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Nanoparticle-mediated tumor vaccine for personalized therapy: preparing tumor antigens *in vivo* or *ex vivo*?

Qian Li^{a,b}, Jia Li^b, Sha Song^b, Wujun Chen^b, Xin Shen^{a,c*}, Suming Li^{e*}, Dongming Xing^{a,b,d*}

^a Cancer Institute, Affiliated Hospital of Qingdao University, Qingdao, 266071, China

^b Qingdao Cancer Institute, Qingdao, 266071, China

^c CP Pharmaceutical (Qingdao) Co., Ltd., Qingdao, 266426, China

^d School of Life Sciences, Tsinghua University, Beijing, 100084, China

^e Institut Européen des Membranes, IEM-UMR 5635, Univ Montpellier, ENSCM, CNRS, 34095 Montpellier, France.

Corresponding author E-mail addresses: shenxin@cppqd.com, suming.li@umontpellier.fr,
xdmtinghua@hotmail.com

Abstract

Tumor vaccine, focusing on tailoring individual tumor antigens, has gained much attention in personalized tumor therapy. Recently, breakthroughs have been made in the development of tumor vaccines thanks to the progress in nanotechnology. We will summarize nanoparticle-mediated tumor vaccines for personalized therapy in this review. ROS/heat generating nanoparticles and molecules could induce immunogenic cell death and tumor antigen release *in vivo*. This strategy often includes chemotherapy, radiotherapy, photodynamic therapy, photothermal therapy, magneto-thermal therapy, etc. On the other hand, *ex vivo* technologies have been applied for processing of tumor cells/tissues to form effective tumor antigens, in which nanotechnology have shown very good prospects in delivering tumor antigens. In *in vivo* and *ex vivo* strategies, nanotechnology also could improve immune effect through enhancing uptake by targeting cells, reducing therapeutic drugs/agents, further encapsulating immuno-modulatory molecules or combining with other therapy treatments. Thus, therapeutic vaccines based on nanoparticles have the potential to enhance immune response and reduce side effects.

Keywords: tumor vaccine, antigen release, antigen delivery, personalized therapy, nanoparticles

1. Introduction

As every tumor has its own unique composition of mutations with only a small fraction shared between patients, there is a huge demand in developing personalized therapy.¹ Therapeutic tumor vaccines represent a viable option for personalized therapy that aims to treat late stage disease by using a patient's own immune system. Several therapeutic tumor vaccine strategies and formulations have been evaluated in recent years in different tumor settings involving thousands of tumor patients.^{2, 3} However, therapeutic tumor vaccines have not made proportionate clinical effect. The only FDA-approved tumor vaccine, Provenge[®] showed a limited 4.5-month improvement in overall survival compared to the placebo. Nevertheless, this major breakthrough not only provides a new treatment modality for tumor management, but also paves the way for rationally designing and optimizing future vaccines with improved anti-tumor efficacy. Compared with the other types of tumor treatments, tumor vaccine therapies are considered to be a type of specific, safe, and well-tolerated tumor treatment, and they have the potential to avoid drug resistance and obtain durable treatment responses due to the long-term immunologic memory.⁴

The limited efficacy could be described to the low specificity of target tumor antigens in the vaccine formulation. Tumor antigens need to be sufficiently distinct from self-antigens to break the immunological tolerance that physiologically blocks undesired auto-immune reactivity against normal cells. Thus, preparing appropriate tumor antigens is one of the central factors in the development of tumor vaccines.⁵ *Ex vivo* technologies have been used to look for effective tumor antigens for a long period, and several strategies including tumor lysate based vaccine,^{6,7} tumor cell

membrane based vaccine^{8,9} and neoantigen¹⁰ have shown the advantage of personalization. Moreover, tumor killing strategies have been introduced into tumor vaccines for inducing tumor antigen release *in vivo*, and these strategies usually include photodynamic therapy, chemotherapy, radiotherapy, hyperthermia, etc.¹¹⁻¹³ However, these treatments suffer from limited induction of immune response for different reasons such as un-stability of tumor cell contents, poor dendritic cells (DCs) uptake of tumor antigens, inefficient antigen cross-presentation.^{6, 14-16}

The use of smart nanosystems represents an opportunity for improving immune responses. Generation of anti-cancer immune response consists in a number of key steps as follows¹⁷: Introduce tumor antigens, antigen presenting cells (APCs) present tumor antigens, activated APCs present tumor antigens to T cells, activated effector T cells kill tumor cells releasing epitope spreading. As tumor antigen plays the key role in tumor vaccines, various nanoparticles have been applied in tumor antigen preparation (Figure 1). Nanoparticles with the function of generating ROS/heats could induce antigen release *in vivo*,¹⁸⁻²⁰ while nano-delivery systems demonstrate very good prospects in processing and delivering tumor antigens *ex vivo*.^{21, 22} In *in vivo* and *ex vivo* strategies, nanotechnology also could improve immune effect through enhancing uptake by targeting cells, reducing therapeutic drugs/agents, further encapsulating immuno-modulatory molecules or combining with other therapy treatments. In this review, we will summarize the application of nanoparticles for improving the efficiency of the tumor vaccines.

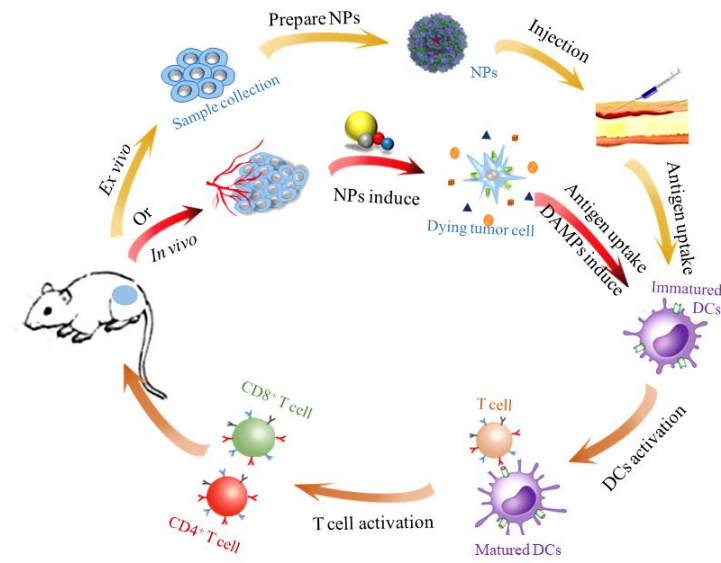


Figure 1. Preparation of tumor antigens through *in vivo* or *ex vivo* strategies and their immune responses

2. Inducing tumor antigens *in vivo*

In situ tumor vaccine, which could generate tumor vaccines *in vivo*, is a newly defined form of tumor therapeutic method based on immunogenic cell death (ICD).^{13, 19, 23} Molecules essential for the induction of ICD are called damage-associated molecular patterns (DAMPs).²⁴ DAMPs have the ability to recruit APCs and stimulate optimal antigen presentation to T cells. *In situ* tumor vaccine also triggers the release of tumor antigens including neoantigens derived from idiosyncratic mutations.²⁵⁻²⁷ DAMPs secreting and neoantigen releasing provide *in situ* tumor vaccines the ability to generate systemic anti-tumor adaptive immune responses. The production of ROS and the resulting induction of **endoplasmic reticulum (ER)** stress are vital for the intracellular trafficking and subsequent surface/extracellular emission of DAMPs/danger signals.^{12, 28} Generation of ROS is related to most of the currently known ICD inducers such as chemotherapy, radiotherapy, photodynamic therapy (PDT), photothermal therapy (PTT) and magneto-thermal therapy (MTT), as

shown in Figure 2. Photodynamic therapy efficiently generates ROS through PDT action,²⁹ while others generate ROS through collateral pathways.^{12, 26, 28} Beside ROS, the heat generated through the process of hyperthermia also could induce ER stress. A small increase in temperature can cause protein unfolding, entanglement and aggregation leading to an imbalance in proteostasis. This can result in increased degradation of aggregated/misfolded proteins, and cellular effects such as ER stress, cellular apoptosis and necrosis.^{30, 31}

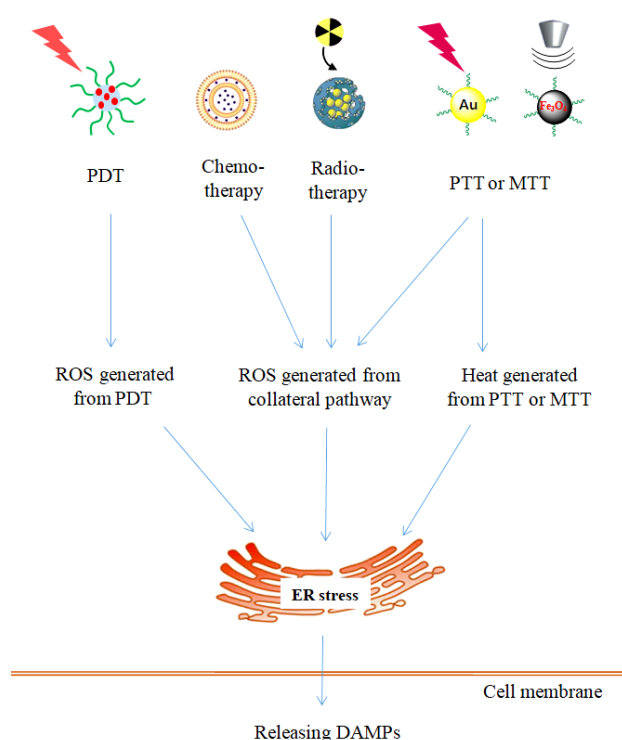


Figure 2. Schematic depiction of *in vivo* strategies inducing ER stress and releasing DAMPs

2.1. Photodynamic therapy

Photodynamic therapy (PDT) is a clinically used, minimally invasive therapeutic treatment on neoplastic pathologic tissues. PDT agents (also named photosensitizers) absorb visible non-thermal light within the phototherapeutic window ($\lambda > 600$ nm) forming excited state of photosensitizer, and

generate ROS through Type I and/or Type II photochemistry.^{32,33} Although PDT has not yet become the mainstream of tumor intervention mainly due to its insufficient therapeutic efficacy and limited light penetration for deep tumor tissues,^{34,35} PDT has returned to the spotlight for its noticeable immune activity. ICD-associated immunogenicity can be evoked through ROS produced ER stress.³⁶ The ROS production and ER stress are crucial for instigation of the danger signalling pathways responsible for the trafficking and emission of DAMPs. Compared to chemotherapy and radiotherapy, PDT generates ROS more directly and does not cause severe side effects such as leucopenia and gastrointestinal reactions.^{37,38} Thus, PDT can induce tumor cells to produce more DAMPs and are more effective at inducing ICD.²⁸ Antigen releasing also enhances anti-tumor immune responses.³⁹ The released DAMPs and tumor antigen signal APCs maturation accompanied by infiltration of inflammatory cells. These tissue changes further stimulate the inflammatory cells to release proinflammatory cytokines such as IL-1 β , TNF- α , IL-6, IL-10, etc.^{40,41} The infiltration of inflammatory cells and production of proinflammatory cytokines supply powerful tools in eliciting *in situ* tumors, but as importantly, it has the potential to kill the residual or metastatic tumor cells.

In an effort to enhance the effectiveness of antitumor PDT, many studies have been conducted to develop photosensitizers with longer wavelength absorption,⁴² solve the problem of tissue hypoxia^{43,44} and selective photosensitizer delivery to target cells^{45,46}. These approaches not only improved the efficiency of directly killing tumors, but also facilitate the antitumor immune response following PDT. Several molecules generating ROS have been proved powerful PDT agents in tumor immune therapy. These molecules include chlorin e6 (Ce 6), hypericin, silicon(IV) phthalocyanine, zinc(II) phthalocyanine, **pyropheophorbide-a**, etc.^{12, 43, 47} For example, Chen et al. reported a Ce6 encapsulated hybrid protein oxygen nanocarrier (C@HPOC) made up from human serum albumin

and hemoglobin (Figure 3a).⁴³ The nanocarrier realized the tumor-targeted co-delivery of photosensitizer and oxygen, which remarkably relieved tumor hypoxia. C@HPOC showed more efficient PDT and enhanced infiltration of CD8⁺ T cells in tumors. Moreover, oxygen-boosted PDT of C@HPOC induced immunogenic cell death, with the release of DAMPs to activate dendritic cells, T lymphocytes, and natural killer cells in vivo. Notably, C@HPOC-mediated immunogenic PDT could destroy primary tumors and effectively suppress distant tumors and lung metastasis in metastatic triple-negative breast tumor model.

Combination therapy that could better balance immune activation and suppressive signals presents great potential in tumor immunotherapy. Yang et al. designed a smart nanovesicle with ICD inducing ability as synergist for photosensitizers.⁴⁷ The smart nanovesicles were self-assembled from a pH-responsive block copolymer of polyethylene glycol with a cationic polypeptide, and co-loaded with 2-(1-hexyloxyethyl)-2-devinyl pyropheophorbide-a (HPPH) and indoximod. ICD was induced through HPPH-mediated PDT and preapoptotic exposure of calreticulin caused by nanovesicles. Indoximod modulated tumor microenvironment via enhancing P-S6K phosphorylation for CD8⁺ T cell development. This work exploited the nanocarrier's potential to induce ICD for the host's immunity activation. The "all-in-one" smart nanovesicles allow the design of multifunctional materials to improve tumor immunotherapy efficacy.

Nanomaterials, which could generate ROS under light, also aroused much attention in PDT-generated vaccine. Nanoparticles generating ROS include black phosphorus quantum dots (BPQDs), gold nanocages, benzoporphyrin-based metal-organic frameworks (TBP-MOF), etc.^{42, 48, 49} Li et al. prepared a BPQD vesicles (BPNVs) from poly(ethylene glycol)(PEG) grafted BPQDs and ROS sensitive poly(propylene sulfide) (PPS) (Figure 3b).⁴⁹ CpG oligodeoxynucleotides (CpG ODNs)

was loaded as immuno-adjuvant in the cavity of the BPNVs. Upon NIR laser irradiation, high levels of ROS are generated from BPNVs to trigger the release of BPQD and CpG into deep tumors. The potent ICD effect and local accumulation of CpG induced an antitumor immune response, which also effectively inhibited distant tumor growth and lung metastasis.

The Fenton reaction, which converts hydrogen peroxide to reactive oxygen radicals, has attracted much interest in enhancing PDT performance. It usually utilizes small amounts of Fe^{2+} and Fenton-like reaction utilizes Cu^+ and its complexes acting as a catalyst. Recently, these catalysts have been introduced into PDT-based immune-therapy. For example, Wang et al. designed an ultra small nanoagent (3.1 ± 0.4 nm) fabricated from PEG modified Cu_{2-x}Se nanoparticles, β -cyclodextrin, and Ce6 under ambient conditions.⁵⁰ The resultant nanoplatform showed excellent PDT performance through Fenton-like Haber–Weiss catalyst under near-infrared irradiation. The antitumor immune responses against the metastasis of breast tumor were robustly evoked through eliciting ICD to release DAMPs and induced proinflammatory M1-macrophages polarization. Thereby the nanoparticle-based nanoplatform offers a promising way to prevent metastatic breast tumor via immunogenic effects through its excellent PDT performance.

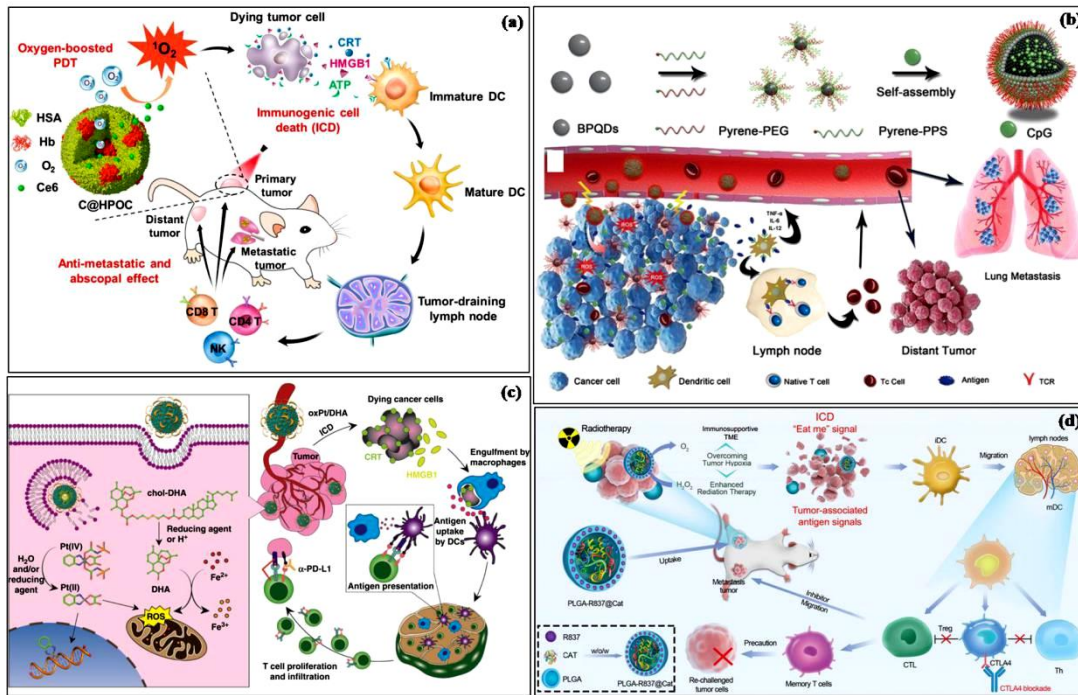


Figure 3. ROS-generating nanoparticles to induce tumor vaccines (a) Ce 6 encapsulated C@HPOC nanoparticles made up from human serum albumin and hemoglobin for oxygen-augmented immunogenic PDT (from reference ⁴³ with permission), (b) Formulation of BPNVs loaded with CpG for PDT-based vaccine (from reference ⁴⁹ with permission), (c) OxPt/DHA nanoparticles synergized with anti-PD-L1 antibody for chemotherapy-based vaccine (from reference ⁵¹ with permission), (d) PLGA nanoparticles loaded with catalase and R837 for radiotherapy-based vaccine (from reference ⁵² with permission).

2.2. Chemotherapy

Conventional chemotherapy relies on direct elimination of tumor cells. As scientific research progress, role of chemotherapy in eliciting anti-tumor immune responses has aroused much attention. Subsequent molecular studies demonstrated that the concomitant production of ROS during chemotherapy is crucial for instigation of ER stress and emission of DAMPs.^{12, 53} Several chemodrugs have been shown to undergo ICD and generate different DAMPs. Such inducers primarily

cause tumor cell death by acting on DNA replication/repair proteins or certain cytosolic proteins. Chemo-drugs acted on DNA replication/repair proteins include anthracyclines (doxorubicin, etc.), mitoxantrone, oxaliplatin, cyclophosphamide,⁵⁴⁻⁵⁸ while bortezomib mainly acted on cytoplasmic proteins.⁵⁹ Accordingly, DNA replication/repair proteins and cytoplasmic proteins represent the site of “focused” effects for chemo-drugs, while the ER represents the site of “collateral” effects. They induce cell death are not directly associated with the ER, but ICD-associated danger signalling is through collateral ER stress effects.

Chemotherapy induced antitumor immune responses was dosage-dependent. A high dose of chemotherapeutic drug would cause side effects such as leukopenia. However, a low dosage of chemotherapeutic drug is inefficient to induce immune response. In order to enhance immune response, nanotechnology has been introduced into chemotherapy-based tumor vaccines. On the one hand, nanoparticle-based drug delivery systems (DDSs) can advantageously enhance therapeutic efficacy by better penetration and controlled release of active ingredients at the target site, producing more serum stability and pharmacokinetics. On the other hand, the DDSs can further encapsulate immuno-modulatory molecules or combine with other therapy treatments. Encapsulating chemotherapeutic drugs into DDSs thus offers a promising way to enhance immune effects.

Materials for preparing nano-carriers usually include organic DDSs and inorganic DDSs. Organic DDSs are usually biodegradable, which includes dendrimers, liposome, micelles, and polymer nanoparticles.^{60, 61} These DDSs are able to encapsulate various chemotherapeutic drugs, and give them advantages such as prolonged circulation time in the body, specific targeting and improved drug solubility.⁶² Some of these organic DDSs have been used in tumor immune therapy. For

example, Duan et al. prepared nanoscale coordination polymer (NCP) core-shell particles to encapsulate oxaliplatin (OxPt) and dihydroartemisinin (DHA) prodrugs (Figure 3c).⁵¹ OxPt and DHA have strong synergy in ROS generation activity. The combined ROS generation is harnessed for immune activation to synergize with an anti-PD-L1 antibody for the treatment of murine colorectal tumours. OxPt/DHA directly converted treated tumours into an *in situ* vaccine through inducing **calreticulin** (CRT) exposure and HMGB-1 release, accompanied by recruiting antigen-presenting DCs and macrophages, facilitating cancer cell phagocytosis, enhancing antigen presentation, and finally increasing intra-tumoural infiltration of CD8⁺ T cells to significantly potentiate checkpoint. The favourable biodistribution and tumor uptake of NCPs allow for repeated dosing to afford 100% tumor eradication and generating long-term anti-tumor immunity.

Inorganic materials, such as calcium carbonate (CaCO₃), calcium phosphate (Ca(H₂PO₄)₂), tri calcium phosphate (Ca₃(PO₄)₂), hydroxy apatite (Ca₅(PO₄)₃OH), calcium peroxide (CaO₂), colloidal gold, silicon, iron oxide and layered double hydroxide have been applied in modern pharmaceutical and medication areas.^{63,64} Inorganic nanoparticles have been widely investigated in the field of drug delivery due to their controllable size, ease of preparation, simple functionalization, and high stability. Although studies investigating the mechanisms underlying the anti-tumor immune response to inorganic DDSs are in a nascent stage, some reports focus on their synthesis for inducing tumor vaccine. For example, Ruan et al. reported a combined therapeutic regimen based on gold nanoparticles-enabled chemotherapy and blockade of PD-L1 immune checkpoint.⁶⁵ The legumain-responsive gold nanoparticles could passively target the glioma site and enhanced accumulation of DOX and hydroxychloroquine (HCQ) at the glioma site. HCQ inhibited the DOX-induced cytoprotective autophagy, which resensitized glioma cells to DOX and inhibited the formation of

autophagy-related vasculogenic mimicry by glioma stem cells. *In vivo* studies showed gold nanoparticles plus anti-PD-L1 antibody could further enhance antiglioma effect and efficiently prevent recurrence. The effectiveness of this strategy presents a potential avenue to develop a more effective and more personalized combination therapeutic regimen for glioma patients.

2.3. Radiotherapy

External beam-based cancer radiotherapy is a mainstream cancer treatment strategy that has been extensively used in clinic to treat 65–75% of local solid tumors at different stages. α -, β - and γ -rays from radioactive decays as well as X-rays have been applied locally onto tumors to kill cancer cells. However, radiotherapy is not able to inhibit the growth of distantly spreading tumors and limited by hypoxia inside solid tumors. Clinical treatment of tumors has found that some patients with disseminated tumor experienced systemic tumor regression after irradiation of a single tumor site. Aside from causing tumor cell death by acting on DNA replication and repair proteins,⁶⁶ radiotherapy passing through living tissues could generate ROS. The effects of ROS are amplified by the interaction with ER stress pathways, resulting in the translocation of CRT to the cell surface (a DC “eat-me” signal) and the release of danger signals such as HMGB1 and ATP, which are essential for the promotion of CD8⁺ T cell anticancer responses. Primed CD8⁺ T cells contribute to subsequent residual tumor cell elimination in the tumor bed as well as nonirradiated tumor deposits at distant sites of disease.^{13, 28, 67} Thus, combinational radio-immune oncotherapy could be considered as next generation cancer radiotherapy strategies.

Emerging applications of local radiotherapy has provided radiation oncologists with a method for converting malignant cells into endogenous anticancer vaccines. However, radiotherapy alone is

unlikely to induce or sustain an immune response that is therapeutically useful based on clinical evidence. Nanomaterials could enhance immune response by priming with immune-modulators, tumor antigens or oxygen suppliers.^{11,52,68} For example, Chen et al. reported poly(lactic-co-glycolic acid) (PLGA) based core-shell nanoparticles for tumor immunotherapy (Figure 3d).⁵² R837 was loaded in the PLGA shell, while catalase with the ability to decompose H₂O₂ to O₂ was loaded in the inner core. The formed nanoparticles could greatly enhance radiotherapy efficacy by relieving the tumor hypoxia and modulating the immune-suppressive tumor microenvironment. After combination with anti-CTLA4 (α CTLA4) checkpoint blockade, radiotherapy using R837 loaded PLGA nanoparticles showed excellent distant tumors control through an augmented abscopal effect.

2.4. Photothermal therapy

Photothermal therapy (PTT), a minimally invasive procedure using heat for attacking various types of tumor cells, has emerged as promising ICD inducer. PTT involves irradiation of light-absorbing agents to an excited state under near-infrared (NIR) light in the tumor site where it releases vibrational energy (heat) and kills the tumor cells. Recently, PTT has shown the ability to generate antitumor immunological effects by producing tumor-specific/associate antigens and DAMPs from ablated tumor cell residues. It has been demonstrated that the key event of hyperthermia appears to be protein denaturation and aggregation.³¹ The correct structure and conformation of proteins is essential for their function in the cell. A small increase in temperature can cause protein unfolding, entanglement and aggregation, thus leading to an imbalance in proteostasis.⁶⁹ Increased degradation of aggregated/misfolded proteins could lead to ER stress, inactivation of protein synthesis, cell cycle arrest and inhibition of DNA repair processes.³¹ Thus, tumor cells go through apoptosis or necrosis, resulting in release of tumor antigens and DAMPs.⁷⁰

Moreover, it has also been reported that hyperthermia promotes mammalian aerobic metabolism, induces oxidative stress and generates ROS rapidly.^{30, 71} ROS acting as danger signals induces immune response. Thus both heat and ROS generated in hyperthermia could contribute to ER stress and DAMPs release.

As an effective therapy for tumor, the PTT based immunotherapy has shown great promise. PTT agents ablate tumor cells with heat generated from the absorbed optical energy by light-absorbing agents that accumulate in the tumors. A number of PTT agents have been developed for immune therapy, including indocyanine green, prussian blue, gold nanoparticles, carbon nanotubes, graphene oxide, CuS nanoparticles, and Bi₂Se₃ nanocage.^{20, 70, 72-75} Low penetration depth and limited immune stimulation are still the major obstacles for PTT.⁷² To achieve a relatively harsh environment for efficient ablation of tumors, photothermal heating to temperatures over 50 °C is required, which will also damage normal tissues and induce intolerable pain to patients. Huang et al. reported the combination of mild PTT under 45 °C and anti-PD therapy. The authors prepared a lipid gel depot with a favorable property of thermally reversible gel-to-sol phase transition for encapsulation NIR photothermal agent new indocyanine green (IR-820) and PD-L1 antibody (α PD-L1) (Figure 4a).⁷⁴ Manually controlled NIR irradiation regulates the release of α PD-L1 and, more importantly, increases the recruitment of tumor-infiltrating lymphocytes and boosts T cell activity against tumors. *In vivo* antitumor studies on 4T1 and B16F10 models demonstrate that symbiotic mild photothermal-assisted immunotherapy is an effective and promising strategy for treating “cold” tumors.

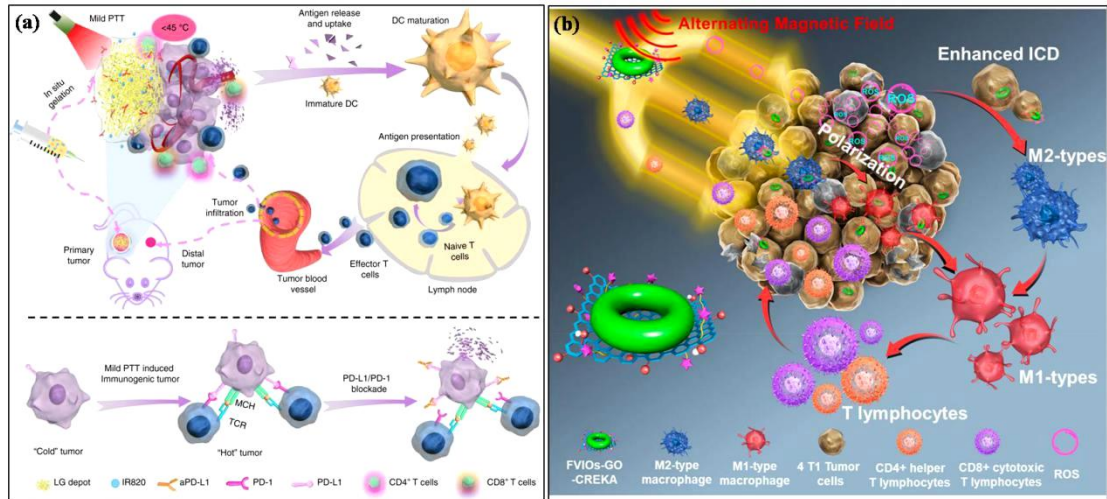


Figure 4. Heat-generating nanoparticles to induce tumor vaccines. (a) Thermal responsive lipid gel loaded with IR820 and $\alpha\text{PD-L1}$ for PTT based immune therapy (from reference ⁷⁴ with permission), (b) Iron oxide nanoring and graphene oxide hybrid nanoparticles for magneto-thermodynamic based immune therapy (from reference ⁷⁶ with permission).

2.5. Magneto-thermal therapy

Magneto-thermal therapy (MTT) is a favorable non-invasive technique for tumor therapy that selectively heats up tumor tissue by coupling alternating current magnetic fields to magnetic nanoparticles. Magnetic nanoparticles transform alternate current magnetic field energy into heat by different magnetic loss mechanisms, such as Néel and Brownian losses.⁷⁷ Compared to traditional hyperthermia therapy or NIR laser-based hyperthermia, MTT has advantages of higher penetration ability of magnetic field in the tissues and enhanced accumulation of magnetic nanoparticles in the tumor via magnetic targeting strategy for tumor treatment. It has been reported that MTT could trigger an antitumor immune response mediated by both CD8^+ and CD4^+ T cells. This leads to elimination of both heat-treated primary tumors and unheated distant tumors and

subsequent rechallenge rejection.^{78, 79} For most biomedical applications, magnetic nanoparticles are made from ferrimagnetic iron oxides.⁸⁰ Iron oxides nanoparticles are an attractive option as a base platform for developing constructs that enable potentially synergistic mechanisms of cancer cell killing to be more effective.¹⁹ For example, Liu et al. propose a magneto-thermodynamic (MTD) approach by leveraging the dual action of the heating effect and reinforced ROS-related immunologic effect to efficiently eliminate tumors at a physiological tolerable temperature (Figure 4b).⁷⁶ The authors prepared a vortex-domain iron oxide nanoring and graphene oxide (FVIOs-GO) hybrid nanoparticle. Such nanopatform has high thermal conversion efficiency and significantly amplified ROS level under an alternating magnetic field (AMF). The amplified ROS generation acted as the dominant factor in provoking a strong immune response below 40 °C in a hypoxic tumor microenvironment. This nanopatform also leads to the exposure of CRT on the 4T1breast cancer cell surface, direct promotion of macrophage polarization to pro-inflammatory M1 phenotypes, and further elevation of tumor-infiltrating T lymphocytes.

2.6. Dual-modal ICDs for inducing tumor vaccine

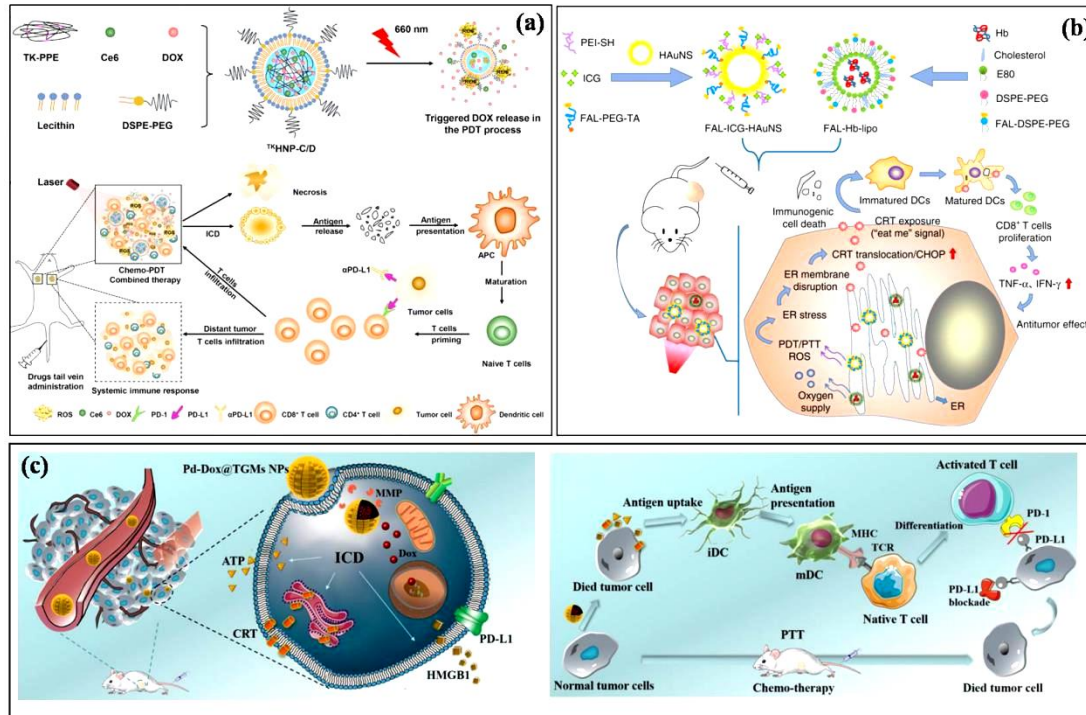


Figure 5. Two ICD inducers combined together for inducing tumor vaccines (a) ROS-sensitive hybrid ^{75}K HNP-C/D nanoparticles for chemo-PDT based tumor vaccine (from reference ⁴⁶ with permission), (b) ER-targeting FAL-ICGH AuNS together with FAL-Hb lipo for PDT-PTT based tumor vaccine (from reference ³⁶ with permission), (c) Pd-Dox@TGMs NPs together with anti-PD-L1 antibody for chemo-PTT based tumor vaccine (from reference ⁸⁶ with permission).

As therapeutic targeting is complicated by multiple facets of the tumor microenvironment, multiple mechanisms are needed to kill the tumor cells.^{81, 82} Different therapeutic modalities combined together could provide opportunities to exploit the advantages and offset the disadvantages of each therapeutic modality, leading to additive or even synergistic therapeutic effects. For example, the combination potentially increases the antitumor efficacy at lower doses of drugs/agents and lower-power light irradiation, enhances permeability in tumor sites and

improves the local immune suppressive state.^{27, 81, 83} Thus combining different ICD modalities together could minimize potential toxicity to non-malignant tissues and enhance immune responses. The details are shown as follows.

The combination of chemotherapy and PDT (chemo-PDT) could induce synergistic therapeutic effects. Chemotherapy could address the limitation of light penetration and might also enhance the sensitivity of cancer cells to PDT. PDT with the ability of accelerating chemo-drug release from the endo/lysosomes by light irradiation could help to overcome chemo-resistance.⁸¹ The chemo-PDT might also enhance treatment efficacy owing to inhibition of drug-efflux P-glycoprotein pumps in multidrug resistance cells resulting from ROS generation by the photosensitizer.^{27, 83, 84} Recently, chemo-PDT has been introduced into ICD based immune therapy. For example, Hu et al. reported the use of chemo-PDT with ROS-sensitive lipid-polymer hybrid nanoparticles ^{TK}HNP-C/D to potentiate the antitumor efficacy of α PD-L1 (Figure 5a).⁴⁶ ^{TK}HNP-C/D not only induced PDT under 660nm irradiation but also rapidly degraded the hydrophobic TK-PPE core to boost intracellular DOX release, thus promoting an efficient cascade of chemo-PDT to inhibit tumor growth. More importantly, the cascade chemo-PDT could evoke anticancer immune responses and efficiently synergize with α PD-L1 to generate an abscopal effect, which could simultaneously inhibit primary and distant tumor growth.

The combination of PDT and PTT (PDT-PTT) has potential synergistic effects. The heating effect of PTT can enhance the intracellular delivery of photosensitizers as well as improve local blood flow and increase the oxygen concentration in tumor tissues, thus resulting in a higher PDT efficacy. Additionally, ROS generated during PDT can disrupt heat-shock proteins, thereby negating their protective effects in tumor cells during PTT.²⁷ Recently, PDT-PTT has been introduced into immune

therapy and shown enhanced anti-tumor efficacy. For example, Li et al. reported a type of nanosystem consists of ER-targeting pardaxin (FAL) peptides modified-, indocyanine green (ICG) conjugated- hollow gold nanospheres (FAL-ICGHAuNS), together with an oxygen-delivering hemoglobin (Hb) liposome (FAL-Hb lipo), designed to reverse hypoxia (Figure 5b).³⁶ Compared with non-targeting nanosystems, the ER-targeting system induces robust ER stress and calreticulin (CRT) exposure on the cell surface under NIR light irradiation. CRT, a marker for ICD, acts as an 'eat me' signal to stimulate the antigen presenting function of dendritic cells. As a result, a series of immunological responses are activated, including CD8⁺ T cell proliferation and cytotoxic cytokine secretion. In conclusion, ER-targeting PDT-PTT promoted ICD-associated immunotherapy through direct ROS-based ER stress and exhibited enhanced anti-tumor efficacy

The combination of PTT and chemotherapy (Chemo-PTT) produces synergistic therapeutic effects. Chemotherapeutics could address the limitation of light penetration in PTT and might also enhance the sensitivity of tumor cells to hyperthermia. The heating effect of PTT can enhance local blood flow, delivery of the chemo-drugs and oxygen concentration in tumor tissues.^{27, 85} Recently, chemo-PTT has been introduced into ICD based immune therapy. For example, Wen et al. reported a new therapeutic system (Pd-Dox@TGMS NPs) made from Dox and a photothermal reagent palladium nanoparticles (Pd NPs) into amphiphilic triglycerol monostearates (TGMS) (Figure 5c).⁸⁶ It was proved that co-delivery of Dox and Pd NPs not only effectively killed CT26 cells through chemotherapy and photothermal therapy but also promoted the release of dangerous signaling molecules, such as HMGB-1, CRT, and ATP, improving the immunogenicity of dead tumor cells. The effective ICD induction mediated by Pd-Dox@TGMS NPs boosted the PD-L1 checkpoint blockade effect, which efficiently improved the infiltration of toxic T lymphocytes at the tumor site

and showed excellent tumor treatment effects to both primary and abscopal tumors.

3. Preparation of tumor antigens *ex vivo*

Looking for effective tumor antigens has been pursued and studied for over a hundred years. As every tumor has its own unique composition of mutations with only a small fraction shared between patients,¹ tumor antigens have been designed from **multiple tumor associated antigens** to **tumor specific antigens** for a single patient. Several strategies have shown the advantage of personalization, including tumor lysate based vaccine,^{6,7} tumor cell membrane based vaccine^{8,9} and neoantigen^{87,88}. Tumor lysate and tumor cell membrane could be obtained from human-derived cells/tissues. Using *ex vivo* tumor cells/tissues maintains biological property and molecular heterogeneity of the original tumor in the patient.⁸⁹ On the other hand, neoantigens are identified through *ex vivo* detection of patient's normal cells and tumor cells with genomic sequencing technology. All these three strategies need *ex vivo* technologies to manipulate tumor cells/tissues. With the progress of nanotechnology, nano-delivery systems have shown very good prospects in delivering tumor antigens. These nanovaccines can be designed with diverse features, such as co-delivery with adjuvants, **targeting to lymph nodes and APCs**, reliable protection and efficient delivery for antigens, tunable antigen release and effective cross-presentation, etc.

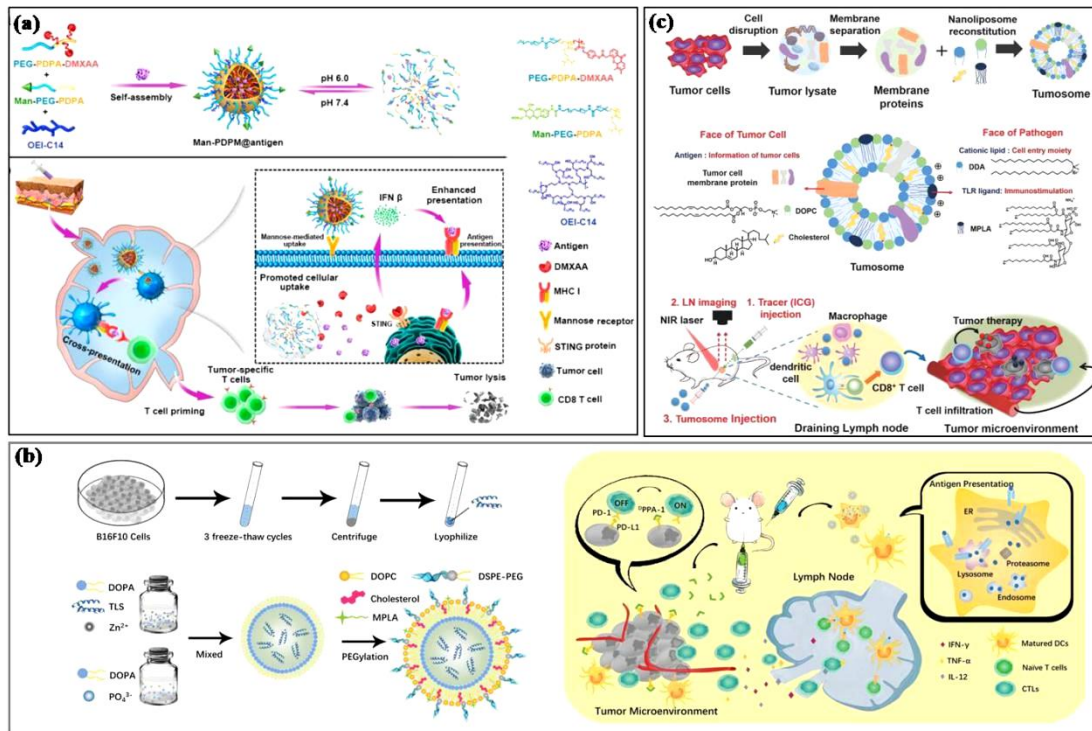


Figure 6. Nanoparticle-based vaccine delivery systems:(a) Combination immunotherapy with neoantigen-based nanovaccine (Man-PDMP@antigen) and anti-PD-L1 antibody (from reference ¹⁴ with permission), (b) Tumor lysate-loaded LZnP nanovaccine combined with PD-L1 antagonist ^DPPA-1 to induce antitumor immunity (from reference ⁹⁷ with permission), (c) Multifaceted immuno-modulatory nanoliposomes prepared from tumor cell membrane and MPLA to induce antitumor immunity (from reference ¹⁰¹ with permission).

3.1. Protein/peptide

Tumor-specific antigen (also called neoantigens), as antigen resulting from somatic mutations present in individual cancers, becomes reality due to next-generation sequencing technologies and computational analysis. Neoantigens, the protein sequences not present in normal tissues, have potential advantages including decreased central immune tolerance, and improved safety profile.⁹⁰ Neoantigens typically have a high predicted binding affinity to MHC molecules and the ability to

induce both CD8⁺ and CD4⁺.⁹¹ Despite promising, neoantigen-based cancer vaccines suffer from insufficient activation of the DCs, which limited their therapeutic performance in clinic. The nano-delivery systems have attracted increasing attention for their roles as antigen protectors and carriers. With further functionalization, these particles could facilitate the access of antigens to APCs, alter intercellular trafficking and enhance accumulation at the lymph nodes.

There is a general need for antigen delivery vehicles that induce a more potent, antigen-specific CD8⁺ cytotoxic T cell (CTL) response. Induction of such a response requires antigen presentation via MHC-I. Exogenous antigens are endocytosed by APCs and degraded into peptides within compartments of the endo/lysosomal processing pathway. Peptides generated in these compartments are predominantly presented on the cell surface via MHC-II complexes, giving rise to CD4⁺ T cell responses.⁹² Elaborately designed carriers have been demonstrated to evade lysosome degradation and stimulate cross-presentation. It has been reported that nanoparticles bearing positively charged polymers/peptides can facilitate antigen escape from the lysosome, reflecting a “proton sponge” effect, and upregulate MHC I complex expression.^{93, 94} Although these positive results are encouraging, harsh preparation conditions, complicated modification processes, and the biosafety concerns of the added materials have slowed their progress. To solve the aforementioned problems, Wang et al. fabricated hierarchical ovalbumin@CaCO₃ nanoparticles.⁹⁵ This type of nanoparticles can efficiently ferry cargo antigen to dendritic cells, blast lysosomes for antigen escape to the cytoplasm and induce autophagy through the LC3/Beclin 1 pathways. These outcomes cooperatively promote antigen cross-presentation, elicit CD8⁺ T cell proliferation, ignite a potent and specific CTL response, and finally achieve prominent tumor therapy effects.

DCs are the key coordinators in tumor immunotherapy and the pivotal cell subsets to connect

innate and adaptive immunity for antigen uptake and presentation, and eventually cross-priming the naive T-cells for activating the tumor-specific CTLs. Besides antigen degradation by lysosome of APCs, limited accumulation at the lymph nodes and cellular uptake by DCs also seriously slowed the progress of neoantigens. Zhou et al. reported a type of nanovaccines constructed from an acid-activatable micellar nanoparticle, neoantigen, and a stimulator of interferon genes (STING) agonist, 5,6-dimethylxanthenone-4-acetic acid (DMXAA) (Figure 6a).¹⁴ **Functionalization with mannose (Man) enhanced cellular uptake by macrophages and B cells in the lymph nodes due to the APC-targeting ability of the mannose receptor, while the acid-responsiveness facilitated cytosol release of the neoantigens.** Meanwhile, the STING agonist activated the STING pathway in the DCs to elicit interferon β secretion and to boost T cell priming with the neoantigen. The nanovaccine dramatically inhibited tumor growth and occurrence of B16-OVA melanoma and 4T1 breast tumors in immune-competent mouse models. Combination immunotherapy with the nanovaccines and anti-PD-L1 antibody demonstrated further improved antitumor efficacy in 4T1 breast tumor model.

3.2. Cell lysate

Tumor cells have been shown to express antigens that can be recognized by immune cells and induce immune responses. Whole-cell tumor vaccines have been studied for several decades as they present many advantages compared to single-target vaccines.⁹⁶ First, whole tumor cells provide multiple characterized and uncharacterized tumor antigens that can be targeted by both the innate and adaptive immune systems. Second, whole-cell vaccination does not need the laborious experiences for antigen identification. Third, whole-cell vaccination may greatly decrease the probability of tumor escape. Furthermore, processing whole tumor derived repertoires of tumor antigens and presenting them through both MHC class I and class II pathways in the presence of

costimulatory molecules lead to a stronger overall anti-tumor response and long-term CD8⁺ T cell memory via CD4⁺ T cells.⁹⁶⁻⁹⁸ However, soluble tumor lysates containing antigens and cytokines are inherently unstable and tend to result in poor DC uptake, inefficient antigen cross-presentation, and limited induction of immune response.^{6, 7, 99, 100}

Encapsulating tumor cell lysate into biomaterial-based nanoparticles has been proposed as a promising strategy to enhance immune responses. Biomaterial encapsulation not only could protect the antigens from degradation during *in vivo* administration, but also enhance antigen delivery and subsequent induction of T cell mediated immune responses.^{6, 99} Surface of biomaterials could be modified with ligands or antibodies that are specifically recognized by DCs and used for DC targeting. For example, Shi et al. reported whole tumor cell lysates combining mannose-chitosan nanoparticles as nanovaccine for tumor immune-therapy.⁶ The result demonstrates the possibility of using mannose as effective DC targeting ligand by conjugation of mannose with biomaterials to facilitate DC maturation, antigen uptake and presentation. Furthermore, by encapsulating tumor cell lysates generated from B16 melanoma cells, mannose-chitosan-tumor cell lysates nanoparticles could effectively stimulate cellular and humoral antitumor immunities.

Tumor lysate is usually used in tumor vaccine based on *ex vivo* modifying DCs. Promising experimental data have indicated that such DC-based vaccines can sufficiently generate primed DCs. However, challenges still remained with these primed DCs, including the poor migration efficiency of primed DCs to lymph node, the labor intensive productive process, and the burdensome medical expenditure. Nanotechnology presents good potential in fulfilling these requirements through enhancing antigen delivery and dealing with immune tolerance accompanied with tumor development. For example, Hu et al. reported a type of lipid zinc phosphate hybrid nanoparticles

(LZnP NPs) loaded with MPLA and B16F10 melanoma cell-derived tumor lysate for vaccination (Figure 6b).⁹⁷ LZnP nanovaccine can efficiently prime DCs and induce cytotoxic T lymphocytes response. To regulate immune tolerance, the PD-L1 antagonist, d-peptide antagonist (^DPPA-1), is involved in treatment. Tumor lysate-loaded LZnP nanovaccine with ^DPPA-1 exhibits the anticipated tumor inhibition on therapeutic and prophylactic melanoma models with extended survival time.

3.3. Cell membrane

There are many antigenic motifs on the cell surface, and cytotoxic T lymphocytes (CTLs) usually recognize tumor cells via the tumor cell membrane. Isolation of individual membrane factors enabled coating of nanoparticles with cell membranes. To date, the tumor cell membrane based nanovaccines have exhibited great potential in cancer immunotherapy. Combining both synthetic components and natural cell membrane yields a unique class of biomimetic materials, which could prolong circulation time and enhance selective targeting ability, ultimately leading to significant therapeutic potential.²¹ More importantly, biomimetic nanoparticles are able to inherit the homologous property from cancer cells, and are considered as an effective strategy for personalized cancer treatment.^{89, 102} The preparation and performance evaluation of these nanoparticles were mostly based on cell lines and cell-line-based xenograft mouse models. However, there are significant differences between cell lines and human-derived cells. Rao et al. reported gelatin nanoparticles (GNPs) coated with head and neck squamous cell carcinoma (HNSCC) patient-derived tumor cell (PDTC) membranes.⁸⁹ The resulting PDTC@GNPs efficiently targeted homotypic tumor cells and tissues in patient derived xenograft models, indicating that tumor cell membrane based nanoparticles could be an effective strategy for personalized cancer treatment.

Cancer immunotherapy mainly focuses on manipulating patient's own immune system to recognize and destroy cancer cells. Vaccine formulations based on nanotechnology have been developed to deliver antigens to APCs and to improve antigenicity by combining with adjuvants. For example, Jin et al. reported biomimetic PLGA NPs coated with human cancer cell membrane fractions (CCMFs).¹⁰³ CCMFs and CCMF-PLGA NPs were capable of inhibiting cancer cell migration toward human mammary fibroblasts. Intravenous injection of CCMF-PLGA NPs significantly reduced experimental metastasis *in vivo*. A higher percentage of CD8⁺ and CD4⁺ cytotoxic T-lymphocyte populations was observed in spleens and lymph nodes. Splenocytes isolated from CCMF-PLGA NP-immunized mice had the highest number of interferon gamma-producing T-cells. These data show that CCMF-PLGA NPs could be promising for disrupting cancer cell–stromal cell interactions and for priming the immune system in cancer immunotherapy. Noh et al. reported multifaceted immune-modulatory nanoliposomes (denoted as “tumosomes”) that use tumor cell membrane proteins as tumor-associated antigens for reshaping the immune response in the lymph node and enhanced antitumor immunity (Figure 6c).¹⁰¹ Two lipid-based adjuvants, monophosphoryl lipid A and dimethyldioctadecylammonium are used as danger signal and cell-invasion moiety, respectively. Tumosomes are able to provide tumor antigens and molecular adjuvants for the priming of a long-term adaptive immune response in tumor draining lymph nodes and in the spleen.

4. Immuno-modulatory molecules

Immuno-modulatory molecules are essential components in vaccine formulations to induce robust immunity against tumor antigens. By combination of immuno-modulatory molecules with nanoparticle mediated therapeutic vaccines, it is possible to achieve complete eradication of tumors

and induce long-term anti-cancer immunity. In nanoparticle-mediated tumor vaccines, several immuno-modulatory molecules have been extensively investigated, including immune checkpoint molecules, nucleic acids, **imidazoquinoline derivatives**, polysaccharides and cytokines (Table 1).

Table 1. Immuno-modulatory molecules used in tumor vaccines

Classification	Molecule	Mechanism	Usage in tumor vaccine
Immune checkpoint molecule	Anti-CTLA-4 antibody	CTLA-4 antagonist	Combined with PDT ¹⁰⁴ , chemotherapy ¹⁰⁵ , radiotherapy ¹⁰⁶⁻¹⁰⁹ , PTT ¹¹⁰
	Anti-PD-1 antibody	PD-1 antagonist	Combined with PDT ¹¹¹ , chemotherapy ^{105, 112} , radiotherapy ^{113, 114} , PTT ¹¹⁵ , OVA ¹¹⁶
	Anti-PD-L1 antibody, PD-L1-blockade siRNA, Peptide	PD-L1 antagonist	Combined with PDT ⁴⁵ , radiotherapy ^{109, 117} , PTT ⁷⁴ , OVA ¹⁴ , chemo-PPT ⁸⁶ Combined with tumor lysate ⁹⁷
	Anti-CD137 antibody	CD137 agonist	Combined with OVA ¹¹⁶
	Anti-OX40 antibody	OX40 agonist	Combined with PDT ¹¹¹
Nucleic acid	CpG ODNs	TLR9 agonist	Combined with PDT ⁴⁹ , chemotherapy ¹¹⁸ , PTT ¹¹⁹ , tumor cell lysate ¹²⁰ , tumor antigen OVA ¹²¹
	Poly (I:C)	TLR3, RIG-I/MDA5 agonist	Combined with chemotherapy ¹⁹ , OVA ¹²³
Imidazoquinoline derivatives	Imiquimod (R-837)	TLR7 agonist	Combined with PDT ¹²² , PTT ^{70, 73} , cell membrane based vaccine ⁸ , OVA ¹²³ , chemo-PTT ¹²⁴ , PDT-PTT ¹²⁵
	Resiquimod (R-848)	TLR7, TLR8 agonist	Combined with chemotherapy ¹²⁶ , PTT ⁷⁵ , OVA ¹²⁷
Polysaccharide	MPL A	TLR4 agonist, induce IFN- γ and IL-12	Combined with tumor cell membrane ¹⁰¹ , OVA ¹²⁸
	Chitosan and its derivatives	macrophage activation, induce IL-12, IL-18, TNF- α and IFN- γ	Combined with radiotherapy ¹²⁹ , OVA ¹³⁰
Cytokine	GM-CSF	enhance antigen presentation, induce IL-2	Combined with MTT ¹³¹ , OVA ¹³²

IL-12	induce IFN- γ , enhance Th1-type response	Combined with hyperthermia ¹³³
IL-2	expansion of memory T cells, NK, B cells	Combined with radiotherapy ¹³⁴ , MTT ¹³¹

Immune checkpoint molecules play a central role in regulating the activities of different immune cell types. These molecules have either stimulatory functions that promote immune cell activation, or inhibitory functions that suppress immune cell activation to dampen inflammation, maintain immune homeostasis, and prevent tissue damage. Tumor cells frequently exploit immune checkpoint pathways by up-regulating the expression of ligands that activates inhibitory receptors on different immune cell types. Thus researchers have focused on targeting immune checkpoint molecules for tumor immunotherapy using either agonists of immune cell stimulatory receptors or antagonists of inhibitory receptors. **Although immune checkpoint molecules have demonstrated some exciting clinical responses, their efficacy is limited by the resistance to treatment of certain types of tumors.** Some of these molecules have been combined with chemotherapy, phototherapy, radiotherapy, or tumor antigen OVA (Table 1). **These combinations allowed to significantly potentiate checkpoint, and to generate long-term anti-tumor immunity, thus showing much promise in treating “cold” tumors.**^{51, 52, 65, 74} Antagonists of CTLA-4 (ipilimumab^{105, 108} and tremelimumab¹⁰⁹), PD-1 (pembrolizumab¹¹⁴ and nivolumab¹⁰⁵), PD-L1 (durvalumab¹⁰⁹ and DPPA-1⁹⁷) have been used for generating tumor vaccines. Agonists of CD137 and OX40 also have been applied in tumor vaccines.^{111, 116}

Nucleic acids, which are derived from DNA or RNA of microbial pathogens, are the main agonists of toll-like receptors (TLRs). TLRs are single, membrane-spanning, non-catalytic receptors usually

expressed in sentinel cells such as macrophages and dendritic cells. They play an important role in the activation of the innate immune system and in shaping the adaptive immune system. TLRs could recognize highly conserved structural motifs known as pathogen-associated microbial patterns which are exclusively expressed by microbial pathogens. However, these DNA/RNA adjuvants alone suffer from insufficient immunogenicity and - in the case of RNA - low stability.¹³⁵ In order to enhance immune responses, these adjuvants have been encapsulated in nanoparticles and combined with other therapeutic strategies (Table 1). In DNA adjuvants, immune-stimulatory sequences (CpG motifs) present in DNA of bacterial origin are used. Synthetic oligodeoxynucleotides containing CpG motifs (CpG-ODNs) could activate TLR9 on variety of APCs to produce various inflammatory cytokines including IL-1, IL-6, IL-8, IL-12, TNF- α , and IFN- γ .¹³⁶ Polyinosinic:polycytidylic acid (poly(I:C)) is a synthetic analog of double-stranded RNA, and acts as agonist of TLR 3 and retinoic acid inducible gene I (RIG-I)-like receptors (RIG-I and MDA5).^{141, 142}

Imiquimod (R837) and resiquimod (R848) are imidazoquinoline derivatives which are one of the most widely investigated groups of TLR agonists. R-837 occurs particularly via the TLR-7 and stimulates the innate immune response through induction, synthesis, and release of cytokines, including interferon- α (IFN- α), interleukin (IL)-6, and tumour necrosis factor (TNF)- α .¹³⁷ R-848 is a TLR7 and TLR8 agonist that induces the upregulation of cytokines such as TNF- α , IL-6 and IFN- α .¹³⁸⁻¹⁴⁰

Polysaccharides belong to a class of natural polymers consisting of glycosidically linked carbohydrate monomers. As vaccine adjuvants, polysaccharides can not only promote antigen-specific immune system, but also enhance the body's natural immune functions.¹⁴³⁻¹⁴⁵

Polysaccharide adjuvants mainly include lipopolysaccharide, glucan, mannan, inulin, chitosan and its derivatives. Some of them have been used as adjuvants in combination with tumor vaccines (Table 1). For example, 3-O-desacyl-4'-monophosphoryl lipid A (MPLA), a nontoxic derivative of the lipopolysaccharide of *Salmonella minnesota* R595, could act as TLR4 agonist and induce the production of IFN- γ and IL-12.¹⁴⁵ Chitin, a linear β -1–4-linked polymer of D-glucosamine and N-acetyl-D-glucosamine extracted from shrimp, and chitosan, which is obtained by partial deacetylation of chitin, exhibit a range of immunological effects, including macrophage activation and production of inflammatory cytokines including IL-12, IL-18, TNF- α and IFN- γ and enhanced antibody production when co-administered with antigens.^{129, 130}

Cytokines, a group of regulatory peptides or glycoproteins with molar masses below 30 kDa, have been widely recognized as crucial factors in tumor development and treatment. They are secreted by various cells of the immune system (innate as well as adaptive) that help to regulate and boost immunity. At the top of the inflammatory cytokine cascade are granulocyte-macrophage colony-stimulating factor (GM-CSF) and some interleukins. A number of cytokines have been used in combination with tumor vaccines. For example, GM-CSF can promote antigen presentation, IL-2 production, antibody secreting, and enhance the function of CD4⁺ and CD8⁺ T cells. IL-2 is required for the development and secondary expansion of memory T cells, and it also supports the growth and expansion of NK cells as well as other immune cells such as B cells at various stages of development or activation. IL-12 induces the production of IFN- γ and stimulates TH1 and NK cell growth, while inhibiting TH2 cell responses.

5. Comparison of *in vivo* and *ex vivo* strategies to prepare tumor antigens

Tumor antigens could be induced *in vivo* or prepared *ex vivo*. There are similarities and differences between two strategies as shown in table 2, and the details are as follows. ①Strategies for inducing tumor antigen release *in vivo* usually include chemotherapy, radiotherapy, PDT, PTT or MTT. The ROS/heat generating from focused effect or collateral effect could induce ICD and antigen release through ER stress. Strategies for preparing tumor antigens *ex vivo* aim at revealing efficient tumor antigens, mainly neoantigen which could induce stronger immune response and realize personalized therapy. ②ROS/heat generating nanoparticles are designed to induce tumor antigen release *in vivo*. Nanotechnology also has been used to prepare tumor antigens *ex vivo* via antigen delivery nano-systems which allows enhance the delivery of tumor antigens to APCs. ③Inducing tumor antigens *in vivo* is accompanied by generating DAMPs that recruit APCs and stimulate optimal antigen presentation to T cells, while the *ex vivo* strategies could not. ④Both two strategies could induce CD8⁺ (cytotoxic) and CD4⁺ (helper) T cells. ⑤Inducing tumor antigen *in vivo* usually has side effects which are induced by chemotherapeutic drugs, photosensitizers or hyperthermia agents. Fortunately, nanotechnology could reduce related side effects through controlling drug release, and enhancing the targeting, etc. *Ex vivo* strategies for preparing tumor antigen are usually less toxic, for the tumor components and delivery systems present good biocompatibility. ⑥Both strategies have been reported for the treatment of “cold” immune tumors which usually have a low level of mutations, express few neoantigens, lack of tumor-infiltration lymphocytes (TILs) and cytotoxic T cells(CTLs).^{74, 146} These tumors do not respond to immune-checkpoint therapy. It has been demonstrated that ICD would be a hopeful way for enhancing CTLs infiltration and converting a “cold” tumor to a “hot” one.¹⁴⁶⁻¹⁴⁹ Moreover, studies also have shown that neoantigens are potent T-cell activators, and are thus promising strategies for turning a “cold”

to a “hot” tumor immune microenvironment.^{150, 151}

Table 2. Comparison of *in vivo* and *ex vivo* strategies to prepare tumor antigens

Property	<i>In vivo</i> strategy	<i>Ex vivo</i> strategy
Mechanism	Chemotherapy, radiotherapy, PDT, PTT, MTT	Looking for efficient tumor antigens
Role of nanoparticles	Enhance ROS/heat generation in tumor sites	Protect and deliver tumor antigens to APCs
Generation of DAMPs	Generate DAMPs	Cannot generate DAMPs
Induction of T cells	Induce both CD8 ⁺ and CD4 ⁺ T cells	Induce both CD8 ⁺ and CD4 ⁺ T cells
Related side effect	Chemo-drugs, PDT/PTT/MTT agents may be toxic or non-biodegradable	Antigen delivery systems have good biocompatibility.
Applicable for “cold” tumors	ICD could increase CTLs infiltration and turn “cold” tumors to hot ones.	Neoantigens are potent T-cell activators, and turn “cold” tumors to “hot” ones.

6. Conclusion and perspectives

Tumor antigens could be induced *in vivo* or prepared *ex vivo*. Both strategies allow to realize personalized therapy to patients, turning a “cold” into a “hot” immunogenic tumor microenvironment and inducing long term immunological memory response. There are also advantages and disadvantages when they are compared to each other. Inducing tumor antigen *in vivo* has the advantage of generating DAMPs, however, chemo-drugs or PDT/PTT/MTT agents may be toxic or non-biodegradable. Preparing tumor antigen *ex vivo* usually has good biocompatibility, but this strategy could not generate DAMPs. Combining immune therapy with nanotechnology could further enhance immunotherapy effect owing to unique advantages of smart nanosystems,

such as increasing immune response at lower doses of drugs/agents and lower-power light irradiation, further encapsulating immuno-modulatory molecules or combining with other therapy treatments. ROS/heat generating nanoparticles have been evaluated for inducing tumor antigen release *in vivo*, while antigen delivery nano-systems have been synthesized for processing tumor antigen *ex vivo*.

Various nanoparticles prepared from inorganic compounds and polymers have been evaluated for improving the outcomes while limiting the systemic toxicity. A large number of strategies (*e.g.* different ICD inducers and tumor antigen delivery systems), nano-formulations and adjuvants, have been reported. However, few comparative data are available on these different approaches, particularly in humans. In addition, translational potential of nanomaterials remains low owing to poor stability, insufficient tumor accumulation, toxicity, and difficulty to scale up. Therefore, fundamental issues need to be addressed, including the most effective type of nanoparticle platforms, adjuvant, dose, administration route and schedule. Last but not least, nanoparticles should be safe, stable, preferably with a single dose and a maximum of two doses, and have the ability to induce long-lived memory B and T cell responses so as to achieve efficient tumor vaccine for personalized therapy.

Conflicts of interest

The authors declare no competing financial interest.

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