Nanoparticle Systems for Cancer Vaccine

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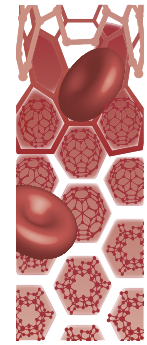

Nanoparticle systems for cancer vaccine

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As effective tools for public health, vaccines prevent disease by priming the body's adaptive and innate immune responses against an infection. Due to advances in understanding cancers and their relationship with the immune system, there is a growing interest in priming host immune defenses for a targeted and complete antitumor response. Nanoparticle systems have shown to be promising tools for effective antigen delivery as vaccines and/or for potentiating immune response as adjuvants. Here, we highlight relevant physiological processes involved in vaccine delivery, review recent advances in the use of nanoparticle systems for vaccines and discuss pertinent challenges to viably translate nanoparticle-based vaccines and adjuvants for public use.

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Since the announcement of the polio vaccine in 1953 by Jonas Salk [1], vaccines have played a major role in preventing infectious diseases, curtailing disease devastation and overall benefiting individual life [2]. Vaccines help to develop immunity by 'imitating' an infection, which can be done by inoculating an individual with nonpathogenic components of a virus or bacteria or with specific peptides, small molecules, and so on. Following inoculation, the adaptive immune system mounts a wide-spread defense against both the inoculant and, ideally, the specific pathogen of interest. The hope is that the inoculation mimics true infection closely enough for the primed adaptive immune responses – 'memory' T and B lymphocytes – to be costimulated by the real pathogen. Despite global reliance and the effective deterrence of epidemics and disease through the use of vaccines, only a few adjuvants and delivery systems are licensed for human use [3]. The limited development of vaccines and adjuvants may be largely due to difficulty developing effective vaccine systems.

Designing an effective vaccine requires two key elements. An antigen, generally in peptide form, is needed to stimulate adaptive immune response. Stimulation of the innate immune system through natural killer (NK) cells is necessary for conditioning a robust and long-lasting adaptive immune response. Thus, an adjuvant, or immune 'potentiator', that can work to recruit NK cell response is also critical for an effective vaccination [4,5]. Together, these components must be directed toward appropriate cells (e.g. $CD4^+$ and $CD8^+$ T cells) of the immune system to ensure concerted innate and adaptive immune stimulation and responses essential for successful vaccination.

Cancer is a leading cause of death in the USA, as new cases are diagnosed in various cancer types each year [6]. Systematically inducing immune responses against cancer cells is a plausible method for targeted cancer prevention (i.e., cancer vaccines). Nanotechnology has been used in various applications, from batteries to drug delivery [7–10]. Nanoparticles and nanomaterials are promising delivery vectors for cancer vaccines; various systems have been used for targeted delivery of antigens to essential cell types, as well as for potentiating innate and adaptive immune responses (i.e., adjuvants) [11,12]. Already nanoparticles have been used to prevent and treat cancer by inducing long-lasting immune responses efficiently [13]. Efficacy of these nanotechnologies is often determined by numerous parameters, including but not limited to: particle size, surface properties (e.g., charge, hydrophilic property), geometry, kinetics and so on [14]. Here, we will focus on various nanoparticles used in vaccine delivery systems grouped by composition and application, as well as, highlight relevant nanoparticle clearance pathways. We will also discuss strategies to avoid fast clearance for effective cellular uptake and vaccine intervention.

Future Medicine

Table 1. Nanoparticle vaccine delivery for various types of cancers.

Cancer type	Nanoparticle	Antigen	Comments	Ref.
Melanoma	PLGA nanoparticle	Ag, Poly(I:C)	Linked with CD40-targeting ligand	[18]
	Liposome	TRP2, α-GalCer	PEGylated liposomes were prepared with DSPE-PEG	[19]
	Carbon nanotube	αCD40, CpG	Codelivery of tumor-derived antigen greatly inhibited tumor growth	[20]
	CPMV nanoparticles	eCPMV	CPMV is of 30 nm and composed of 60 copies of protein units	[21]
Non-small-cell lung cancer	L-BLP25 liposome	MUC1	Clinical trial Stage III	[22]
Breast cancer	PLGA-PEG	OVA, MPLA, CpG	The nanoparticle also showed strong immune responses against melanoma	[23]
	L-BLP25 liposome	MUC1	It is safe but does not show better therapeutic effects	[24]
Prostate cancer	Virus-like particles	PSA	TC-83 vaccine is used	[25]
Cervical cancer	Tumor virus vaccine	HPV	HPV vaccines consist of L1 capsid proteins and are licensed to use since 2006	[26]

CPMV: Cowpea mosaic virus; DSPE: 1,2-distearoyl-sn-glycero-3-phosphoethanolamine; eCPMV: Empty CPMV; MPLA: Monophosphoryl lipid A; OVA: Ovalbumin; PEG: Polyethylene glycol; PLGA: Poly(lactic-*co*-glycolic acid); PSA: Polyethylenimine-stearic acid.

Nanoparticles as vaccine delivery systems

In 2018, approximately 9.6 million cancer-related deaths occurred worldwide. Although significant progress has been made toward understanding cancer pathogenesis, developing cancer therapy able to significantly extend patient life-expectancy has proved challenging [15,16]. Chemotherapeutics and targeting agents can induce remission and decrease overall tumor burden. However, evolving cancer resistance mechanisms and cancer-related sequelae prevent the development of a widely applicable cancer cure. Several surface antigens, or free peptides, have been found to be specifically generated by cancer cell populations. The presence of these distinguishing antigens, or peptides, provides a foundation for developing novel cancer vaccines that can vaccinate patients against certain cancers. The development of a cancer vaccine potentiates the need for robust delivery systems applicable to various diseases and cancers. Nanoparticles can be used as delivery systems for various diseases, including cancer [17]. Nanoparticles can be used to deliver antigens and prime immune cells as vaccines, or as adjuvants to enhance cancer immune response. Nanoparticle-based cancer vaccines and adjuvants have been used to treat a wide array of cancers, and can be targeted toward specific cancers through modification of surface properties and/or composition. Table 1 summarizes nanoparticle vaccines for various types of cancer.

One major obstacle to developing a novel cancer vaccine is the successful delivery of cancer antigens to specific cell populations, NK cells and antigen-presenting cells (APCs), which is critical to the first step of acquired immunity. Antigens are relatively fragile in the blood microenvironment and readily susceptible to degradation. Fast antigen/peptide degradation results in decreased delivery to cells and an ineffective immune response [27]. Thus, there is an urgent need for developing novel delivery vehicles that can sustainably release antigens without attenuating bioactivity.

Using nanoparticles as delivery systems is one way to address ineffective antigen delivery. Nanoparticles are promising vectors for antigen delivery in cancer vaccines due to various advantages, including prolonged biological activity, enhanced bioavailability, antigen protection from degradation and controlled antigen release. Various types of nanoparticles can be used as delivery systems or as adjuvants, such as polymeric nanoparticles, liposomes, micelles, carbon nanotubes, mesoporous silica nanoparticles (MSNs), gold nanoparticles (AuNPs), virus nanoparticles, acting alone or in combination [28] (Figure 1). Adding specific surface targeting moieties can significantly enhance delivery and hone nanoparticles to specific subcellular organelles involved in immune response [29–33]. Active vaccine systems can be formulated by incorporating cell-penetrating peptides, APC-specific cellular epitopes or immune-stimulant lipid moieties [34].

Polymeric nanoparticles

Polymers can consist of either natural or synthetic monomers. Biodegradable polymers have attracted significant interest from the biomedical field due to advantages of biodegradability, biocompatibility, nonimmunogenicity and so on. Various polymers, such as poly(lactic-*co*-glycolic acid) (PLGA), polyethylene glycol (PEG), polycaprolactone, chitosan and dextran, reviewed in [35], have been used in nanocarrier systems. Various polymeric combinations such as PLGA-PEG and PLGA-polycaprolactone have been used as delivery systems [36–38]. Synthetic, the US

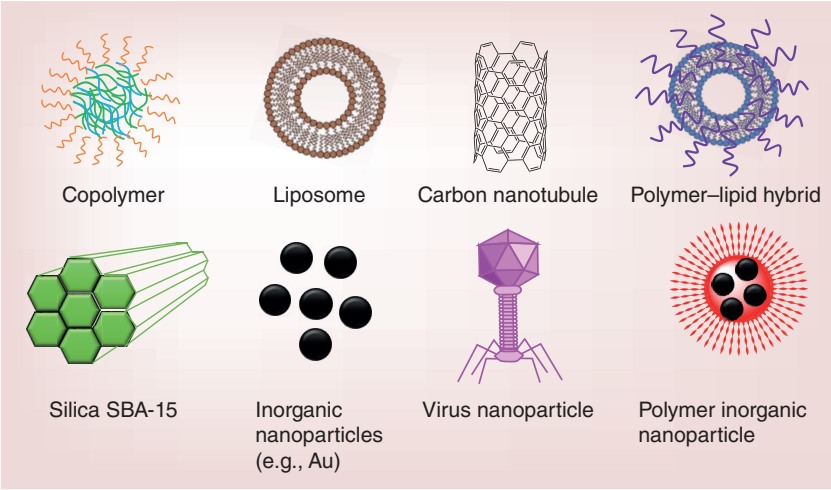

Figure 1. Structural representation of various nanoparticle delivery vehicles for cancer vaccine.

FDA-approved polymers, PLGA and PEG have been extensively studied as carriers for vaccines [39,40]. Dextran, a natural polymer derived from glucose, is also FDA-approved and has desirable biocompatible, biodegradable, cost-effective, highly stable and water-soluble properties. Chitosan can facilitate cellular transport across epithelium via opening tight junctions, and nanoparticles formulated with chitosan are suitable for vaccine release. Cellulose, another natural polymer, is abundant in plants and has been used in many fields, such as bioenergy, cement and drug delivery [44,45]. Nanocrystal cellulose is used in various applications [46–48], and may be useful in designing new cancer vaccine delivery systems.

PLGA nanoparticles showed potent antitumor effects through CD40-targeting in dendritic cells (DCs) when codelivered with ovalbumin (OVA) antigen, Pam3CSK4 and poly(inosinic-polycytidylic acid) (poly(I:C)) adjuvants. Targeted OVA-adjuvants-coloaded-PLGA nanoparticles significantly enhanced maturation and activation of DCs compared with nontargeted nanoparticles vaccines. *In vivo* vaccination of CD40-targeted nanoparticles showed more $CD8^+$ T-cell proliferation and stronger immunological responses in comparison to nontargeted nanoparticles or a mixture of free antigen and adjuvants [49]. A polymeric poly(D,L-lactic-*co*-hydroxymethyl glycolic acid) nanoparticle is reported to codeliver human papilloma virus (HPV) synthetic long peptides and poly(I:C) for inducing effective immunological response to inhibit tumor growth *in vivo*. The HPV-synthetic long peptides-poly(I:C) poly(D,L-lactic-*co*-hydroxymethyl glycolic acid) nanoparticle was not toxic and did not show any autoimmunity that is commonly caused by high concentrations of poly(I:C) [50]. Wen and Dhar reported that PLGA-PEG-triphenylphosphonium (TPP) nanocarrier can effectively deliver α-tocopheryl succinate to mitochondria and greatly enhance the therapeutic efficacy against cancer by upregulating complex V activity [37]. This system can be optimized to elicit robust immune response and inhibit the tumor growth.

Supramolecular polymers are characterized by connections of molecular monomers thorough intermolecular noncovalent (e.g., hydrogen bonding) interactions [51]. Supramolecular nanofibers constructed from small molecules can be effective vaccine nanocarriers with controllable activity upon external stimuli like pH change. Tian *et al.* designed G-NMe structure based on Nap-GFFY-OMe to enhance the immune responses of vaccines. The G-NMe nanocarrier significantly stimulated stronger immune response by delivering HIV DNA vaccine *in vivo* via intradermal or subcutaneous administration than free antigen and showed good safety and biocompatibility profiles both *in vitro* and *in vivo* [52].

Copolymers are made by covalently linking individual polymers. Copolymers combine the advantages of individual polymers and, thus, maximize versatility. Therefore, copolymers are commonly used to prepare vaccine delivery systems. Di-block (e.g., PLGA-PEG) or tri-block (e.g., PLGA-PEG-PLGA or PEG-PLGA-PEG) copolymers can be prepared by chemical conjugation and have been commonly used as therapeutics/vaccine vectors. A pentablock copolymer PDEAEM was constructed from pluronic triblock copolymers via polymerization. The pentablock PDEAEM hydrogel was shown to load and controllably release OVA antigen, and dramatically enhance immune response compared with free OVA protein *in vivo* [53]. PEG-*b*-poly(L-lysine)-*b*-poly(L-leucine) (PEG-PLL-PLLeu) micelles were constructed to codeliver PMP (polypeptide-poly(I:C))/OVA/siRNA vaccine to prevent

10.2217/nnm-2018-0147

tumor growth. The PMP/OVA/siRNA micelle showed enhanced *in vitro* DC maturation and activation, and *in vivo* cytotoxic $CD8^+$ T-cell proliferation and Th1 immune response compared with PMP/OVA and OVA controls. Furthermore, three dosages of PMP/OVA/siRNA nanovaccines in a 3-week period greatly inhibited tumor growth and prolonged the survival of mice [54].

Polymer thermal, physical and chemical properties play significant roles in carrier and biological interactions as delivery systems [55,56]. It is important to note that molecular weight also plays an important role for controlling effective payload release [41–43]. The intrinsic properties of polymer nanoparticles, such as composition, size, shape, surface properties and charge influence the efficacy and intensity of delivery and immune response, respectively. Various shaped polymeric nanoparticles, such as layered nanogels and micelles, have been designed for biomedical application. Polymeric nanogels are nanosized hydrogels characterized by water-swollen and polymeric networks. The properties of polymer nanogel such as solubility can be tuned from a group of hydrophilic and hydrophobic polymers among network chains. Polymeric nanogels can be used as vaccine delivery carriers as well as adjuvants when chemically conjugated with immunostimulants. Li *et al.* reported that dextran nanogels (~200 nm) prepared from methacrylated dextran and trimethyl aminoethyl methacrylate were able to deliver OVA antigen and release OVA in a controlled fashion *in vitro* and stimulated enhanced immunological activation compared with free OVA and OVA-microgels [57]. Li *et al.* further formulated core–shell nanosized hydrogels via layer-by-layer coating with oppositely charged polymers through electrostatic attraction, in which OVA was used as model antigen and showed stronger cytotoxic T-cell activation [58]. Poly(methacrylic acid) nanogel (~200 nm) was constructed using mesoporous silica as a template. PEGylation modification was used to enhance the circulation and lymph node drainage of nanogel, which did not affect the effective internalization of nanogel into DCs *in vitro*. The OVA was used as model antigen in PMA and PEG-PMA nanogels. OVA-PEG-PMA hydrogel nanoparticles stimulated significantly more T-cell proliferation than OVA-PMA nanoparticles when immunized in mouse model [59]. Polymeric hybrid micelles constructed from amphiphilic di-block copolymers, PEG-phosphorethanolamine (PEG-PE) and polyethylenimine-stearic acid (PSA) conjugate were reported to enhance the immunological potency of vaccines for cancer. The hybrid micelles could coload melanoma antigen peptide Trp2 and CpG ODN at a size of approximately 25 nm. Addition of positively charged PSA improved polymeric micelle cellular uptake *in vitro*. Hybrid micelles HM50 showed better targeting efficiency in immune cells of popliteal draining lymph nodes (DLNs) and stronger cytotoxic T-lymphocytes (CTLs) responses than free CpG and Trp2-PEG-PE micelles *in vivo* [60].

Acid-labile polymers may also be useful in delivery systems due to their ability to release antigen in a pH-dependent fashion. Acid-labile polymers are preferentially degraded in acidic environments and, thus can be used for controlled and effective immunological stimulation in the acidic cancer microenvironment. Acid-labile polymers either contain weak acids or bases such as carboxylic acids and amines, or consist of acid-labile linkages facilitating degradable cleavage in response to pH variance in acidic organelles (e.g., lysosomes) [61,62]. The pH-dependent di-block copolymers composed of dimethylaminoethyl methacrylate-*co*-pyridyl disulfide ethyl methacrylate and dimethylaminoethyl methacrylate-*co*-butyl methacrylate-*co*-propylacrylic acid were reported to be effective dual delivery systems for OVA antigens and CpG ODN adjuvants. The amphiphilic copolymers were self-assembled into micelles with a diameter of approximately 30 nm and a nanoparticle formulation of approximately four OVA molecules, approximately 30 strands of CpG ODN and approximately 80 polymer chains, showed the optimal membrane disruption upon pH changes. The OVA-CpG ODN nanoparticles greatly enhanced intracellular uptake of OVA and CpG ODN and demonstrated much stronger immunological responses both *in vitro* and *in vivo* compared with free antigens and CpG ODN controls [63].

Nanoparticle composition can be further modified to enhance delivery and therapeutic efficiency. Cracked cell membranes can be used to modify polymeric nanoparticles for enhanced cellular uptake. Cell membrane-coated polymeric nanoparticles can induce strong tumor-specific immune responses when conjugated with an immunological adjuvant monophosphoryl lipid A (MPLA) [64]. Chitosan is nontoxic and biocompatible, and has been FDA-approved for wound care. Although chitosan is normally immunogenic, modifications can modulate immunogenicity to induce much stronger immune responses in a vaccine system. PEGylation is a common approach for endowing a polymer with tunable functionality, solubility, biodegradability and biocompatibility. Polymer–drug constructs can be prepared by covalent linkage, directly or indirectly by connecting molecules to a therapeutic drug. Similarly, antigen/vaccines can be attached to polymer systems through chemical conjugation. OVA antigen was conjugated to poly(*N*-hydroxypropylmethacrylamide-PDS) poly(HPMA-PDS) polymer through disulfide formation. The OVA-poly(HPMA-PDS) conjugation enhanced the antigen cross-presentation to $CD8^+$

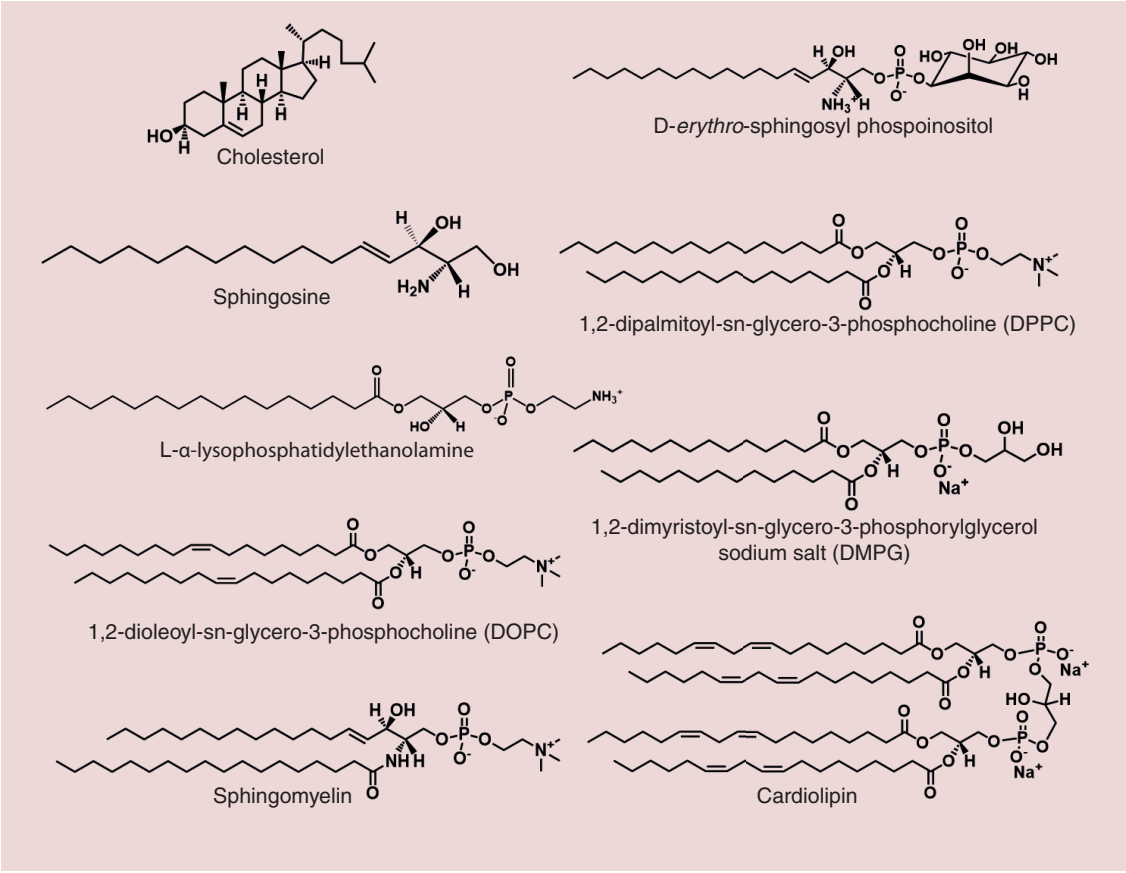

Figure 2. Chemical structure of lipid used for liposome nanoparticles.

T cells [65]. Dendrimers are hyperbranched polymers that possess versatile multivalent surfaces for interacting with surrounding surfaces [66]. E6 and E7 peptide–dendrimer conjugates (alkyne-functionalized 4-arm poly(*t*-butyl acrylate) conjugated to unprotected peptides from HPV E7 and E6) were prepared as vaccine adjuvants for cervical cancer, and showed effective antitumor immune response when implanted in mice [67]. The immune responses elicited by these polymer–peptide systems can be further improved when protected from degradation in a nanoparticle formulation.

Liposomes

The study of liposomes in the immune systems dates back to the early 1970s [68]. Liposomes can be constructed by various lipids such as 1,2-dioleoyl-*sn*-glycero-3-phosphocholine, cholesterol and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC; Figure 2). Several lipids can be used to combine desirable properties for tailored liposomal systems. Liposomes are versatile and can be constructed with desired properties by regulating lipid composition, surface properties, charge, size and so on [69–71]. They are potential delivery carriers reported to increase the immunogenicity of antigens for cancer vaccines and have been used as delivery systems for siRNA, DNA and antigens. Hydrophilic and lipophilic antigens can be loaded into liposomes in a manner such that hydrophilic antigens are trapped into the aqueous inner space while lipophilic components are inserted into the lipid bilayer by adsorption or chemical attachment. Table 2 summarizes the liposome delivery systems for cancer vaccines.

Liposomal surface charge plays an important role in vaccine immune response. Positively charged liposomes induce stronger immune responses than negatively charged liposomes since they can be more efficiently taken up by APCs-like macrophages and DCs. Thus, cationic liposomes are potent carriers for subunit vaccines and stimulate robust immune response at low doses. Liposomal surface charge properties can be chemically modified with functional groups, such as amine (cationic) and carboxyl groups (anionic). Moon and colleagues compared the antigen cross-presentation behavior of cationic liposomes composed of 3β-[*N*-(*N*′,*N*′-dimethylaminoethane)-

10.2217/nnm-2018-0147

Table 2. A summary of liposomal vaccines delivery system.

Liposomal system	Properties	Outcome	Ref.
L-BLP25	Composed of MUC1 peptide, MPLA as adjuvant and three lipids of cholesterol, DMPG and DPPC	The L-BLP25 was of minimal toxicity by Phase I and II clinical trial, and Phase III studies indicated prolonged survival rate of patients with non-small-cell lung cancer	[22]
Polymer-modified liposome	Poly(glycidol) was used to modify liposome, OVA was used as vaccine. DPPC, DOPE and MPLA were used as lipid source	The OVA-loaded polymer-modified liposomes showed higher level of Th1 and Th1 cytokine and antibody production than unmodified liposome and free OVA controls *in vivo* of both mice and chicken models	[72]
c-di-GMP/YSK05 liposome	Used as adjuvant system, the efficiency can be modified by helper lipids (e.g., POPE) and/or polymer (e.g., PEG)	The liposome showed the highest IFN-γ production as of YSK05/POPE/Chol/DMG-PEG2000 (40/25/35/1). The c-di-GMP could undergo endosomal escape and be released in cytosol by liposomal system for effective immune responses	[73]
LCP	Mannose was used to modify the surface, could codeliver both tumor antigen Trp 2 peptide and adjuvant CpG. The LCP nanoparticles were of 50 nm (diameter) and a zeta potential of 25 mV	LCP vaccine nanoparticles showed robust stimulating immune responses to inhibit tumor growth against B16F10 melanoma *in vivo*. The early-stage vaccine demonstrated better tumor inhibition than late-stage vaccine	[74]
Poly(I:C) adjuvanted cationic liposomal system	OVA_{24} peptide was used as vaccine. DOPC and DOTAP were used as lipid source Poly(I:C) was used as immunostimulant	The OVA_{24}-loaded poly(I:C)-liposomes showed more effective immune responses with greater capability to induce DC maturation, higher level of cytokines (e.g., IFN-γ) and stronger $CD8^+$ T cell activation compared with the mixture of OVA_{24} and poly(I:C) vaccination, both *in vitro* and *in vivo*	[75]
MPLA-sTnNPhAc conjugate	STnNPhAc was used as tumor-associated antigens	MPLA-sTnNPhAc conjugate stimulated significant production of antibody and cytokines *in vivo*. DSPC and cholesterol incorporation in the liposomal system could enhance the solubility	[76]
DPPC:Chol liposomes	OVA or photosensitizer tetraphenyl chlorine disulfonate (TPCS2a) was encapsulated. DPPC and cholesterol were used for liposome preparation. The loading of antigens did not change size of liposomal nanocarrier (~350 nm) and surface charge (~ -5 mV)	The liposome delivery released the OVA in the cytosol for immune response. The combination immunization of OVA liposomes and TPCS2a liposomes increased the proliferation of OVA-specific $CD8^+$ T cells. The photosensitizer TPCS2a liposome enhanced OVA-specific T cell cross-priming	[77]
Rha-TEG-cholesterol liposome	MUC1 and Tn were used as antigens. Pam3Cys bacterial lipoprotein was used to enhance the enhance immunogenicity. Rha was used for improving antigen uptake. DPPC and cholesterol were used as lipid sources	The Rha-decorated Pam3Cys-MUC1-Tn liposome showed greater production of antibody response, cytokine IFNγ production and $CD8^+$ cytotoxic T-cell proliferation and activation than non-Rha-modified liposomal system in *in vivo*	[78]
Targeted nanoliposome	PC and phosphatidylglycerol were used as lipid source. R848, Poly(I:C), LPS, Pam3Csk4 were used as immunostimulatory adjuvants. The liposome was designed to target FcRs. LHRH peptide and T-helper epitope (TT) were used as antigens	The targeted peptide and three adjuvants loaded liposome showed significantly stronger immune responses regarding to dendritic cell maturation, cytokine generation and T-cell activation than nontargeted liposomes, and two or one adjuvant/peptide-loaded liposomes in *in vitro*	[79]
Dextran derivative modified liposome	Dextran in the form of MGlu-Dex-endowed liposome with pH-sensitive properties. OVA was encapsulated as antigen. EYPC was used as lipid source	MGlu-Dex-modified liposome delivered OVA into the cytosol by endosomal escape for inducing immune responses. The OVA-loaded MGlu-Dex-liposome could induce the antigen-specific CTLs when administrated in *in vivo* and showed significant tumor inhibition with subcutaneous immunization in mice	[80]

Chol: Cholesterol; CTL: Cytotoxic T lymphocyte; DMPG: 1,2-Dimyristoyl-sn-glycero-3-phosphate; DOPC: 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine; DOTAP: 1,2-Dioleoyl-3-trimethylammonium-propane; DPPC: 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine; DSPC: 1,2-Distearoyl-sn-glycero-3-phosphocholine; DSPE: ,2-distearoyl-sn-glycero-3-phosphoethanolamine; EYPC: Egg yolk PC; FcR: Fc receptor; LCP: Lipid-calcium-phosphate; LPS: Lipopolysaccharides; MPLA: Monophosphoryl lipid A; OVA: Ovalbumin; PC: Phosphatidylcholine.

carbamoyl] cholesterol (DC-Chol) and 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) with tertiary amine groups to negatively charged liposomes composed of EPC/Chol/DSPE-mPEG. The cationic liposomes (CLs) including DOTAP-CLs and DC-Chol-CLs showed antigen cross-presentation/priming of $CD8^+$ T cells in bone marrow-derived DCs by acidic interactions with lysosomes given that amine-functionalization provided buffering capacities and enhanced protection of loaded antigens before reaching targets of interest. In comparison, the anionic liposome EPC/Chol/DSPE-mPEG did not show any antigen intervention [81]. The cationic liposomal CAF09 adjuvant based on DDA- and MMG-1 liposomes containing poly(I:C) is reported to induce strong T-cell immune response. The CAF09 liposome was more stable than other lipid composition constructs like CAF01 based on dimethyldioctadecylammonium (DDA) and trehalose dibehenate (TDB), and CAF05 composed of DDA, TDB and poly(I:C). The CAF09 liposomal system could serve as an adjuvant by inducing antigen-specific $CD8^+$ T-cell response and as a delivery carrier for vaccines to prevent tumor growth after immunization. *In vivo* studies demonstrated that CAF09 liposome induced $CD8^+$ T cells by three doses of immunization and was a superior delivery system when compared with other adjuvants for antigens like HPV E7. The E7/CAF09 immunization showed inhibition of tumor growth and increased survival rate of mice compared with control groups [82]. Cationic

DNA vaccine liposomes constructed by cationic amphiphile is reported to stimulate long-lasting remarkable immune response for melanoma *in vivo.* When conjugated with a mannose-mimicking shikimoyl head group, the liposomal-DNA system with guanidinylation in side chain of lysine amino spacer effectively transfected DCs by targeting DNA in DCs and induced the secretion of cytokines TNF-α, IL-6 and IL-12p70 *in vitro*. The p-CMV-β-gal complexed with liposome system immunization showed enhanced level of cytokine IFN-γ and anti-β-Gal antibodies. The immunization of lipoplexes constructed by mannose-mimicking shikimoyl lipid 1 showed 100% survival rate up to 100 days after post-tumor change and 80% survival up to 6 months with a second tumor challenge [83]. Cationic liposomes modified with polyallylamine were reported to effectively deliver DNA as nanocomplexes for transfection [84], and thus could be potential vaccine-delivery systems for effective cancer prevention and therapy.

Lipid composition influences both delivery efficiency and immune response. Various lipids with tunable composition are used instead of a single lipid when formulating the liposome system in vaccine delivery. Many lipids used in the drug delivery development, such as phosphatidyl ethanolamine (PE), sphingomyelin, cholesterol, DSPC and DSPE, can also be utilized for vaccines. Oleoyl liposome consisting of dioleoyl PC, dioleoyl PE, dioleoyl phosphatidyl glycerol acid and cholesterol in a ratio of 4:3:2:7 has been reported to deliver glypican-3-derived epitope peptide pGPC3 to induce T-cell immune responses and mice immunized with pGPC3-liposome showed significant inhibition of GPC3-expressing tumor growth [85]. A liposomal system Lip-DOPE-P5-MPL composed of five lipids DMPC, DMPG, cholesterol, DOPE and MPLA was constructed as peptide P5 delivery carrier to generate effective vaccine capacity for breast cancer. The peptide P5 encapsulation in liposome was improved with chemical modification by maleimide-PEG2000-DSPE. Lip-DOPE-P5-MPL elicited robust CTL response *in vitro* by releasing P5 peptide to the cytosol of APCs [86].

To target specific immune cell types or subcellular organelles, liposome can be modified by APC-specific ligands, such as fucose, mannose and glucan, and antibodies for active endocytosis, or chemical conjugation by functional groups such as guanidinylated modification for DNA interaction. Lymphatic-targeted mannosylated DOTAP liposomes (LP-Man) were constructed by incorporating mannosylate DSPE-PEG into DOTAP. LP-Man achieved targeting behavior via mannose receptor on APCs and demonstrated significantly higher cellular uptake of OVA compared with nontargeted LP-OVA in mouse bone marrow derived cells (BMDCs) *in vitro* and *in vivo* in spleen and DLNs. OVA-loaded LP-man showed enhanced levels of anti-OVA IgG and IgG subtypes like IgG1 and IgG2b, which could remain in the body system for up to 90 days. OVA-LP-man vaccine induced formation of splenic germinal center, and elevated T-follicular helper and memory T-helper cells for long-term immunological memory [87]. Galactosylated liposome has been reported to target DCs through C-type lectin receptors recognition of carbohydrate structure galactose for antigen presentation. OVA-loaded galactosylated liposome showed remarkable CTL responses and antibody production against tumor growth [88].

Liposomes are considered alternatives to polymeric nanoparticles due to reported low encapsulation efficiency; limited solubility; and propensity for phospholipid degradation, compound leakage and fusion. Overall, the known disadvantages of using liposomes limit their application as vaccine delivery systems. The properties of liposomes can be modified by incorporating new components such as polymers and carbon nanotubes. Modifying liposomes with glycan are reported to increase antigen binding and internalization by DC-SIGN expressed BMDCs. OVA-loaded glycoliposomes demonstrated enhanced cellular uptake (by binding to DC-SIGN), antigen presentation in $CD4^+$ T cells and activation of effector $CD8^+$ T cells when compared with nontreated liposome and free OVA controls [89]. Negatively charged carbon nanohorn is reported to modify cationic liposome nanoparticles for an antinicotine vaccine. Carbon nanohorns act as a scaffold and prevented precipitation or flocculation of cationic liposomes as vaccine delivery vectors. Modified liposomes showed significantly enhanced *in vitro* Th1/Th2 immune response compared with nonmodified controls with no significant histopathologic lesions in major organs [90]. This liposome system may be used to deliver cancer vaccines as well. Lipid-polymer hybrid nanoparticles constructed by DOTAP liposomes and hyaluronic acid are reported to successfully deliver OVA to APCs and showed robust $CD8^+$ T-cell immune and antibody responses both *in vitro* and *in vivo* [91].

Inorganic nanoparticles

Inorganic nanoparticles have been used in various applications, such as bioimaging, sensors, drug delivery and therapeutics, and cancer immunotherapy due to their unique optical, physical, chemical, electronic and magnetic properties. The size, shape and surface properties of inorganic nanoparticles can be facilely manipulated during synthesis and modification process to actively interact with cellular functions. Inorganic nanoparticles are usually

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Table 3. A summary of inorganic nanoparticles used in cancer vaccine system.

Material	Size/zeta potential	Antigens	Outcomes	Ref.
Gold (Au)	15–80 nm -0.7 to -40 mV	OVA, gp100	AuNPs are of nontoxic. PEG linker provided the optimal AuNPs for antigens delivery Peptide-loaded AuNPs showed effective immune responses than free peptide. The size of AuNPs did not alter IFN-γ efficacy *in vitro*	[92]
Silver (Ag)	8–30 nm +48 to +78 mV	H5 DNA	Low toxicity within tested window. The immunization with Ag/H5 in *in vivo* chicken model generated HI antibody and cytokines including IL-15, IL-12β, TNFSF13B and IL-1β	[93]
α-Al_2O_3	60–200 nm	OVA	α-Al2O3-OVA NPs (60 nm) were effective antigen adjuvants by inducing T-cell proliferation and activation both *in vitro* and *in vivo* (mouse)	[94]
Carbon nanotube	120–400 nm (length) +6 to -40 mV	OVA	MWNT-OVA (122 nm) with the negative demonstrated the highest cellular uptake and immune response among all other MWNT-OVA controls in *in vitro* and *in vivo*	[95]
Silica	30–80 nm 0 to +30 mV	OVA, CpG	The CpG-loaded silica nanoparticles enhanced the cellular uptake, TLR activation and immune response of CpG in *in vitro* and *in vivo*. The nanoparticle could codeliver OVA and CpG with size slightly increased for enhanced immune stimulation	[96]
Iron oxide	40–300 nm -25 to 35 mV	DNA	PEI modification provided the versatility of iron oxide NPs for DNA vaccines. The surface and size of the nanoparticles were dependent on the buffer conditions. SPIONs/PEI/DNA-HA complexes induced the stronger humoral and cellular immune response than free DNA and SPIONs/PEI/DNA vaccine both *in vitro* and *in vivo*	[97]
$CaPO_4$	275 nm, +20 mV	TLR ligand poly(I:C)	The nanoparticles showed efficient macrophage THP-1 cellular uptake and robust immunostimulatory effects in *in vivo* mouse model	[98]
Ag@SiO_2	490–500 nm	pF DNA	The nanoparticles were of low cytotoxicity and high stability and induced Th1-type immune responses	[99]

AuNP: Gold nanoparticles; HA: Hyaluronic acid; MWNT: Multi-walled carbon nanotube; NP: nanoparticle; OVA: Ovalbumin; PEG: Polyethylene glycol; PEI: Polyethyleneimine; TLR: Toll-like receptor.

biostable and nondegradable, and several are in preclinical stages as vaccine delivery systems. Furthermore, inorganic nanoparticle trafficking and cargo release can be internally or externally induced by factors like temperature, pH, metabolites, magnetic fields and/or light. Gold, iron oxide, aluminum-based nanoparticles, quantum dots, up-conversion nanoparticles and mesoporous silica are all viable delivery systems for cancer vaccines. Inorganic nanomaterials can form cores and provide scaffolding with unique structural and dynamic properties for other biomaterials, such as polymers and lipids, to construct robust and effective delivery vectors. Table 3 summaries inorganic nanoparticle vaccine delivery systems.

Aluminum-based systems are one of the most common adjuvants in vaccine development for strong cell-mediated immunity. However, high aluminum levels are associated with immune toxicity. To improve the practicality of aluminum in vaccines, it is essential to construct new aluminum-based adjuvants with less toxicity – either decreasing/sequestering total aluminum content, increasing aluminum excretion or decreasing aluminum metabolism – and minimizing associated immunologic side effects. Aluminum-based nanoparticles display enhanced immune activation as adjuvants when used in lower doses compared with conventional aluminum-based adjuvant. Aluminum hydroxide nanoparticles (~100 nm) containing OVA demonstrate stronger *in vitro* immune activities and *in vivo* tumor progress with immunization in lower dose compared with traditional aluminum hydroxide (~9 μm) [100]. Phospholipid bilayer-coated OVA-aluminum nanoparticles showed enhanced *in vitro* APC cellular uptake and *in vivo* CTL immune responses, and less inflammation than the traditional OVA-aluminum nanoparticles [101].

AuNPs are widely used as delivery and/or adjuvant systems for cancer vaccines due to biocompatibility, controllable size and shape, facile synthesis, and self-adjuvant and imaging capability. The size-dependent role of AuNPs on the effect of vaccine delivery is reported by Kang *et al.* OVA-AuNPs, 33 nm in diameter, exhibited the higher uptake efficiency by DCs and stronger T-cell immune responses than smaller-sized AuNPs (10 and 22 nm) [102]. Niikura *et al.* demonstrated AuNPs to be effective vaccine adjuvants for West Nile virus envelope (E) protein both *in vitro* and *in vivo*. The shapes (nanosphere, nanocube, nanorod) and size (20–40 nm) of AuNPs affected the immune responses and antibody production. Sphere40-Es (40 nm spherical AuNP-Es) induced stronger immunological response and higher level of antibodies and cytokines (TNF-α and IL-6) than differently shaped/sized controls, for example, Sphere20-Es [103]. Codelivery of OVA antigen and CpG adjuvant using AuNPs is reported to

stimulate robust antigen-specific responses and inhibit tumor growth for cancer immunotherapy [104]. Glycosylated AuNPs are constructed as synthetic cancer vaccines by conjugating with tumor-associated (Tn) antigen glycans to induce strong immune response and produce antibodies for aberrant mucin glycans. The AuNPs modified with PEG25Tn25 and PEG80Tn2 showed the strongest immune stimulatory antibodies among all PEG-modified formulations *in vivo* [105].

The unique physical and chemical properties of magnetic nanoparticles make them desirable potential vaccine systems for cancer. Magnetic nanoparticles lack the versatility to deliver vaccines for cell-specific targeting due to unstable and inflexible hydrophilic/hydrophobic properties. To address these issues, modification with other components such as polycationic polymer can be used to provide the surface with sufficient capacity for functionalization and antigen binding. Superparamagnetic iron oxide nanoparticles (SPIONs) modified with cationic polyethyleneimine (PEI) polymer are promising DNA vaccine vectors due to their high buffering capacity[106]. Magnetic fields offer the capacity of enhanced cellular uptake and DC maturation. The SPIONs/PEI/DNA-HA gene complexes were constructed to deliver DNA vaccines and showed improved *in vitro* DC transfection and maturation [107]. Magnetic materials can be used for hyperthermal treatment of cancer and/or induce antitumor immune response by converting dissipated magnetic energy to thermal energy heating tumor tissue (over 43°C) but allowing surrounding normal tissues to be unharmed under external stimulation [108]. Generated heat-shock proteins within tumor tissues by magnetic nanoparticle hyperthermia stimulate tumor-specific immune responses for cancer therapy [109]. A combination therapy by magnetite cationic liposomes and immature DCs in an existing tumor site showed significant enhanced CTL and NK cell activity *in vivo*. Heat generated by magnetic nanoparticles induced necrotic tumor cell death and release of *HSP70* for DC maturation. Combination therapy with magnetic nanoparticle system caused more accumulation in lymph node DCs and increased immune response than DC-only controls [110]. With their unique magnetic properties, magnetic nanoparticles can be used to manipulate DC migration into lymph nodes for DC-based immunotherapy. Magnetic nanoparticles are reported to migrate DCs to lymph nodes under magnetic pull force *in vivo*. Fluorescent magnetic nanoparticles (α-AP-fmNPs) were constructed by iron oxide nanoparticles, indocyanine green and fusion peptides (α-AP). External simulation by magnetic pull force significantly enhanced the migration of α-AP-fmNP-loaded DCs than control DCs both *in vitro* and *in vivo*. BMDCs treated with α-AP_{OVA}-fmNP showed enhanced *in vitro* $CD8^+$ T-cell proliferation and cytokine IFN-γ production, and *in vivo* CTL response than nonmagnetic nanoparticle treated and nontreated controls [111].

Mesoporous silicas are solid materials featured by mesoporous structure encapsulation of biomolecules [112,113]. Mesoporous silicas have been intensively studied as drug delivery systems due to the advantage of high surface area, tunable pore size and stable chemical/thermal properties [114]. MSNs can also be used as antigen carriers and/or adjuvants for cancer immunotherapy. Mahony *et al.* reported MCM-41 MSNs to be both OVA antigen delivery vectors and adjuvants *in vivo*. Amino-functionalization of MCM-41 (AM-41) increased binding efficiency and more OVA were bound compared with MCM-41. OVA-AM-41 nanoparticles showed enhanced specific adaptive immune responses than free OVA without eliciting any damage to major organs [115]. The adjuvant activity of MSNs SBA-15 and SBA-16 with recombinant antigen ($HSP70_{212\text{-}600}$) was evaluated *in vivo*. SBA-15 demonstrated higher adjuvant effect than SBA-16, which was comparable to alum, and $HSP70_{212\text{-}600}$/SBA-15 combination-induced Th1/Th2 response and anti-inflammatory cytokine IL-10 in mice [116].

Virus nanoparticles

Viruses are naturally occurring infectious agents consisting of nucleic acid genomes such as DNA and RNA, protein capsids, and probably lipid envelopes derived from host cell membranes. Viruses are commonly defined as viral nanoparticles (VNPs), and virus nanostructures have been used as scaffolds for various materials such as vaccine. A virus-like particle (VLP) is a noninfectious particle lacking genomes with safer and less immunogenic properties compared with a virus. VNPs and/or VLPs play significant roles in the development of vaccines with the added advantage of easy modification and functionalization.

Several VLPs are commercially available for vaccine in clinical settings, such as HPV and hepatitis B virus. HPV-16/18 L1 VLP AS04 vaccine showed long-term vaccine efficacy for HPV-16 and -18 with good safety profile [117]. HPV-16/18 vaccine has prevented 70% of cervical cancers [118]. 9-valent HPV VLPs (9vHPV) vaccine increases the long-term prevention of cervical cancer up to 90% by dealing with four HPV types (6, 11, 16, 18) and five oncogenic types (31, 33, 45, 52 and 58) [119].

Cowpea mosaic virus (CPMV) VLPs are reported as vaccines for treating metastatic lung cancer. Empty CPMV (eCPMV) VLP system induced neutrophil recruitment to the lung of B16F10 tumor-bearing mice for stimulating

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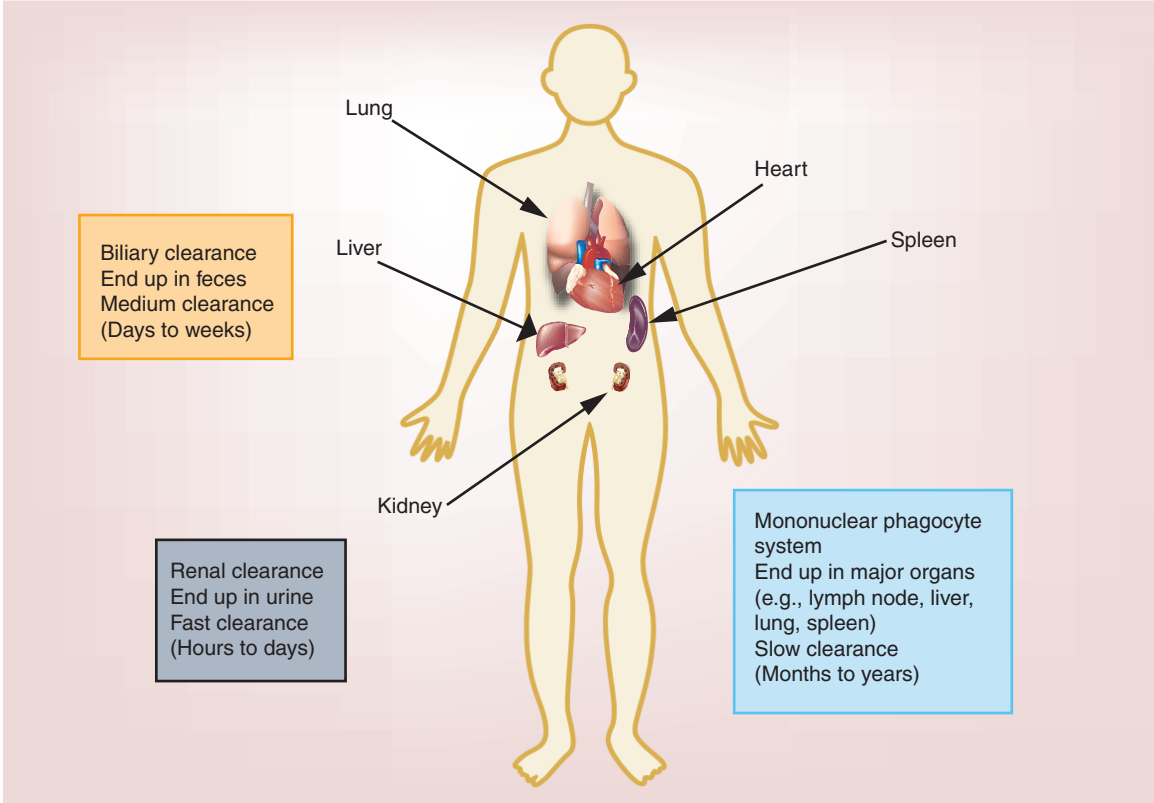

Figure 3. Secretion pathway of nanoparticles.

antitumor immune responses. Treatment with eCPMV particles resulted in prolonged survival and inhibited tumor growth compared with phosphate-buffered saline (PBS) treated control *in vivo* [120]. This VLPs may be modified or loaded with antigens/vaccines for enhanced immunotherapy for other cancers metastasized to the lung.

The chemical and physical properties of VNPs and/or VLPs can be influenced by various factors. VNPs or VLPs can be enhanced by modification, functionalization and/or encapsulation into a secondary nanoparticle with a targeting ligand for APCs. A study on the structural properties of hepatitis B core protein VLP indicated that stability of VLPs could be improved by introducing covalent disulfide bridges and loss of surface negative charges resulted in low solubility and poor nanoparticle assembly [121]. Epstein-Barr virus (EBV)-based VLPs in patients showed induction of T-cell populations in Phase I trial, warranting further study in Phase IB and II trials [122]. Self-assembled EBV nanoparticles are reported to elicit robust antibody response. EBV nanoparticles were modified by conjugating viral trimeric glycoprotein to ferritin structures with fusion of amino terminal by *H. pylori* ferritin forming a hybrid to prevent autoimmunity. The structurally designed EBV nanoparticle vaccine demonstrated strong cytotoxic T lymphocyte (CTL) activities and CR2BS-specific antibodies by precisely targeting to B cells via gp350 *in vivo* [123]. A VLP system made of pyruvate dehydrogenase E2 protein nanoparticle showed great potential as a vaccine delivery platform for cancer. E2 nanoparticles could codeliver melanoma-associated gp100 epitope and CpG (CpG-gp-E2) by conjugation and showed significantly enhanced *in vitro* CTL activities and cytokine IFN-γ secretion than free peptide and CpG formulation. *In vivo* studies showed that CpG-gp-E2 immunization increased the melanoma epitope-specific $CD8^+$ T-cell populations in DLNs and spleen than free peptide and CpG, and effectively inhibited tumor growth [124].

Nanoparticle clearance pathways

One important concern for designing nanoparticle vaccine carriers is clearance, degradation and metabolism in the body. Clearance and/or excretion of nanoparticle is necessary to reduce side effects after delivering and/or releasing antigens to a specific site. Nanoparticles can be cleared through the mononuclear phagocytic system (MPS), renal/urinary system and/or biliary clearance (Figure 3).

Mononuclear phagocytic system

The immune system plays an important role in nanoparticle clearance. MPS, or reticuloendothelial system (RES), is primarily composed of monocytes and macrophages. Macrophages participate in the clearance of nanoparticles by phagocytosis and accumulate mainly in the lymph nodes, spleen and/or liver [125]. The surface properties, size, shape and nature of materials play critical roles in the clearance pathways of nanoparticle. Polyvinyl pyrrolidone (>120 kDa) is reported to be phagocytosed by macrophages for clearance [126]. Kupffer cells can retain nanoparticles with a diameter more than 100 nm [127]. The modification of intrinsic surface properties (e.g., hydrophilic or hydrophobic) of magnetite nanoparticles by lipids is reported to have no effects on alveolar macrophage clearance due to the interaction of surface lipid and proteins [128]. Nanoparticle clearance by phagocytic cells can be greatly enhanced by modifying with surface ligands/proteins such as opsonins for recognizing the phagocytic cell [129]. Modifying quantum dots with anionic dihydrolipoic acid, cationic cysteamine or PEG coating to enlarge their hydrodynamic diameter over 5.5 nm prevented renal excretion and increased uptake by MPS with large macrophage populations in the lung, spleen and liver [130].

Renal clearance

Renal clearance is the primary method for eliminating small molecules or nanoparticles. Small nanoparticles less than 10 nm in diameter can undergo glomerular filtration and be excreted in the urine. Large nanoparticles (>10 nm) can be designed to be biodegradable and thus, degrade into small molecules after completing vaccine delivery. Inorganic nanoparticles are usually stable and nonbiodegradable; alternatively, inorganic nanoparticles can be designed to be filtered through kidney for excretion to avoid long-term accumulation and toxicity from decomposition by regulating size, surface properties and shape of nanoparticles. Small-sized AuNPs (<10 nm) can be eliminated via renal function. The glutathione-coated AuNPs with a diameter of 2 nm showed more effective renal clearance (10–100-fold more) than bis(*p*-sulfonatophenyl)phenylphosphine and cysteine coating [131]. Glutathione-coated copper nanoparticles (~2.7 nm) showed faster renal clearance than the smaller dissociation fragments and Cu(II)-GSSG complex in the first 2 h after intravenous administration in mice [132]. Small Pd nanosheets with a diameter of approximately 4 nm underwent renal clearance *in vivo* after photothermal treatments [133]. CdSe/ZnS core/shell QDs with a diameter of less than 5.5 nm is reported to be rapidly excreted in the urine, and modification of size and surface charge of QDs can reduce or avoid renal clearance [130]. Ligand chelate-coated AuNPs (Au@DTDTPA) consisting of a gold core and a dithiolated derivative of diethylenetriaminepentaacetic acid (DTDTPA) shell was tested to be safe in *in vivo* rats model, since Au@DTDTPA NPs could be excreted via renal clearance with primary accumulation in the kidneys and bladder in biodistribution studies [134].

Biliary clearance

Biliary secretion is commonly used for nanoparticle clearance. Generally, intravenously administered carriers show biodistribution mainly in the liver, which makes these delivery systems less effective for therapeutic purposes. The properties of nanoparticles such as surface charge, size and composition play an important role in determining the biliary clearance excretion. Nanoparticles larger than 150 nm can be cleared through the hepatobiliary system [135]. Biliary clearance is reported to be inversely related to size. For AuNPs ranging from 3 to 200 nm in diameter, increasing nanoparticle size increased percentage of AuNPs undergoing biliary clearance. Surface charge modification of AuNPs by amine or carboxyl group can enhance liver accumulation after intravenous administration [136]. Single-walled carbon nanotubes with a length of 100 nm and diameter of 1–2 nm mainly accumulated in the liver and were excreted by biliary system. Short single-walled carbon nanotubes of less than 50 nm showed a small percentage of biliary excretion and could undergo renal clearance [137]. Nanoparticle clearance may vary in different *in vivo* models, and thus the model selection of *in vivo* studies should be considered when designing nanoparticles for clinical application. Gadolinium–perfluorocarbon nanoparticles are reported to be rapidly eliminated through biliary system in less than 5 min in rats, which was markedly different from results seen in larger animal models and humans [138].

Strategy to escape rapid clearance

Fast clearance of nanoparticles may lead to low efficiency in cancer vaccines. Recently, Wilhelm *et al.* suggested that less than 1% of nanoparticles is delivered to tumor sites for therapeutic intervention; MPS and renal clearance accounts 99% of nanoparticle clearance [139]. Modifying nanoparticles to escape clearance is a possible strategy to enhance circulation and avoid fast clearance before cargo release to targeted sites. However, since many studies

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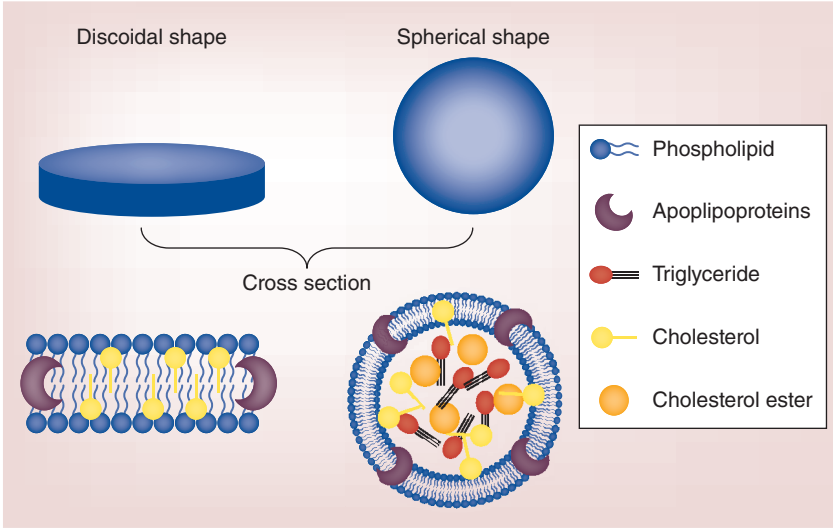

Figure 4. The designing factors and vaccine intervention of nanoparticles.

mostly focus on improving nanoparticle uptake and targeting efficacy, approaches to escape nanoparticle clearance after successful cellular uptake are not well investigated for enhancing therapeutic intervention.

Tuning properties of nanoparticles

The molecular weight of nanocarriers is an important factor for controlling effective payload release [41–43]. It is important to tune the properties of nanoparticles for robust immune response and long-term immunity (Figure 4). Surface coating with specific ligands or introducing new components, such as PEG and polysaccharide, can be used to escape or reduce the extent of fast clearance. Chitosan-coating is reported to enhance insulin circulation *in vitro* when delivered by solid lipid nanoparticles to bypass MPS-mediated phagocytosis after intestinal uptake [140]. PEGylated liposome containing polycation-DNA (LPD) nanoparticles showed reduced liver uptake and enhanced favorable tumor delivery of siRNA in *in vivo* model compared with naked LPD. PEGylated LPD nanoparticles reduced their clearance by MPS by preventing surface opsonization with serum proteins and thus favored specific targeted delivery [141]. PEG modification can enhance nanoparticle circulation and slow renal clearance even for nanoparticles within glomerular size-threshold (5 nm) [142]. PEGylated MSNs (PEG10k–MSNs) showed significantly reduced phagocytosis (0.1%) compared with nonmodified MSN controls (8.6%) by preventing nonspecific binding with serum proteins *in vitro* [143].

Neutral zwitterionic groups composed of positive and negative charges can resist nonspecific protein adsorption through charge and hydrogen bonding interactions. Thus, zwitterionic coating can be used to prevent serum protein adsorption in blood circulation for extended circulatory lifetimes, and minimal engulfing by macrophages and MPS clearance. Zwitterion-coated iron oxide nanoparticle is constructed by ligand zwitterionic dopamine sulfonate, which is much smaller than PEG–lipid ligand, and shows low nonspecific binding with serum proteins in *in vivo* model [144]. Glutathione is an intracellularly synthesized antioxidant in tissues/cells that prevents cellular damage caused by reactive oxygen species [145]. Glutathione-coated AuNPs are reported to reduce the serum protein binding when incorporated into SPION (iron oxide) nanoparticles [146]. PEG-poly(propylene sulfide) (PEG-PPS) was surface-coated onto ultrasmall SPIONs with various surface charges and sizes. PEG-PPSylated nanoparticles with a size of more than 100 nm were internalized by macrophages via CD204 receptor-mediated endocytosis, and smaller nanoparticles (<40 nm) had less nonspecific macrophage uptake. Threefold increased uptake was observed for 100-nm nanoparticles with ζ potentials from -3.5 to -0.8 mV and fourfold reduced uptake was found for 40-nm nanoparticles with ζ potential range from -9.0 to -3.5 mV [147].

Constructing biomimetic nanoparticles

Biomimetic nanoparticles leverage naturally occurring cellular recognition processes and uptake without causing immunogenicity and toxicity. High-density lipoprotein (HDL) is a natural nanoparticle composed of lipids and proteins. HDL is associated with low cardiovascular disease risk by participating in reverse cholesterol transport [148].

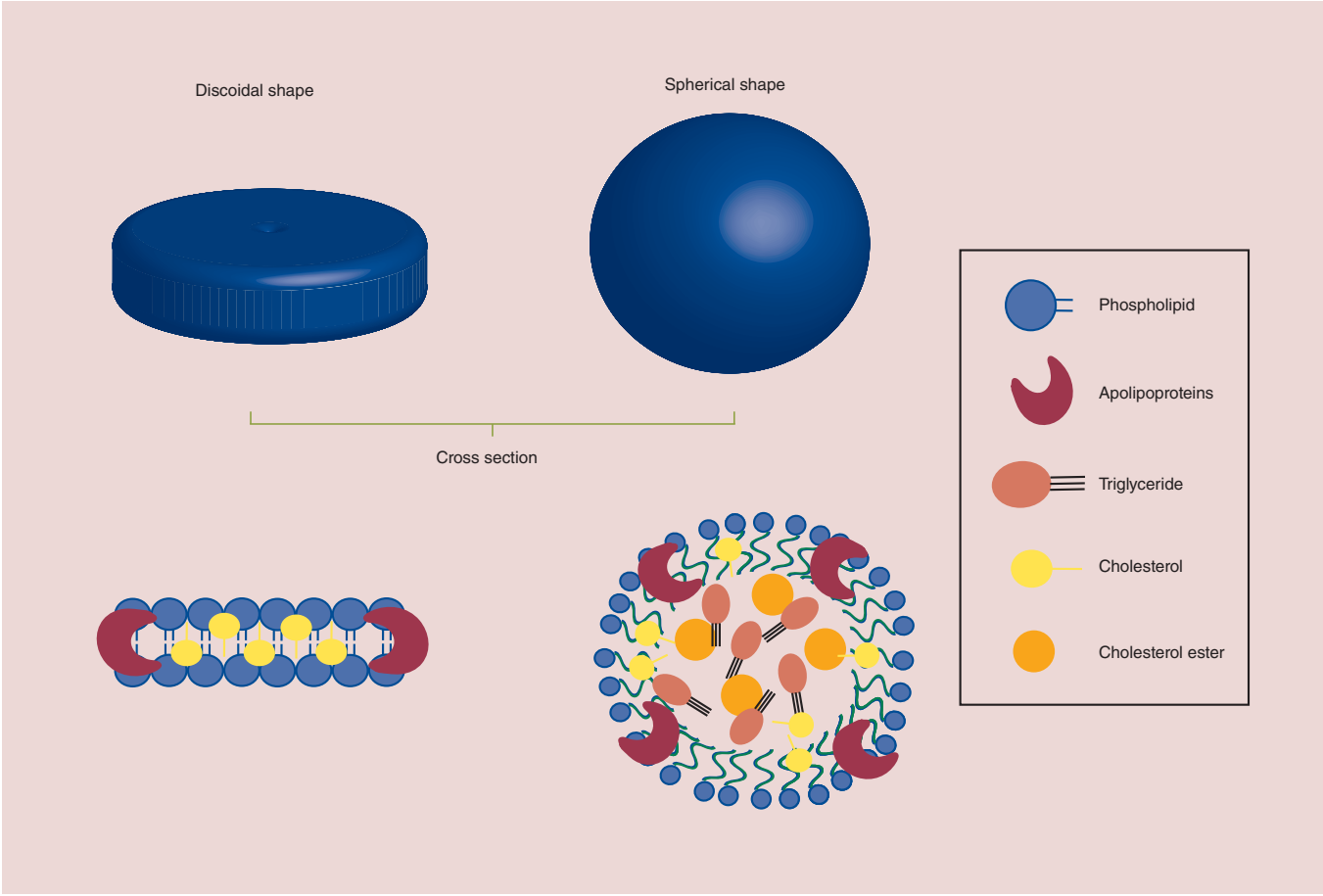

Figure 5. Shape and structural diagram of natural high-density lipoprotein nanoparticle.

HDL-like particle or reconstituted HDL can be prepared by reconstituting phospholipids and apolipoprotein A-I (ApoA1) extracted from human plasma to mimic the shape and structure of natural HDL. The HDL or HDL-like nanoparticles have demonstrated potential for application in drug delivery systems, and details have already been reviewed [149]. The discoidal bilayer structure and 3D spherical shape of HDL (nascent and mature HDL, respectively) have attracted the most interest in constructing HDL-like nanoparticles (Figure 5) [150]. Spherical HDL NPs are reported to be more efficient at delivering cargos (e.g., glioblastoma-targeting drug) than discoidal HDL NPs, with a higher cellular uptake and tissue penetration *in vitro* and *in vivo* [151]. HDL NPs are reported to incorporate inorganic nanoparticles such as gold, iron oxide or quantum dots as core material to replace the hydrophobic core of natural HDL [152]. Magnetic nanostructures were surface coated by DPPC and NBD-PC (phospholipid source) and human ApoA1 to mimic the function of HDL and showed cholesterol efflux capacity comparable to human HDL [153], suggesting safe properties and potential long-term circulation in the blood system. Given the potential capability of modifying the components of HDL, it is possible to encapsulate vaccines/antigens in the core, insert it into the double lipid layers or attach to the surfaces by chemically modifying apopA1 in HDL or HDL-like nanoparticles.

Specific targeting is critical to improve vaccine immune response. Nanoparticles can attain targeting capability by using specific targeting ligands or constructing nanoparticles of a specific composition. Zhang and colleagues demonstrated that HDL-mimicking nanoparticles, α-Ap-FNP, which consist of a fusion peptide α-Ap and phospholipids containing a near-infrared fluorescent dye, efficiently targeted to DLNs and successfully delivered Ap to DCs through scavenger receptor class B1 (SR-B1)-mediated pathway. *In vivo* B16F10-bearing mouse model indicated that α-Ap(gp100)-NP-CpG nanovaccine significantly enhanced secretion of cytokine IFN-γ and inhibited tumor growth compared with α-Ap(gp100) and chol-CpG group [154]. They further modified the biomimetic HDL nanoparticles with M2pep to dual target siRNA to tumor-associated macrophages in melanoma. The dual

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targeting biomimetic nanoparticles were able to deplete M2-like tumor-associated macrophages, and inhibit the progression of melanoma tumors [155].

HDL nanoparticles have also been reported to target organelles. Zhang *et al.* reported a spherical HDL biomimetic peptide-phospholipid nanocarrier constructed by apoA-I-mimetic peptide, DMPC and cholesterol oleate to directly deliver payloads to the cytosol. Cytosolic delivery to cancer cells was confirmed *in vitro* and *in vivo* showing that nanocarrier bearing natural HDL receptor SR-B1-targeting ligand mainly accumulated in tumor, with threefold more of accumulation in SR-BI$^+$ tumor compared with SR-BI$^-$ tumor [156]. In another study, HDL-mimicking nanoparticle is reported to target mitochondria for effective reverse cholesterol transport. The spherical HDL-mimicking nanoparticles composed of cholesteryl oleate, PLGA and apoA-I mimetic peptide were modified by mitochondria-targeting ligand TPP. Highly efficient cholesterol efflux was demonstrated in TPP-modified HDL-mimicking nanoparticles compared with nontargeted control both *in vitro* and *in vivo* [157]. This HDL-mimicking system may be potential delivery nanocarrier for vaccines to achieve effective immune response in cancer.

Synthetic HDL (sHDL) nanodiscs are reported as delivery vectors for antigen peptide and CpG adjuvant to induce robust immune response [158]. The sHDL nanodiscs were of discoidal shape and composed of phospholipids and ApoA1 mimetic peptide 22A to avoid autoimmunity. Various antigen peptides such as $OVA_{257-264}$ were successfully surface coated to sHDL nanodiscs through a cysteine-serine-serine linker, and CpG was attached via cholesterol linker. Nanodiscs demonstrated versatility; varying phospholipid source (e.g.,1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine [POPC] provided turbid suspension, DPPC for clear sHDL suspension) and antigen cargos did not compromise size (~10 nm) and stability. Composite nanodisc, sHDL-Ag (SIINFEKL)/CpG, significantly enhanced and sustained antigen presentation in BMDCs with approximately ninefold greater at 24 h and approximately fourfold higher at 48 h, than free Ag(SIINFEKL)+CpG. Maturation and antigen presentation of BMDC by sHDL-Ag/CpG promoted *in vitro* CD8$^+$ T-cell activation. *In vivo* vaccination with sHDL-Ag/CpG effectively prevents tumor growth by stimulating remarkably stronger CTL responses (41-fold) than free CSSSINFEKL-CpG group. When combined with αPD-1, sHDL-Adpgk/CpG immunization showed strong neoantigens-specific immune responses and remarkable tumor inhibition in mice (88%) compared with Adpgk+CpG+αPD-1 group (25%). The combined immunization could completely prevent the tumor recurrence when challenged with subsequent inoculation of murine colon carcinoma MC-38 cells. They further modified sHDL nanodiscs by optimizing the phospholipid composition for withalongolide A 4,19,27-triacetate delivery, which showed effective tumor inhibition in adrenocortical carcinoma model [159].

Low-density lipoprotein (LDL) is another group of lipoproteins and is characterized by a monolayer surface composed of phospholipids and a single molecule of apolipoprotein B-100 (ApoB-100). LDL has been recognized as a potential drug delivery nanoparticle since 1980s because of its biocompatible, biodegradable and long circulation properties [160,161]. Similar to HDL, LDL cores can be replaced by other molecules such as fluorescent probe during reconstitution [162]. Fatty acids are reported to be successfully introduced into the hydrophobic core of LDLs through the core-loading method for targeted delivery of therapeutics to the tumor site *in vivo* [163]. Besides, phospholipid monolayer insertion and covalent attachment to surface apolipoprotein can be used to introduce external molecules/components (e.g., vaccines) into the LDL nanoparticles.

Similarly, the addition of cell membranous components can be used to optimize vaccine delivery. Polymeric PLGA nanoparticles coated with B16−F10 melanoma membrane were able to effectively deliver immunological adjuvant MPLA and induce strong tumor-specific immune response *in vitro* [64].

Developing nanoparticles with unique properties

The unique properties of nanoparticles can be manipulated by external conditions (e.g., light irradiation, magnetic field or temperature) to improve vaccine uptake and release payload in a controlled fashion. Altering nanoparticle environment by applying a magnetic field or light irradiation with a specific wavelength can alter nanoparticle surface properties to avoid rapid clearance and enhance the cellular uptake for immune response.

Photodynamic therapy focuses on exciting photosensitizers to generate reactive oxygen species for tumor removal under light of specific wavelength. Upconversion nanoparticles (UCNPs) with tunable emission and high photostability potentiate their application in photodynamic therapy for cancer. Near-Infrared UCNPs offer deep tissue penetration without causing toxicity; and UCNPs with long circulation times can be additionally used for nanoparticle tracking *in vivo*. OVA antigen-loaded UCNPs were reported to be efficient vaccines for cancer both *in vitro* and *in vivo* [164]. The core of Yb and Er-doped NaY/GdF4 UCNPs was shelled with PEG-PEI polymer-OVA antigen complex. OVA-UCNPs improved maturation of DCs through effective delivery of OVA visualized by

FITC labeling *in vitro,* and OVA-UCNPs-pulsed DC vaccine greatly enhanced cytotoxic T-cell responses in mice compared with DC and OVA-pulsed DC control groups. The light irradiation at 980 nm enabled the tracking of implanted OVA-UCNPs-pulsed DCs through strong upconversion luminescence emission signal, which indicated the localization of injected OVA-UCNPs DCs in DLNs after 36-h postadministration. The strong *in vivo* immune responses induced by OVA-UCNPs-pulsed DC vaccine may be attributed to deep tissue penetration of OVA through UCNPs under near-infrared irradiation. UCNPs, modified with aminosilane for prolonged release and protection, were used in a DNA vaccine. The NaYF4:Yb/Er@silica(UCNPs)/DNA vaccine stimulated stronger *in vivo* immune responses to protect from viral challenge in guinea pigs than those immunized with DNA vaccine alone [165].

Conclusion

Nanoparticles are useful tools toward enhancing antigen presentation and stimulating robust immune responses as adjuvants for effective vaccination against cancer. Various type of nanoparticles including polymeric nanoparticle, liposomes, virus and inorganic nanoparticles have attracted great interest in the vaccine research fields. Virus and/or virus-like nanoparticles are one of the mostly studied nanoparticle systems in the development of vaccines and are the main impetus for the increasing interest in cancer vaccine development. The success of cancer vaccines, like HPV, inspires the development of other cancer vaccine types by incorporating structural antigens and/or multiple antigens for inducing robust immune response that can target and destroy existing cancer cell populations or prevent the growth or progression of future tumors. Natural nanoparticles or their mimics might be good alternatives as vaccine delivery systems by taking advantage of native functions involving cellular recognition and uptake, and circulation, and avoiding clearance mechanisms common to conventional nanoparticle systems. Fast clearance is still a major challenge for adopting more widespread use of nanoparticle systems. Mechanistic pathways involved in nanoparticle excretion from cells/tissues/bodies after cellular uptake/targeting need further investigation. The nanoparticles can be tuned through modulating surface properties, size, shape and composition to enhance the immune responses against cancer. The inexpensive, robust, reproducible and reliable methods of large-scale nanoparticle synthesis are required to the usage of nanoparticles in the clinical settings in the future.

Future perspective

Nanoparticle-based vaccine delivery for cancer is still in its infancy. Extensive studies are needed to prove the reproducibility, rigor and applicability of reported findings to the clinic. The fundamental mechanisms underlying how nanoparticle physical properties affect biological interactions are still poorly understood, and real-time tracking of nanoparticles during uptake and clearance *in vivo* is generally not well characterized. Fast clearance may account for most nanoparticle degradation and ineffective nanotherapeutic intervention. New methods must be developed to address problems concerning poor reproducibility with uniform size and shape, loss of unique properties due to aggregation, fast clearance and unstable properties before wide-spread public and medical use. Nanoparticles should be designed to resist nonspecific protein binding, escape fast clearance and display no toxicity. Thus, nanoparticle surface modifications need to be balanced for simultaneously optimal vaccine delivery and self-clearance. Delivery and release of vaccines should be directed or targeted via surface modification, and so forth, toward specific cells or sites of action for effective cancer vaccines. Nanoparticle delivery systems are expected to be biodegradable and/or biocompatible, nontoxic and controllable vaccine delivery with the ability to be completely cleared from the body – thus, avoiding any systemic toxicities attributed to gradual accumulation, such as heavy metal accumulation. Hybrid nanoparticle delivery system, such as polymer-lipid hybrid and polymer-inorganic nanoparticle hybrid, can be a possible approach to enhance the cellular immunity by synergistic effects from individual components.

Clinically, virus and virus-like nanoparticles have made great progress and several types of these nanoparticles are being licensed to use worldwide. However, other types of nanoparticle-mediated delivery systems are still in infantile and receive relatively poor outcomes. The reasons may be due to the following: cancer biology may have already induced immune-checkpoint suppression, which is often seen in many drug-resistant cancer types. The nanoparticle vaccine may have induced the response, but the intensity is far from satisfying. To fully leverage immune response, activation or stimulation may require a lasting and gradually adjusted pattern that adapts to a patient's specific disease progression. The nanoparticle delivery into the tumor environment may have elicited multiple effects compromising the immune responses. Multiple studies have demonstrated the possibility of increasing Treg activity while reducing antigen expression. This means that nanoparticle-mediated cancer vaccines may need long, personalized, enhanced immune response activation to exert its potential therapeutic effect in clinical usage. An easy

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research direction might be to extract immune cells or those from tumor microenvironment to test the effects from different nanoparticle mediation first from multiple patient and cancer types. The existing *ex vivo* humoral/cellular response may provide insight for prognosis and serve as a proper reference for further nanoparticle evolution.

Executive summary

Nanoparticles as vaccine-delivery systems

- Nanoparticle delivery systems are potential carriers for cancer vaccines to elicit robust immune response and inhibit tumor growth.
- Polymeric nanoparticles are biodegradable and highly modifiable delivery systems that have shown potent antitumor effects by targeting dendritic cells and mitochondria.
- Aluminum-based systems are one of the most common adjuvants in vaccine development for strong cell-mediated immunity.
- Virus and virus-like nanoparticles like the HPV vaccine are currently being licensed for use worldwide.

Nanoparticle clearance pathway

- The nanoparticles can be cleared through immune system such as mononuclear phagocytic system, renal/urinary or biliary clearance.
- The surface properties, size, shape and nature of materials play critical roles in the clearance pathway of nanoparticle.
- Renal excretion is a primary method for excretion of small molecules or nanoparticles with glomerular threshold of size less than 10 nm.
- Biliary secretion is commonly used for nanoparticle clearance.

Strategy to escape rapid clearance

Surface properties, size, shape and composition are important factors in designing nanoparticles with enhanced cellular uptake and decreased nonspecific binding.

Natural nanoparticles are alternative delivery systems that leverage native biological functions to avoid fast clearance.

The unique properties of nanoparticles can be regulated by external environment to enhance the vaccine uptake and release in a controlled fashion.

Conclusion

- Various types of nanoparticles including polymeric nanoparticle, liposomes, virus and inorganic nanoparticles have attracted great interest in the vaccine research fields.
- Nanoparticles are promising tools to enhance the antigen presentation and robust immune responses stimulation for effective vaccination against cancer.

Future perspective

- New strategies are urgent in need to address problems concerning poor reproducibility with uniform size and shape, loss of unique properties due to aggregation, fast clearance and unstable properties before widespread public and medical use.
- Nanoparticle-mediated cancer vaccine may need long, personalized, enhanced immune response activating to exert its potential therapeutic effect.

Financial & competing interests disclosure

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