ability. The obtained NP-R@MM vaccine alone could be used as a preventive vaccine to inhibit tumor growth, and its combined use with anti-PD-1 could effectively treat B16-OVA melanoma (Fig. 4). And the cancer cell membranes are specific between different patients and different types of cancer, which makes it a possible strategy to develop personalized tumor vaccines based on this vaccine. Inspired by the homologous targeting of cell membrane-coated nanoparticles [135], a recent study has used DC membranes to achieve efficient delivery to LNs [136]. The inefficient delivery to LNs is one of the main factors that have precluded cancer vaccines from succeeding as immunotherapy. It is reported that DCs membrane-based DCM/HCtSA/OVA micelles can target and retain LNs because a large number of DCs reside there to ensure effective uptake and maturation of DCs [136]. Moreover, the modification of histidine allowed the system to exhibit a strong pH response ability to promote the cytoplasmic release of antigen. In fact, the homologous targeting of cell membranes deserves to be further exploited for drug-targeted delivery.

3.3. Biogenic nanocarriers as drug delivery systems

Biogenic nanocarriers are nanomaterials derived from living organisms. These materials are usually biocompatible and degrad-

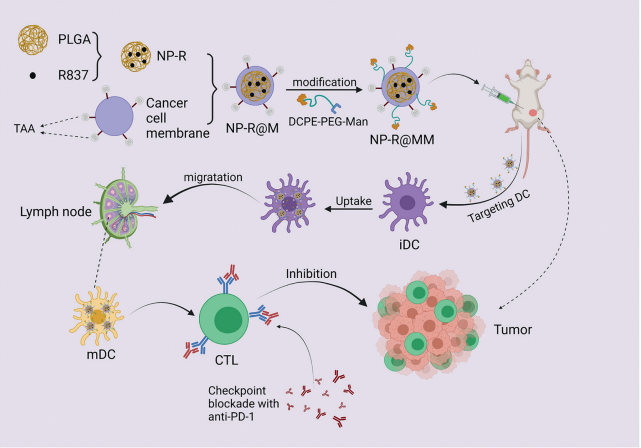

Fig. 4. The structure and mechanism schematic illustration of the cancer cell membrane-coated and mannose-modified nanoparticles loaded with adjuvant (NP-R@M-M). Cancer cell membranes provided adequate antigens for the vaccine system, R837 adjuvant potentiated immunogenicity, and mannose modification further enhanced the uptake of nanoparticles by DCs. This figure was created using BioRender.

able, and have many biological characteristics that can be applied to cancer vaccines, such as homologous targeting and immunostimulation. Nowadays, research in this area mainly focuses on extracellular vesicle (EV), including exosome and outer membrane vesicle (OMV).

Exosomes are double-layer lipid vesicles with a diameter of 30–150 nm secreted by various cell types such as immune cells and tumor cells, which have shown a good prospect for vaccine delivery [113,137]. Exosomes have been proven to have a variety of biological activities and are essential for the transmission of signals between cells [138]. The lipid properties and the type of receptors in the membranes of exosomes and donor cells are similar, so exosomes from different donor cells have the potential to be modified into vaccine formulations with different functions [137,139]. Tumor-derived exosomes (TEXs) can release signal substances that are conducive to tumor development and metastasis in the tumor immune microenvironment [140]. However, more and more evidence indicated that TEXs can trigger an effective antitumor immune response due to their rich antigens provided they are appropriately administered [141]. A recent study indicated the ability of cancer-derived exosomes as carriers to deliver antigens or adjuvants [142]. High-mobility group nucleosome-binding protein 1 (HMGN1) is a powerful endogenous adjuvant that can enhance the ability of DC to activate T cells, but its inefficient delivery in the body limits its application [143]. Zuo *et al.* [142] loaded the functional N-terminal domain of HMGN1 (N1ND) onto TEX *via* an exosomal anchor peptide (CP05) to obtain TEX-N1ND, and realized the co-delivery of the powerful adjuvant N1ND and TAAs to DCs. This platform significantly inhibited the growth of large tumors. And durable antitumor immune responses were induced in in three different subcutaneous syngeneic tumor models Hepa1-6 HCC, Panc02 pancreatic cancer, and 4T1 breast cancer and in orthotopic hepatocellular carcinoma models. Because the source of TEXs can be patients, the TEX-based delivery platform is theoretically biocompatible and safe, and if its preparation process can be further improved, it will have a very promising clinical future. In addition to tumor-derived exosomes, immune cell-derived exosomes have been studied for use in cancer vaccines. It has been reported that exosomes from macrophages can serve as immune adjuvants for cancer vaccines [144], but the relatively more extensive research is on dendritic cell-derived exosomes (DEXs). DEXs carry MHC I and MHC II, costimulatory molecules (CD80, CD86) and adhesion molecules (ICAM1), which can induce the activation of $CD4^+$ and $CD8^+$ T cells [145]. Previous evidence has shown that exosomes derived from DCs expressing alpha-fetoprotein can induce effective antigen-specific immune responses and cause tumor regression in hepatocellular carcinoma mice [146]. From the perspective of the tumor microenvironment, the specific manifestations are the increase of immunostimulatory cytokines and infiltrating $CD8^+$ CTLs, and the decrease of immunosuppressive cytokines and Treg cells. Although exosomes are promising tools for immunotherapy of cancer, there are still challenges in translating findings into clinical outcomes. At present, most of the exosomes are separated from cell culture supernatants and complex biological fluids by centrifugation [147,148]. On the one hand, there is a lack of standardized and unified production methods; on the other hand, yield and purity need to be considered, which limits the clinical translation of exosomes. In conclusion, immunotherapy for exosomes, while promising, is still in early clinical stages and has a long way to go.

Outer membrane vesicles are non-replicating double-layer lipid vesicles naturally released by Gram-negative bacteria, with a diameter in the range of 20–250 nm [149,150]. Similar to exosomes, OMVs are the key to communication and group behavior between bacteria. Due to the particle properties and surface composition (lipopolysaccharides, membrane proteins), OMVs have excellent immunostimulatory properties as immune adjuvants but are usually accompanied by cytotoxicity [151,152]. Previous evidences suggested that OMVs derived from Bacillus pseudomallei strain 1026b or Bp82 possess naturally-attenuated lipid A, which shows insignificant cytotoxicity as adjuvant [153,154]. On this basis, Prior's research group evaluated the adjuvant effect of Bp82-derived OMV [155]. They found that OMV can activate DC at a lower concentration than heat-inactivated or live-attenuated bacteria both *in vitro* and *in vivo*. Moreover, compared with the traditional adjuvant combination alum and CpG, OMV is more dominant in driving antibodies. Another interesting finding is that when mixed with antigen peptides/proteins, OMV can deliver antigen to APC and induce a specific immune response, which is important for the application of OMV in vaccines. In addition to being as adjuvants, OMV also shows good prospects as carriers. Previous research proved that it is possible to present tumor antigens on OMV derived from *E. coli* by genetic engineering methods to obtain recombinant OMV vaccines [156]. However, due to tumor heterogeneity and individual differences, the efficacy of single antigen-modified OMV-based vaccine, whether for different patients with the same tumor or at different stages of the same patient, is open to question. To solve this problem, Professor Nie's team recently developed an OMV-based vaccine that can display antigens flexibly [157]. The protein Plug-and-Display system can be expressed on the surface of OVA through genetic engineering techniques which consisted of protein catchers that can capture specific antigen proteins. Tagged antigenic proteins can spontaneously bind to protein catchers through isopeptide bond formation. Through such a system, different antigen proteins were combined with different protein catchers and displayed on the surface of OMV simultaneously to trigger a synergistic antitumor response. Notablely, the synthesis of the antigen and carrier in this system does not interfere with each other, so a neoantigen library can be established in advance, and when needed the appropriate antigen-carrier pairs can be quickly selected, which is valuable for the mass production of OMV-based vaccines. Overall, whether OMV is used as adjuvant or carrier or both, it has shown a multifaceted potential in the field of cancer immunotherapy. However, there are still challenges to overcome before it can actually be used in the clinic, such as mass production, toxicity and lack of quality standards.

3.4. Nanocarriers as adjuvant in cancer vaccine

In addition to serving as an antigen delivery platform for cancer vaccines, some nanomaterials have inherent adjuvant properties while serving as carriers. In particular, the adjuvant effect of cationic nanoparticles has been extensively studied and applied in various preclinical experiments. Generally, due to the anionic nature of cell membranes, cationic nanoparticles are more easily taken up by cells than neutral or anionic particles [158]. They can generate a potent antigen-specific T cell response by activating DC, which is manifested as an up-regulation of costimulatory molecules, and in some cases, increased production of proinflammatory cytokines [159]. Furthermore, most antigens are negatively charged, so cationic nanoparticles have inherent advantages in loading antigens [160].

Cationic liposomes are one of the most popular and widely used cationic nanoparticles as carriers or adjuvants for cancer vaccines. Cationic liposomes are easily absorbed by APC to improve the induced humoral and cellular immune response [161,162]. The adjuvant effects of cations appear to be diverse, involving multiple immunostimulatory pathways. The previous research by Yan *et al.* [163] proved for the first time that cationic liposomes cause the transcriptional upregulation of several chemokine genes (CCL2, CCL3, and CCL4) in mouse bone marrow-derived cells (BMDCs) by activating the extracellular-signal-regulated kinase (ERK) pathway. On this basis, they further researched and found that DOTAP, as an active DC stimulator, interacted with the DC membrane to cause the generation of reactive oxygen species which can activate multiple signaling pathways including ERK and p38, leading to the production of chemokines, cytokines and costimulatory molecules [164]. Recent research has pointed out that DOTAP stimulated endosomal TLRs through Myd88 to induce type I IFN is the primary mechanism for its adjuvant effect *in vivo* [165]. In addition, cationic liposomes have been demonstrated to promote cross-presentation by increasing the pH of lysosomes in DCs and reducing antigen degradation [166]. In conclusion, cationic liposomes show excellent delivery ability and adjuvant effect, which allows us to see a great prospect of its application in cancer vaccine. Compared with cationic liposomes, there are fewer studies on the immunostimulatory abilities of cationic polymers, but some still stand out among them. Chitosan (CS), a cationic polysaccharide which is derived from deacetylation of chitin, is extensively used as vaccine delivery vehicles [167,168]. CS nanoparticles have been reported to induce type I IFNs by engaging the cGAS-STING pathway, which in turn mediates DC activation and cellular immunity, thereby exerting an adjuvant effect [169]. Research by Shi *et al.* showed that both CS nanoparticles and mannose-modified CS nanoparticles for targeting DC could delay tumor growth in mouse models of melanoma to a certain extent [170]. More about the immunomodulatory potential of CS in cancer treatment is elaborated in the review by Lima *et al.* [171]. In addition to natural polymers like CS, some synthetic polymers such as PEI and polylysine may activate APC through TLR4 signal to exert adjuvant effect [172,173]. Therefore, CS, PEI, and polylysine have been the most widely used cationic polymers for modification [174–176]. Wusiman's team modified the surface of PLGA with the above three cationic polymers and found that it can significantly improve the ability of antigen loading [177]. Meanwhile, the surface modification of cations greatly enhanced the proliferation of lymphocytes in immunized mice and increased the ratio of $CD4^+/CD8^+$ T cells. Among the three cationic polymers mentioned above, the PEI modified preparation (PEI-AHPP/OVA) induced the highest level of humoral and cellular immune response, showing the great potential of a novel and effective adjuvant. As an inorganic particle, aluminum hydroxide (alum) is an FDA-approved adjuvant commonly used in vaccines, which shows a positive zeta potential in water [178]. After injection, alum induces the body's inflammatory response and causes the production of inflammatory cytokines, which is considered to be the immune activation mechanism of alum adjuvant [179]. Although alum is safe and used in many vaccines, it induces a relatively weak immune response.

Many other materials besides cationic nanoparticles have also been proved to be immunostimulating and used in vaccine development. While mesoporous silica is widely used as an antigen delivery platform, it has also been shown to serve as an adjuvant to a certain extent. In experiments by Mahony *et al.*, it was found that, compared with free OVA, mesoporous silica nanoparticles combined with OVA can induce a stronger adaptive immune response without causing damage to major organs [180]. Carbon nanotubes, which are also inorganic nanomaterials, have been demonstrated to possess immunostimulatory effects. Research by Hassan *et al.* showed that the covalent binding of OVA and CpG and its loading on multi-walled carbon nanotubes can improve antigen presentation *in vitro* and increase the intensity of specific immune responses *in vivo* [111]. Compared to the mixture of unconjugated OVA and CpG or OVA-CpG, their binding to multi-walled carbon nanotubes significantly increased the intensity of the immune response *in vitro* and *in vivo*. Moreover, multi-walled carbon nanotubes as a carrier can enhance the synergistic antitumor effect derived from CpG and αCD40, which further proves the adjuvant effect of carbon nanotubes. Bio-based nanomaterials, such as VLPs, extracellular vesicles or membrane-coated nanoparticles, have inherent immunostimulatory properties due to their biological characteristics, so they can be used as self-adjuvants for cancer vaccine delivery. For example, highly organized structure of VLPs, lipopolysaccharides and membrane proteins on the surface of outer membrane vesicles, *etc.*, resemble pathogen-associated structural patterns that can be effectively recognized by the immune system. However, the common problem of such vectors is their uncertain cytotoxicity and difficulties in mass production, which may be the focus of future research in this area.

4. Clinical trials of nanocarrier-based cancer vaccines

Nanomaterials in clinical trials for cancer treatment mainly focus on the delivery of anti-cancer drugs such as paclitaxel, camptothecin, and some ICB monoclonal antibodies. Nanomaterials used in cancer vaccines are often complex in design and need to be modified, making it difficult to achieve quality control and mass production, which has also led to fewer results in clinical trials than traditional cancer drugs. However, there are still some nanovaccines that stand out from preclinical studies, which are summarized in Table 2. The most notable in the table is the clinical trial of the Epstein-Barr virus (EBV) gp350-ferritin nanoparticle vaccine (NCT04645147). In preclinical studies, the vaccine was shown to induce high levels of neutralizing antibodies in mice and non-human primates [181]. Currently this vaccine is undergoing phase I clinical trials to test its efficacy for the treatment of Epstein-Barr virus infection, involving 500 patients. EBV is associated with a variety of cancers such as Hodgkin's lymphoma, non-Hodgkin's lymphoma, nasopharyngeal cancer, and gastric cancer [182], so we included it as preventive vaccine in cancer vaccine-related clinical trials. In addition, a PLGA nanoparticle loaded with antigens and adjuvants is undergoing phase I clinical trials (NCT04751786) to evaluate the effect of treating advanced solid tumors. New York Esophageal Squamous Cell Carcinoma-1 (NY-ESO-1) cancer-testis antigen peptides and alpha-galactosylceramide (α-GalCer)-derived iNKT cell activator IMM60 are important components of this vaccine. NY-ESO-1 protein, an immune target found in many cancers, has previously been combined with ISCOMATRIX® adjuvant in phase II clinical trials (NCT00199901) for the treatment of melanoma. Although from the result point of view, the patient's survival period had not been improved, it had triggered a signif-

Table 2
Cancer nanovaccines in the clinical stage.

Nanobiomaterials		Cancer	Active components	Assisted ingredient	Enrollment	Trial number (Phase)
Polymer	PLGA	Melanoma	Tumor lysate, GM-CSF, CpG	-	23	NCT01753089 (Phase I)
	PLGA	Advanced solid tumor	Antigen peptide NY-ESO-1, iNKT cell agonist IMM60	-	15	NCT04751786 (Phase I)
	PEG-PEI-cholesterol lipopolymer	Epithelial ovarian, fallopian tube or primary peritoneal cancer	IL-12 DNA plasmid vector GEN-1	Pegylated liposomal doxorubicin hydrochloride	16	NCT01489371 (Phase I)
Liposome	Lipid nanoparticle	Relapsed/refractory solid tumor malignancies or lymphoma	mRNAs encoding human OX40L, IL-23, and IL-36γ	Alone/Durvalumab	126	NCT03739931 (Phase I)
	Lipid nanoparticle	Relapsed/refractory solid tumor malignancies or lymphoma ovarian cancer	mRNA encoding human OX40L	Alone/Durvalumab	117	NCT03323398 (Phase I/II)
	Lipid nanoparticle	Non small cell lung cancer	TUSC2 (a tumor suppressor gene)	Pembrolizumab	156	NCT05062980 (Phase I/II)
	Lipid nanoparticle (DOTAP)	Non-small cell lung cancer	TUSC2 (a tumor suppressor gene)	Osimertinib oral tablet	74	NCT04486833 (Phase I/II)
Inorganic material	Gold nanoparticle	Gliosarcoma Recurrent glioblastoma	Spherical nucleic acid (NU-0129)	-	8	NCT03020017 (Early Phase I)
Bio-based material	VLP	Melanoma	Multi-antigen of modified canarypox virus (ALVAC[2]), GM-CSF	-	23	NCT00613509 (Phase II)
	VLP (Bacteriophage lambda system)	Prostate cancer	HAAH peptides	-	12	NCT03120832 (Phase I)
	D123-ferritin	Epstein-Barr virus (EBV) infection, infectious mononucleosis	EBV gp350-ferritin	Matrix-M1	500	NCT04645147 (Phase I)
	Irradiated human plasmacytoid dendritic cells (PDC)	Non-small cell lung cancer	A distinct synthetic peptide encoded by a lung tumor antigen mRNA	Pemetrexed/ Pembrolizumab	64	NCT03970746 (Phase I/II)
-	Not specified	Breast cancer	Pathotropic nanoparticles bearing a dominant negative cyclin G1 construct (Rexin-G)	-	20	NCT00505271 (Phase I/II)
	Not specified	Sarcoma	Pathotropic nanoparticles bearing a dominant negative cyclin G1 construct (Rexin-G)	-	36	NCT00505713 (Phase I/II)

icant antibody response in the majority of patients, which is encouraging for subsequent further research. Previous studies have demonstrated that the combination of α-GalCer adjuvant with OVA showed superior immune efficacy in mice compared to classical TLR ligand adjuvant [183]. On this basis, NY-ESO-1-derived peptide together with IMM60 was encapsulated in polymer PLGA nanoparticles as a novel cancer vaccine. Nanoization of vaccines and the addition of new adjuvants are expected to induce a better immune response in clinical trials. Liposomes are widely used in gene-based cancer vaccines, such as the two clinical trials on mRNA shown in Table 2 (NCT03739931, NCT03323398). In these two clinical trials, mRNA-2752 and mRNA-2416 were encapsulated in lipid nanoparticles, respectively, for intratumoral injection alone or in combination with Durvalumab to evaluate the efficacy of patients with relapsed/refractory solid tumor malignancies or lymphoma. Both mRNAs encode human OX40L, which is a ligand for OX40, a costimulatory receptor in the tumor necrosis factor receptor (TNFR) superfamily that can enhance adaptive immune responses. These two clinical studies aimed to test the safety and tolerability of corresponding mRNA vaccines in cancer patients.

As these examples in the table show, nanomaterials based on polymers and liposomes occupy a large proportion of cancer vaccines in clinical trials. Polymers and liposomes are one of the nanomaterials with the longest research time and the widest application range. Complete and mature preparation method, characterization method and quality standard have been established for them. In addition, as a promising nano-delivery vehicle, research on VLPs has previously achieved such achievements as the HPV vaccine, which is encouraging for the development of other VLP-based cancer vaccines. At the same time, it can be seen that the effect of cancer vaccine as a single-agent treatment is not ideal, and it is mainly combined with other treatment methods such as ICB to fight cancer. And cancer vaccines are more likely to appear in the clinic as adjunctive therapy after surgery or chemotherapy. Currently, in most preclinical studies of cancer nanovaccines, preparation of the formulation is time-consuming and labor-intensive. And the rate of disease progression in cancer patients often requires that the lead time for treatment should not be so long that cancer vaccines with overly complex preparation processes are often of little clinical value. In contrast, vaccines with mature preparation processes are disappointing for immunogenicity and toxicity in the clinical setting. Therefore, more use should be made of FDA-approved biocompatible materials for drug delivery and focus on process simplification. Also, the differences between laboratory animal models and the human immune system make the results of preclinical studies often not reproducible in humans. Although the current clinical performance of cancer nanovaccines is not promising, it is believed that as complex nanoparticle preparation processes are simplified and mature, more and more attractive nanovaccines will enter clinical trials and achieve good results.

5. Conclusions and perspectives

In recent years, immunotherapy has always been a research hotspot in cancer treatment, and there have been successful cases such as CAR-T and ICB. As an important branch of immunother-

apy, cancer vaccines activate the immune system in a safe and effective way to induce antitumor immune response. In addition, cancer vaccines are used as adjuvant therapy in combination with other immunotherapies to improve therapeutic efficacy. The development of nanotechnology is expected to maximize the potential of vaccines in cancer immunotherapy. We discussed the application of various nanocarriers, both synthetic and bio-based, in cancer vaccines. TAAs or neoantigens, together with adjuvants, are encapsulated in nanocarriers or bound to the vector through interactions, and eventually targeted to APC, thus greatly increasing the immunogenicity of the antigen. Meanwhile, appropriate carrier selection or chemical modification ensures the controlled-release of vaccine components. There are already many studies active in preclinical and clinical trials that are attracting a lot of attention.

Although cancer nanovaccine has achieved some encouraging results in recent years as a potential therapeutic approach, many challenges have to be overcome in the process of translational research. Here we list several issues to be addressed: (1) Many vaccines achieved satisfactory results in preclinical studies, but most of them failed in clinical trials. The reason may be the difference in the immune system between laboratory animal models and humans, although humanized mice can compensate for these differences to some extent. Therefore, the development of novel experimental animal tumor models that extend disease mechanisms more similar to humans is critical to the successful clinical translation of cancer vaccines. And because of the relatively large variety of animal models for tumors, it needs to be selected according to the specific scientific question. In addition, the trend of personalized cancer treatment makes it imperative to establish patient-derived xenograft models. (2) Nanoparticles used in cancer vaccine design should be non-toxic and resistant to non-specific protein binding and rapid clearance, which is the basis for cancer nanovaccines to exert their efficacy in the body. In addition, mass production capacity and inter-batch quality control are prerequisites for the development of vaccines into clinical practice and widespread commercialization. The clinical data in Table 2 tell us that most of the vaccines in the clinical trial phase use more established materials that have proven their safety in other areas, such as PLGA, liposomes and VLP. This requires that we should use FDA-approved components whenever possible in the development of cancer vaccines. Also, more efficient and mature production technologies and uniform quality testing standards should be developed. The success of cancer vaccines requires a multidisciplinary effort. (3) Because of the awareness of tumor heterogeneity and individual differences, personalized cancer vaccines based on neoantigens have received extensive attention in recent years. Optimization of the identification of tumor-specific antigen sequence has been shown to be a key step in the development of personalized vaccines. However, the existing technology is still difficult to achieve efficient and accurate antigen sequencing. Therefore, the advantages of cross-discipline should be better exploited by combining biological sciences with artificial intelligence computer simulation techniques for more accurate sequencing and identification of new antigens. (4) Some nanomaterials are inherently immunogenic, which helps them to serve as carriers while also exerting adjuvant effects. However, at the same time, they are accompanied by problems of cytotoxicity and unwanted immune response. Therefore, how to balance the immunogenicity and toxicity of materials is also a problem that needs to be resolved.

Additionally, some of the cancer treatments that utilize advanced emerging technologies are interesting. For example, the concept of using nanorobots to deliver drugs to treat diseases is gradually being realized [184–186]. Perhaps the combination of these emerging technologies with chemotherapy or immunotherapy will give us unexpected results. Altogether, there are many possibilities to be explored.

In conclusion, cancer vaccine is an integral part of immunotherapy, which could be either used as a single-drug therapy or as combined administration and has shown encouraging results in the preclinical studies. For now, cancer vaccines based on nanomaterials appear to be in their infancy, and the clinical applications still get barriers in the road though. It is believed that with the development of various fields such as materials science, immunology, biology, safer and more efficacious nanovaccines might be developed as the next generation of immunotherapy for cancer in the near future.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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