Nanomedicine-boosting tumor immunogenicity for enhanced immunotherapy

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Abstract

Immunotherapy has revolutionized oncology remarkably and gained great improvements in cancer therapy. However, tumor immunotherapy still encounters serious challenges, especially certain tumors barely respond to immunotherapy. The lack of immunogenicity and subsequent insufficient antitumor immune activation is a pivotal reason. Here, a general introduction and the strengthening strategies of immunogenicity of tumor for enhanced immunotherapy are reviewed. Specifically, nanotechnology nowadays is playing important roles in increasing the antitumor efficacy of various treatments including immunotherapy. This review highlights how nanomedicines integrating one or more anticancer therapeutic methods (e.g., cancer vaccines, chemotherapy, phototherapy and radiotherapy) to increase the tumor immunogenicity for rousing T cell related immune responses and achieving inspiring antitumor efficacy. Given the sophisticated immune evasion mechanisms, rational designed nanodrugs with combinational formulations are summarized to improve therapeutic efficacy in synergistic ways. Nanoplatforms taking advantage of the distinct features of tumor tissue or tumor cell with stimuli-responsiveness and targeting functions were introduced to successfully accelerate tumor accumulation of drugs and greatly promote therapeutic efficacy with low-dose administration and programmed drug release. Finally, the related challenges and personal perspectives of nanomedicines for tumor immunotherapy are concluded.

Key words: tumor immunotherapy; immunogenecity; Nanomedicine; combination therapy

1. Introduction

Table 1. Relative abbreviations in the manuscript

Full name	Abbreviation
Chimeric antigen receptor	CAR
T cell receptor	TCR
Immune checkpoint blockade	ICB
Programmed cell death ligand 1	PD-L1
Programmed cell death	PD-1
Metal organic framework	MOF

Natural killer	NK
Immunogenic cell death	ICD
Stimulator of interferon genes	STING
Dendritic cells	DCs
Tumor associated antigens	TAA
Cytotoxic T lymphocytes	CTLs
Heat shock proteins	HSPs
Adenosine triphosphate	ATP
High-mobility group box 1	HMGB1
Calreticulin	CRT
Antigen presenting cells	APCs
Myeloid-derived suppressor cells	MDSCs
Photodynamic therapy	PDT
Photothermal therapy	PTT
Reactive oxygen species	ROS
Tumor-associated antigens	TAAs
Chemodynamic therapy	CDT
Sonodynamic therapy	SDT
Matrix metalloproteinase	MMP
Cytotoxic T lymphocytes	CTLs
Cytosine-phosphate-guanine	CpG
Tumor associated macrophages	TAMs
Fc-gamma receptors	FcγRs
Immune complexes	ICs
Pathogen-associated molecular patterns	PAMPs
Pattern recognition receptors	PRRs
Cyclophosphamide	CTX

Indoleamine 2,3-dioxygenase	IDO
Red blood cell membrane	RBCm
Tumor microenvironment	TME
Catalase	CAT

Cancer is one of the greatest threats to human health from which millions of people die every year. Since William B. Coley first used bacterial toxins as an immunotherapy agent to deal with bone and soft-tissue sarcoma[1], immunotherapy has aroused great concern among scientists, offering alternatives for cancer treatment[2]. Immunotherapy is becoming the new pillar of cancer treatment and the Nobel Prize in Physiology or Medicine 2018 was awarded to Janes Allison and Tasuku Honjo. Immunotherapy aims to activate patients' own immune system to fight against tumor[3]. Compared to traditional cancer treatment modalities, immunotherapy could work in a subset patients with advanced tumor and mediate immune protection against recurrence and metastasis[2, 4].

With the fast development of immunotherapy, a lot of approaches have been developed to inhibit tumor growth, including cancer vaccines, chimeric antigen receptor (CAR) T cell and immune checkpoint blockade, etc. Cancer vaccines are usually composed of tumor specific antigens and adjuvants. Tumor specific antigens, recognized as "non-self" by patient's immune system can bind to T cell receptors (TCR) with high affinity and elicit antigen specific adaptive immunity[5]. It has been confirmed in some phase I clinical trials that the cumulative rate of metastasis was reduced after vaccination, leading to sustained progression-free survival[6, 7]. Adjuvants could stimulate distinct immunity and therefor enhance anticancer immune response. Despite the feasibility of vaccines, there are still some challenges remained. First, the soluble formulation may result in chaotic distribution in the body, restricting vaccine immunogenicity against tumor[8, 9]; second, it is almost impossible to eradicate tumors with only one antigen derived immune response due to the diversity of tumor subpopulations; third, the immunosuppression and immune evasion of tumors may inhibit immune responses, decreasing the efficacy of vaccines [5].

Besides vaccines, CAR-T cell therapy is another attractive strategy for tumor immunotherapy. These kinds of autologous therapies require ex vivo cell engineering by genetical modification to obtain T cells expressing chimeric antigen receptors, and the engineered T cells were then transfused back into patients to attack tumors [10]. In 2017, US Food and Drug Administration (FDA) approved B-cell antigen CD19 targeted CAR-T cell therapy for the treatment of child acute lymphoblastic leukemia. CAR construction enable T cells to bind cancerous B cells specifically to induce apoptosis, resulting in high remission rate of 82.5% [11, 12]. Though CAR-T cell therapy could

induce durable remission [13-15], there still remains great challenges like severe immune-related side effects, high cost and potential risks arising from viral transduction [16-19]. Additionally, CAR-T cell therapy is not efficient enough in other hematological and solid malignancies[20, 21].

In addition to the above therapies, immune checkpoint blockade (ICB) has achieved encouraging results in recent years[22-24]. Tumor cells could escape from immune responses by overexpressing immune checkpoints like programmed cell death ligand 1 (PD-L1), CD47, CTLA-4, LAG-3 and TIM-3 et. al. PD-L1 could inactivate cytotoxic T cells via binding to programmed cell death 1(PD-1) on T cell surface[25]. The overexpressed CD47 ("don't eat me signal") on tumor cells could help them escape from phagocytosis and impair antigen presenting function of macrophages, facilitating immune evasion[26, 27]. By blocking such immune checkpoints, ICB reverses tumor-mediated immunosuppression other than stimulating cytotoxic T cells directly, overcoming tumor immune resistance[28, 29]. Clinical studies showed that pembrolizumab and atezolizumab (monoclonal antibodies against PD-1 and PD-L1, respectively) both have high response rate (40%~50%) for several cancers[30-32]. However, challenges still exit. First, the nonspecific distribution in body could induce severe immune-related side effects in some organs[33, 34]. Second, ICB monotherapy may result in drug resistance and decrease durable response rate[35]. The last but not the least, according to clinical data, only a few patients respond well to ICB, depending on immunogenicity of tumors[36, 37]. Nonimmunogenic tumors, also termed "cold tumors", feature a small amount of T cells infiltration or low expression of PD-L1, thus, barely respond to ICB therapy[38, 39]. Therefore, converting "cold tumors" into "hot tumors" (immunogenic tumors with increased T cell infiltration) could be a rational solution to deal with this problem, in which immunogenicity plays a critical role.

Despite the efforts devoted to immunotherapy, there are still challenges impeding its applications, especially the low tumor immunogenicity which greatly inhibit the activation of efficient immune responses. Only with high tumor immunogenicity can tumors respond well to immunotherapy. Although simply improving tumor immunogenicity is not sufficient for effective tumor immunotherapy, which also need robust effective T cells and may further overcome the immune-suppressive tumor microenvironment, improving tumor immunogenicity is certainly a key point to potentiate immunotherapy. With the development of nanotechnology, nanotheraputics with improved pharmacokinetics properties such as selected organ accumulation and longer circulation time have been widely studied nowadays. Nanoplatforms, including micelles[40, 41], liposomes[42], vesicles[43], protein nanoparticles[44, 45], metal organic frameworks (MOFs)[10, 46] and other inorganic nanoparticles, have been extensively studied for the delivery of various drugs to increase tumor immunogenicity and inhibit tumor growth. Different from traditional nanomedicines which target tumor cells directly, nanoplatforms tailored for cancer immunotherapy have more alternative

targets (T cells, macrophages, lymphoid tissues etc.)[47-49]. In clinic, the US Food and Drug Administration has approved nanomedicine formulations Doxil/Caelys and Abraxane for substantial patient benefit, which could reduce toxicity as well as improve survival quality. The combination of nanotechnology and immunotherapy would bring many advantages and create great potential for future treatment.

In order to evoke potent tumor-specific immune responses, it is vital to elicit the immunogenicity of cancer cells to differentiate it from normal cells precisely. This review focuses on the tumor immunogenicity and gives a comprehensive understanding of its generation and strengthening methods. Notably, we highlight the promising strategies in combination with nanotechnology for eliciting more potent tumor immunogenicity to improve immunotherapeutic efficacy, which are expected to provide promising approaches for successful cancer treatment.

2. Immunogenicity

2.1 The role of immunogenicity

Immunogenicity occurs when people's immune system recognizes an agent as foreign, followed by the generation of cellular and/or humoral immune response. It is identified as the ability to elicit immune response for immunotherapy, which plays a highly significant role in cancer treatment. Immunogenicity always associates with medical use of proteins, peptides, polysaccharide, nucleic acid and so on. In addition, foreign bacteria, viruses are also able to induce severe immunogenicity and elicit robust immune responses[50]. When proteins originating from animals were first used as therapeutics[51], the foreign origin was regarded as the main reason of immunogenicity. Then, it was confirmed that factor VIII and growth hormone originated from human tissues could also induce immunological response[52], inspiring the potential of endogenous substance. These immunogenic biotherapeutics are able to stimulate the immune system with no need for covalent binding to any endogenous molecules.

Tumor consists of various cell types, including origin cells with genetic mutations and a large number of other cells (e.g., endothelial cells, fibroblasts and different kinds of immune cells). There might be inadequate immune cells infiltrates at the start, but with the boost of immune response, other immune cells may participate in this process, such as macrophages, natural killer (NK) cells, and most importantly, T cells which attack tumor cells directly[53]. However, tumors have various phenotypes and one of them is distinguished by immunogenicity which plays a highly important role in immune evasion mechanism. Tumors with high immunogenicity which have immunogenic microenvironment including infiltrated T cells, memory T cells, cytokines (for example granzyme B, IFN-γ) and high PD-L1 expression as shown in

Figure. 1 are usually termed "hot tumors"[53]. While tumors with low immunogenicity which have nonimmunogenic microenvironment and lack the above components are termed "cold tumors". It has been proved by numerous reports and becomes a consensus that only hot tumors could respond well to immunotherapies, while cold tumors are able to escape from immune attacks. However, there are still no common methods to accurately quantify tumor immunogenicity yet. But the judgement of tumor immunogenicity can be analyzed qualitatively by the representative T cells and cytokines and sometimes assisted with clinic outcomes. Some types of tumors, such as triple negative breast carcinoma, pancreatic cancer and prostate cancer are always found to be cold tumors with low immunogenicity.

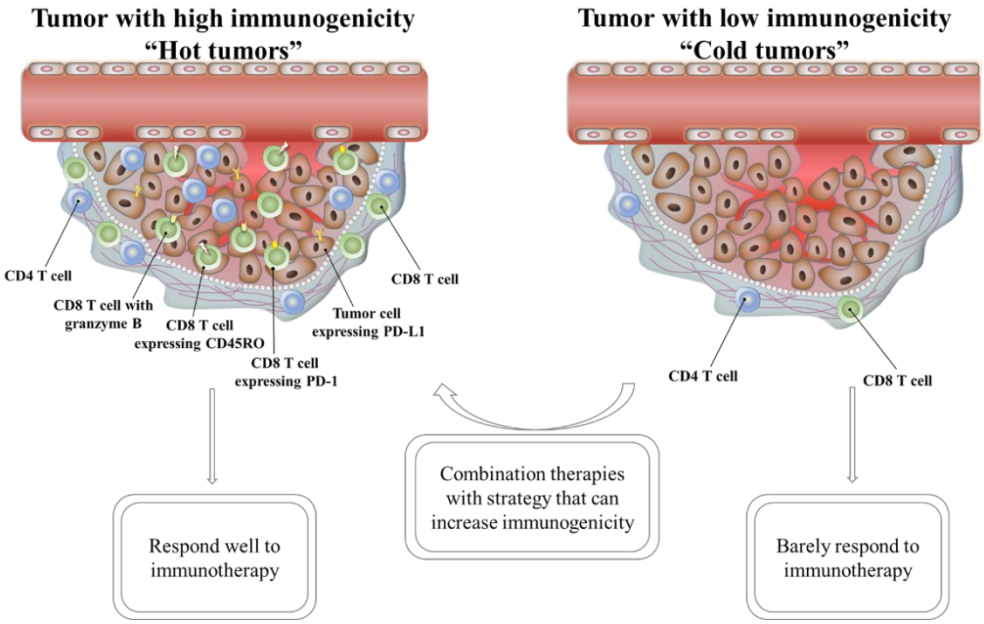

Figure.1 Potential characteristics of "hot" tumors with high immunogenicity and "cold" tumors with low immunogenicity. "Hot" tumors have the highly immunogenic microenvironment, including infiltrated T cells, memory T cells, cytokines (for example granzyme B, IFN-γ) and high PD-L1 expression. "Cold" tumors with low immunogenicity have nonimmunogenic microenvironment and always lack the above components.

The efficacy of immunotherapy usually relies on tumor immunogenicity. Though ICB has been confirmed to be a pretty good strategy for cancer treatment, it is proved that only tumors with high immunogenicity could respond well to checkpoint inhibitors and elicit durable clinical benefit. The residual patients bearing "cold tumors" barely respond to ICB due to the lack of T cell infiltration ascribed from low immunogenicity. For example, in one study, patients with melanoma, which is considered as "hot tumor", experienced progression on ipilimumab, nivolumab resulted in a 32% overall response

rate[54]. However, in another phase I trial of patients with PD-L1 negative tumors (cold tumors), only a response rate of 17% was obtained with anti-PD-1 antibody nivolumab[55]. Given the dynamic feature of immune responses against tumors and the complexity of modulating the expression of various immune checkpoints, it is insufficient to apply monotherapy for cancer treatment. In terms of ICB, one of the advantages is that a large number of suppressed T cell clones could be stimulated bypassing the requirement for antigen specificity, while the benefit could be negated when tumor-specific T cells generation is inhibited due to the low immunogenicity in the first place. Thus, it is highly important to combine different therapies to potentiate immunogenicity before ICB, making "cold tumors" more susceptible to immunotherapy.

Besides ICB, the efficacy of cancer vaccine is also highly correlated with immunogenicity. Most tumor neoantigens (tumor specific antigens) have undetectable immunogenicity, and the situation would be further complicated for cancers such as glioblastoma and pancreatic cancer with low tumor mutation burdens. Effective cancer vaccines rely on robust and durable neoantigen-specific immune responses, ascribed to the delivery of highly immunogenic agents to lymph nodes. Therefore, it is urgently desired to potentiate the immunogenicity of neoantigens for potent cancer immunotherapy. In some cases, cancer vaccines based on nucleic acids, peptides, proteins, etc. are not able to evoke robust immune responses due to insufficient production of immunogenicity with these antigens. Some other strategies have been applied to potentiate the efficacy of cancer vaccines: (1) codelivery of adjuvants to create a more immunogenic microenvironment and further activate distinct innate immunity and neoantigen-guided tumor-specific adaptive immunity[56, 57]; (2) synergistic regulation of a variety of immune signaling pathways and (3) multiepitope antigens that are able to stimulate a wide spectrum of immune responses.

Moreover, tumor immunogenicity could not only affect the therapeutic efficiency on orthotopic tumors, but also influence the efficiency on recurrence and metastasis, which are great challenges remained in cancer treatment. For example, a large number of patients would suffer from recurrence after surgery, leading to a decreased long-term survival rate. Traditional therapies such as chemotherapy and radiotherapy barely show any efficiency on metastatic tumors and tend to prompt immunosuppressive effect which would weaken the efficacy of monotherapy. So far, a variety of strategies, especially immunotherapies, have been utilized to not only treat in situ tumors, but also elicit anti-tumor memory effect and abscopal effect against recurrence and metastasis. However, these effects are rare in tumors with low immunogenicity. Therefore, it is highly reasonable to increase tumor immunogenicity to accelerate the eradication of tumors as well as the inhibition of recurrence and metastasis.

In general, high tumor immunogenicity is the prerequisite of efficient immunotherapy. Pretreatment of tumors, especially "cold tumors", to promote

immunogenicity and T cell infiltration could provide intriguing possibilities to immunotherapy.

2.2 Strategies to induce and strengthen tumor immunogenicity

Overall, overcoming low-immunogenicity is one of the biggest challenges of tumor immunotherapy. Up to today, several strategies have been applied to elicit immunogenicity, for example, cancer vaccines, certain kinds of chemotherapies, radiotherapy, photodynamic therapy, photothermal therapy and chemodynamic therapy, et.al. In general, we would divide them into two categories: methods by delivering exogeneous immunogenic antigens, and methods inducing immunogenic cell death (ICD) to release endogenous immunogenic neoantigens (**Figure. 2A**).

In the former case, these immunogenic antigens could be proteins, peptides, tumor cell lysate, nucleic acids and neoantigens [58], acting as cancer vaccines. In some cases, adjuvants (for example, cytokines, chemokines, Toll-like receptors agonists, stimulator of interferon genes (STING) agonists) would be co-administrated with these antigens to further improve the tumor immunogenicity. Once reaching lymph nodes, these immunogenic antigens and adjuvants could be recognized by host immune systems, promoting antigen cross-presentation, dendritic cells (DCs) maturation and cytotoxic T lymphocytes (CTLs) activation to induce strong immune responses (**Figure. 2A**).

In the latter case, different from most programmed cell death which is nonimmunogenic, ICD is able to stimulate immune responses and has been widely studied during the past decades. Most ICD inducers, for example, chemotherapeutic agents, 7A7 (a kind of antibody targeting epidermal growth factor receptor) and cardiac glycosides, are classified as type I ICD inducers, primarily targeting cytosolic proteins, nucleic proteins and plasma membranes. Type II ICD inducers preferentially target the endoplasmic reticulum. When undergoing ICD, dying tumor cells could release cellular antigens and endogenous danger signals such as heat shock proteins (HSPs), adenosine triphosphate (ATP), high-mobility group box 1 (HMGB1) protein and calreticulin (CRT)[59]. ATP could promote DCs recruitment via the interaction with P2RX7. HMGB1 could facilitate DC maturation via the interaction with TLR2, TLR4 and RAGE. CRT acts as an ‘eat me’ signal to stimulate the antigen presenting function of dendritic cells via the interaction with CRT receptors. Matured DCs would lead to T cell priming through the binding of CD80/86 with CD28 as well as MHC1 with TCR antigen (Ag-TCR), inducing antigen-specific T cell responses to boost tumor eradication[60-62]. Clinical and preclinical studies confirmed that certain types of chemotherapy, radiation therapy, photothermal and photodynamic therapy can stimulate tumor specific adaptive immunity via inducing ICD[63]. Meanwhile, all of these strategies could induce immune memory effect to fight not only against orthotopic tumors, but also distal tumors and recurrence (**Figure. 2B**).

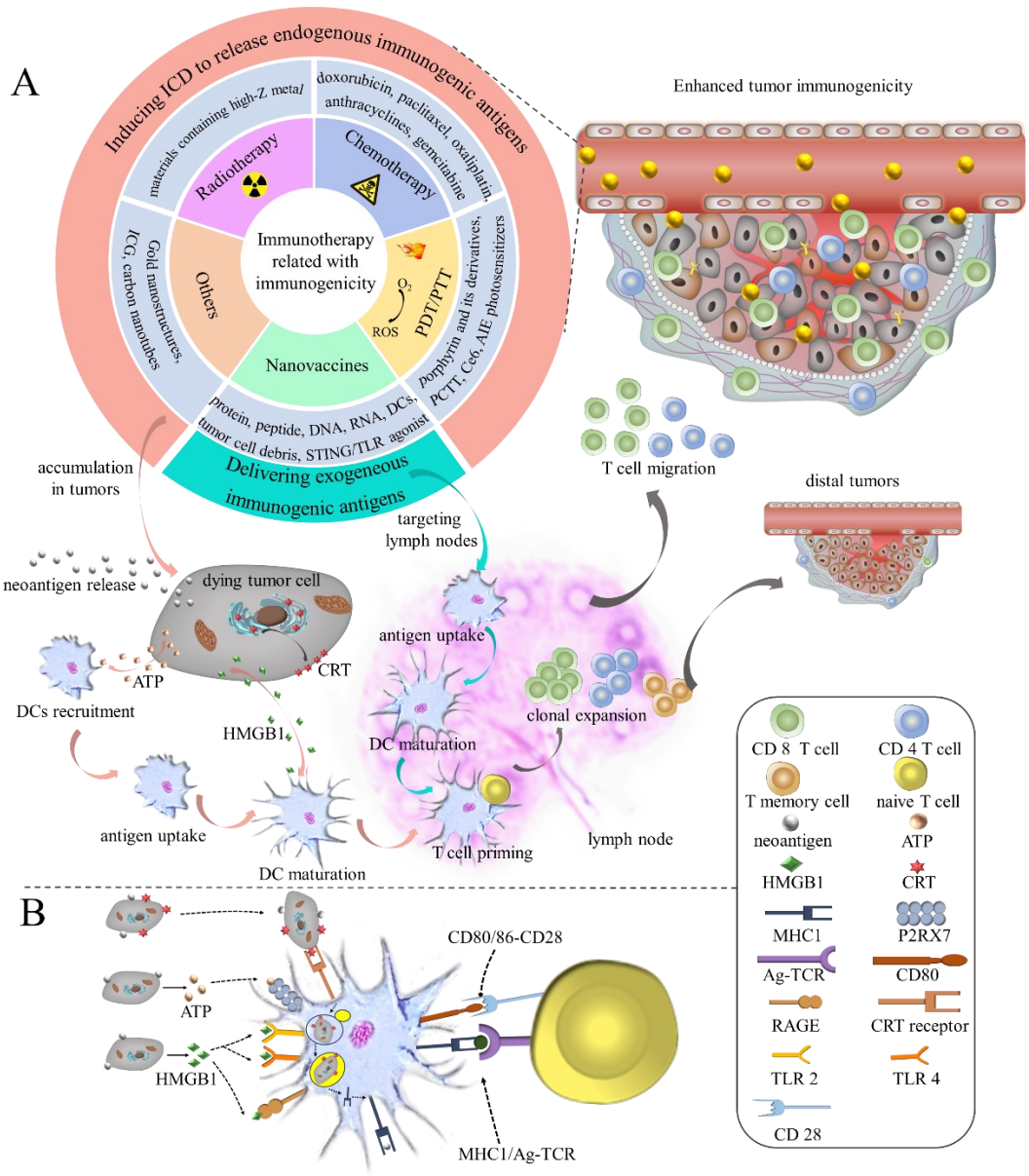

Figure. 2 (A) Strategies used to improve tumor immunogenicity via (1) delivering exogeneous immunogenic antigens which mainly target lymph nodes to promote DC maturation and (2) inducing ICD to release endogenous immunogenic agents such as neoantigens, ATP, and HMGB1 to promote DC maturation. Both strategies could promote T cell priming and clonal expansion of T cells, leading to the suppression of both orthotopic and distal tumors. (B) Simplified mechanism of T cell priming via the release of ATP and HMGB1 as well as the exposure of CRT. ATP could promote DCs recruitment via the interaction with P2RX7. HMGB1 could facilitate DC maturation via the interaction with TLR2, TLR4 and RAGE. CRT acts as an 'eat me' signal to stimulate the antigen presenting function of dendritic cells via the interaction with CRT

receptors. Matured DCs would lead to T cell priming through the binding of CD80/86 with CD28 as well as MHC1 with TCR antigen (Ag-TCR).

2.2.1 Cancer vaccines

Highly immunogenic cancer vaccines are able to activate and recruit T cells and NK cells to recognize and combat tumor cells. In the 1980s, it was proved that antigens from human melanomas could elicit T cell responses[64], inspiring the use of vaccine to attack cancer through mobilizing immune system. So far, cancer vaccines have become an attractive strategy to elicit immunogenicity for robust and durable antitumor immune responses, popular in both prophylactic and therapeutic modalities. A variety of sources of antigens are available for antitumor vaccines, such as proteins, whole-cells, DCs and nucleic acids et. al. Dendritic cell vaccines are one of the most commonly developed categories of cancer vaccines. They are prepared from DCs isolated from patients and then engineered for the expression of tumor-associated antigens (TAAs). After transfusion back to patients, they can activate T cells directly to eliminate cancer cells[65]. Sipuleucel-T, a kind of dendritic cell vaccine, has achieved approval for the treatment of prostate cancer in 2010[66]. Vaccines based on nucleic acids such as DNA and RNA have aroused great concerns recently. The exogenous DNA or mRNA is delivered to lymph nodes and then taken up by antigen presenting cells (APCs), inducing antigen expression and subsequent T cell activation[67]. mRNA has some advantages over DNA due to its easier production, longer half-life after modification and no integration into genomes. Another important cancer vaccine is based on neoantigens that are specific presented in cancer cells, thus could avoid off-target side effects[68]. Moreover, thanks to the capability of encompassing numerous neoantigens, these vaccines are good choices for the treatment of heterogeneous tumors. To sum up, the most significant principle of vaccines is to improve the tumor immunogenicity, which is closely related to antitumor efficacy. Besides, to further accelerate the immune cascades, adjuvants are usually co-administrated with immunogenic antigens, especially pattern recognition receptor agonists such as TLR agonists and STING agonists. In some cases, adjuvants without antigens were also used to activate DCs for the stimulation of immune responses.

2.2.2 Chemotherapy

Cytotoxic drugs have been used to kill cancer cells directly for decades, and recently it has been confirmed that certain drugs are effective in eliciting immunogenicity by expression of tumor-specific antigens and MHC-I molecules on cancer cell surface[69]. In addition, chemotherapy could potentiate immunotherapy in some other pathways, for instance, chemotherapy-induced stress could upregulate NK

cell stimulatory ligands (such as NKG2D)[70] and downregulate NK cell inhibitory ligands[71], subsequently activate NK cells for immunotherapy. Chemotherapy can also induce presentation of death receptors (such as TRAIL and mannose-6-phosphate receptor), making tumors more susceptible to immune responses[72]. The boost in inflammatory cytokines could also activate angiogenic networks and convert nonimmunogenic microenvironment into immunogenic one, prompting tumor recruitment of cytotoxic T cells. Casares N. and colleagues first formally demonstrated that the anthracycline is able to induce ICD both in vivo and in vitro, suggesting rational design of chemotherapy to improve the immunogenicity[73]. In recent decades, doxorubicin, paclitaxel, oxaliplatin, gemcitabine, taxane, mitoxantrone and bortezomib have also been confirmed to induce ICD and immunogenicity clinically via various mechanisms[74]. For example, anthracyclines could promote calreticulin transferring from intracellular to cell surface for the exposure of phagocytic signals to DCs[75]. Gemcitabine could reverse imperfect cross-presentation of TAAs and prompt the cross-priming of $CD8^+$ T cells. Cyclophosphamide and taxane deplete T_{reg} cells and myeloid-derived suppressor cells (MDSCs)[76]. Other mechanisms include upregulating co-stimulatory factors (B7-1) or downregulating co-inhibitory factors (B7-H1) to strengthen effector T cell (T_{eff}) activity[60]. In mice models, chemotherapy-led ICD mostly relies on the exposure of endoplasmic reticulum chaperones dependent on eIF2A phosphorylation[58, 77, 78]. Most of these ICD derived manifestations have also been highlighted in human cancer cells, demonstrating the immunogenic chemotherapy[79, 80].

2.2.3 Radiotherapy

Radiotherapy is another strategy to promote endogenous neoantigen presentation and T_{eff} responses, eliciting immunogenicity by a variety of mechanisms[81]. Notably, radiation dose and fractionation level play an important role in inducing abscopal effects. Compared to single-dose radiotherapy, fractionated radiotherapy is more possible to induce ICD[82, 83]. Several groups demonstrated that 7.5 Gy or higher fraction sizes were required to facilitate antigen presentation[84, 85]. Additionally, high-dose radiotherapy elicits limited immunogenicity due to the unexpected activation of enzyme-dependent DNA digestion[86]. Owning to the defects in DNA repair mechanism, tumor cells are more vulnerable to radiotherapy. Exposure to radiation would lead to the release of intracellular peptides through radical-induced protein degradation, as well as the overexpression of proteins associated with protein breakdown[87]. Radiation is able to alter the peptide repertoire, benefiting antitumor immune responses. It promotes neoantigen presentation via upregulating the expression of normally silent genes. And with the formation of immunoproteasome induced by IFN, radiation-mediated inflammatory responses could alter peptide epitopes processing and presentation. Radiation-induced inflammatory responses also enable activation of DCs via

chemokines, pro-inflammatory cytokines[88], as well as cytosolic DNA detection mediated by stimulator of interferon genes. Furthermore, radiotherapy could enhance T cell infiltration ascribed to vascular normalization and secretion of inflammatory cytokines such as TNF-α and IFN-γ. All the above-mentioned mechanisms would benefit immunogenicity, prompting tumor recession.

2.2.4 Phototherapy

Noninvasive photodynamic/photothermal therapies (PDT/PTT) are emerging strategies to improve immunogenicity for the benefit of immune responses. Photosensitizers of PDT would undergo photochemical reaction with light excitation, generating reactive oxygen species (ROS) such as O_2^-, $^1O_2$, HO• and H_2O_2 et.al. These cytotoxic ROS would damage malignant cells via oxidizing proteins, amino acids, lipids as well as disrupting plasma membranes and subcellular organelles[89]. More importantly, tumor cells debris and cytosolic components would be released after light excitation and act as tumor antigens to induce DCs maturation, activate cytotoxic T lymphocytes and boost their infiltration into tumor regions, resulting in the increase of tumor immunogenicity[90]. Additionally, PDT induces in situ accumulation of neutrophils that can destroy cancer cells through the release of lysosomal enzymes and toxic substances, as well as trigger subsequent macrophages and monocytes invasion. Then, the secreted chemokines and inflammatory cytokines could stimulate immune responses to eradicate residual tumor cells[91]. Moreover, PDT can also upregulate the expression of stress-induced proteins, resulting in dendritic cell activation and tumor antigen presentation to T cells[92]. PTT can cause tumor ablation through hyperthermia, and then lead to the release of TAAs and endogenous signals such as heat shock proteins and DMAPs under certain conditions[93, 94], increasing tumor immunogenicity and facilitating immune responses and even immunological memory. All these positive signals from phototherapy could benefit immunogenicity and facilitate tumor eradication.

2.2.5 Others

Besides the above-mentioned strategies, other treatments such as chemodynamic therapy (CDT) and sonodynamic therapy (SDT) can also induce immunogenicity via similar mechanisms of PDT to potentiate immunotherapy. CDT and SDT are emerging noninvasive therapies that can generate ROS through Fenton chemistry and sonosensitizers, respectively[95, 96], to increase immunogenicity for better therapeutic efficacy. Compared to phototherapy strategies that are restricted by tissue penetration depth, mental catalysts in CDT and ultrasonic irradiation in SDT are able to reach deep region of soft tissues to trigger tumor ablation. In addition, Michail and colleagues found that respiratory hyperoxia could reverse the hypoxia-adenosinergic immunosuppression in tumor microenvironment and stimulate increased intratumoral

recruitment of T cells; meanwhile, it could also reduce inhibition of endogenously developed or adoptively transfered tumor-reactive CD8 T cells, decrease immunosuppressive molecules and weaken immunosuppression by T_{reg}, transferring cold tumors into immunogenic hot tumors[97]. Moreover, gene therapy or cytokines, such as trail, IL-2 or IL-12, can also improve the tumor immunogenicity and activate the immune system[98]. Other physical treatments such as high hydrostatic pressure has also been reported to induce ICD and elicit immunogenicity[99].

3. Nanomedicine boosting immunogenicity for immunotherapy

3.1 Nanomedicine and immunogenicity

During the past few decades, synthesized and naturally derived nanoparticles with distinct physical and chemical properties have been widely studied in cancer immunotherapy[100, 101]. These nanomedicines are able to improve antitumor immunotherapy through the following aspects: 1) nanomedicines enable efficient loading of hydrophobic drugs; 2) nanoscale-size helps the drugs to escape from rapid renal elimination that small molecules usually undergo; 3) the mostly used PEG modification of nanomedicines could avoid drugs clearance by the mononuclear phagocytic system; 4) nanomedicines in certain size tend to accumulate in tumor regions by the EPR effect; 5) modified with certain ligands, nanomedicines could target cancerous cells as well as some vital immune cells that overexpress specific moieties through ligand/receptor interaction; 6) given the distinct microenvironment in tumor regions (e.g. moderate acidity, high concentration of matrix metalloproteinase (MMP) and ROS, high concentration of GSH inside tumor cells), some nanomedicines are designed to respond to tumor microenvironment for programmed drug release. Due to the prolonged circulation in blood stream and more efficient accumulation in tumor sites, nanomedicines could enlarge therapeutic window and decrease immune-related side effects caused by drug distribution in normal tissues.

To maximize the tumor immunogenicity, it is of great importance to deliver as much immunogenicity inducers to tumor regions or immune cells as possible. Nanomedicines play a significant role in the delivery process, because immunogenicity inducers, such as tumor cell debris, DNA, mRNA, hydrophobic drugs, photosensitizers and sonosensitizers, cannot be transported to tumor or immune cells efficiently owing to various in vivo obstructions (poor solubility of hydrophobic agents, enzymatic degradation of biomolecules and lack of accumulation in target sites). All these obstacles could be overcome by nanomedicines to some extent, realizing the efficient delivery, accumulation and even on-demand release of one or multiple different kinds of therapeutic agents in tumor regions, and greatly increasing tumor immunogenicity and promoting therapeutic efficacy. More importantly, nanomedicines could not only target tumor regions, but also enable immune cell/tissue targeting owing to their

efficient uptake by immune cells like macrophages, DCs and monocytes[102], providing more opportunities for cancer treatment. Therefore, it is highly reasonable to boost immunogenicity and subsequent immune response with nanomedicines.

3.2 Arsenal of nanomedicines to improve immunogenicity

To overcome the low tumor immunogenicity that restrict the efficacy of immunotherapy, numerous nanomedicine-mediated therapies have been applied to potentiate immunotherapy which in return bring about memory effect and distal effect to fight against recurrence and metastasis. It has been confirmed that PDT and chemotherapies that increase immunogenicity through inducing ICD could only elicit acute immune responses, and the concentration of biomarkers would decrease to normal level several days after treatment[103, 104]. Moreover, monotherapy tends to cause resistance of tumors and accelerate immunosuppressive microenvironment, inhibiting immune responses and tumor remission. Therefore, it is necessary to introduce other strategies, especially immunotherapy, to cooperate with the enhanced immunogenicity, facilitating the ablation of tumors and inhibiting metastasis and recurrence. In view of the importance of improving the tumor immunogenicity, several classical nanomedicine-mediated therapies have been reviewed to potentiate immunotherapy.

3.2.1 Cancer nanovaccines to improve immunogenicity

Immunogenicity elicited by vaccines depends on two key factors: antigenicity and adjuvanticity. To design a competent cancer vaccine, it is of great significance to develop rational formulations of immunogenic antigens and adjuvants to invoke robust immune responses. However, the soluble formulation may result in chaotic distribution in the body, restricting vaccine immunogenicity against tumor. A variety of studies have confirmed that neoantigens delivered by nanoplatforms are able to promote antigen cross-presentation and cytotoxic T lymphocytes (CTLs) activation to induce stronger immune responses compared to free neoantigens. Additionally, nanoplatforms could realize the codelivery of immunogenic neoantigens and adjuvants to enhance immune responds potency and reduce side effects by facilitating retention and prolong vaccine activity in draining lymph nodes[105], avoiding repeat local injection which is invasive and tends to cause antigen tolerability[106].

Proteins, peptides, DNA, mRNA and tumor cell derived moieties are commonly considered to be useful antigens to induce immune responses, while the deficient immunogenicity limits their clinical applications[107]. Therefore, it is necessary to introduce some immunological adjuvants, including Toll-like receptors agonists[108] , hydrogels[109] and engineered proteins[110] et. al. Chen and coworkers[111] developed a simple vaccine where polyethylenimine was used for the co-delivery of antigen OVA

and the adjuvant unmethylated cytosine-phosphate-guanine (CpG) (**Figure. 3A**). PEI could enhance nanovaccine uptake in DCs, leading to efficient DC maturation and antitumor immunity. Meanwhile, hyaluronidase was introduced to facilitate the permeability of tumor tissue via breaking down the tumor extracellular matrix. In some cases, two adjuvants were utilized to further improve immunogenicity and vaccine efficacy. Lim and co-workers[112] synthesized multifaced tumosomes (a kind of immunomodulatory nanoliposomes shown in **Figure. 3B**) via the co-assembly of immunogenic tumor cell membrane proteins and two lipid-based adjuvants, acting as TAAs and pathogen characters, respectively. The highly antigenic tumosomes mimicked the key features of biological objects such as shape, size and surface molecular organization, and were able to increase tumor immunogenicity and reshape immune response in lymph nodes, inhibiting tumor growth.

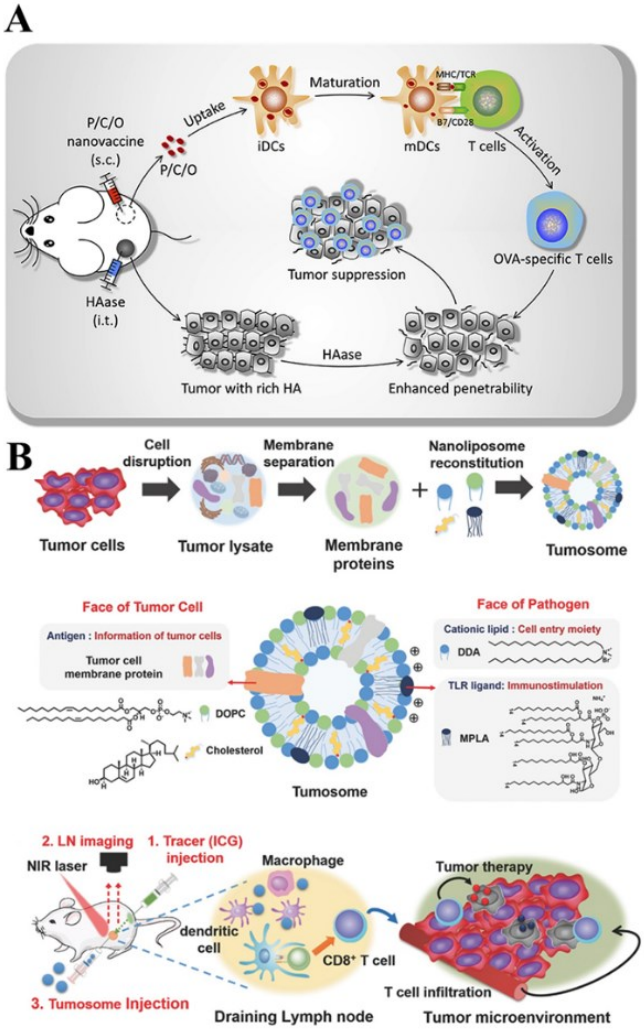

Figure. 3 (A) The schematic diagram of the enhanced cancer immunotherapy by combining nanovaccine with HAase[111]. The co-delivered antigen OVA and the adjuvant unmethylated cytosine-phosphate-guanine (CpG) could lead to efficient DC maturation and antitumor immunity. Hyaluronidase could facilitate the permeability of

tumor tissue via breaking down the tumor extracellular matrix (B) Schematic diagram of multifaceted immunomodulatory nanoliposomes (tumosomes) containing immunogenic tumor cell membrane proteins and two lipid-based adjuvants to increase tumor immunogenicity and reshape immune response [112].

Vaccines usually interact with immune cells, so nanomedicines that could target to these immune cells may greatly increase the efficacy of vaccines. DCs are significant immune cells that connect innate and adaptive immunity, acting as vaccine targets. DCs display various receptors, such as Fc-gamma receptors (FcγRs) that can bind to Fc domain of IgG and subsequently induce antigen uptake and antigen presentation[113]. In view of this, Lim and co-workers[114] prepared highly immunogenic antigen-antibody immune complexes (ICs) mimicking vaccine nanoparticles (NPs), where ICs were able to combine and cross-link FcγRs via Fc portion of antibodies[115]. These vaccine NPs could target DCs and prompt DCs migration to draining lymph nodes (**Figure. 4A**). In this kind of vaccine NPs, termed PLGA(IC/CpG) NPs, PLGA core containing adjuvant CpG oligodeoxynuleotides was coated with OVA proteins (as model antigen) to modulate DCs, and OVA antibodies were then introduced to form OVA–OVA antibody ICs to realize DCs targeting. DCs treated with PLGA(IC/CpG) were then injected to mice to promote migration to lymph nodes as well as T cell priming for the increased immunogenicity and antitumor immunity. With FcγRs-mediated antigen uptake and CpG-induced immunostimulation, the secretion of IL-6 (7.29-fold), IL-12 (11-fold) and TNF-α (12.3-fold) are dramatically enhanced in DCs as well as homing capability and cross-presentation. Besides, DCs could recognize pathogen-associated molecular patterns (PAMPs) through the involvement of pattern recognition receptors (PRRs). PAMP-PRR recognition is one of the most important host defense mechanisms[116]. Polysaccharides on microbial cell walls can be recognized via PRRs (for example, TLRs and mannose receptors) on DCs, eliciting potent immune stimulation[117]. Inspired by microbe, Moon and co-workers[43] constructed hollow sugar-capsules coated with mannanose or dextran polysaccharide for engaging DCs (**Figure. 4B**), and studied their immunogenicity and potential as a delivery platform for mRNA-based vaccines. PEI was coated and cross-linked on carboxylated silica nanoparticles, serving as the backbone for sugar-capsules. mRNA was then loaded efficiently through 1-3 cycles of layer-by-layer assembly of mRNA and PEI, endowing the particles with high immunogenicity. Subsequently, polysaccharide-CHO was introduced to form the external flexible polysaccharide layer by amine−aldehyde reaction. The immunogenic hollow sugar-capsules were finally obtained by the removal of silica templates. The combination of flexibility and PAMP-PRR recognition can not only prompt targeting to lymph nodes, but also exhibit inherent immunostimulatory properties, eliciting immunogenicity for robust T cell responses.

Moreover, macrophages are another kind of immune cells that play an important role in immune system. Vaccines with macrophages targeting abilities would further increase the efficacy of tumor inhibition. In mice bearing metastatic tumors, CD8 T cells could be activated for the specific ablation of M2 macrophages via the fabricated legumain-based DNA vaccine, leading to the blockages of angiogenesis and metastasis[118]. Shiku and co-workers[119] fabricated cholesteryl pullulan (CHP) which could self-assemble in water to form cross-linked nanogels with diameter of ~ 50 nm. Due to the small size and uncharged surface, CHP nanogel could travel to draining lymph node and then reach medulla where it is vastly engulfed by macrophages. With the presence of TLR agonist, these macrophages could efficiently cross-prime the vaccine specific T cells.

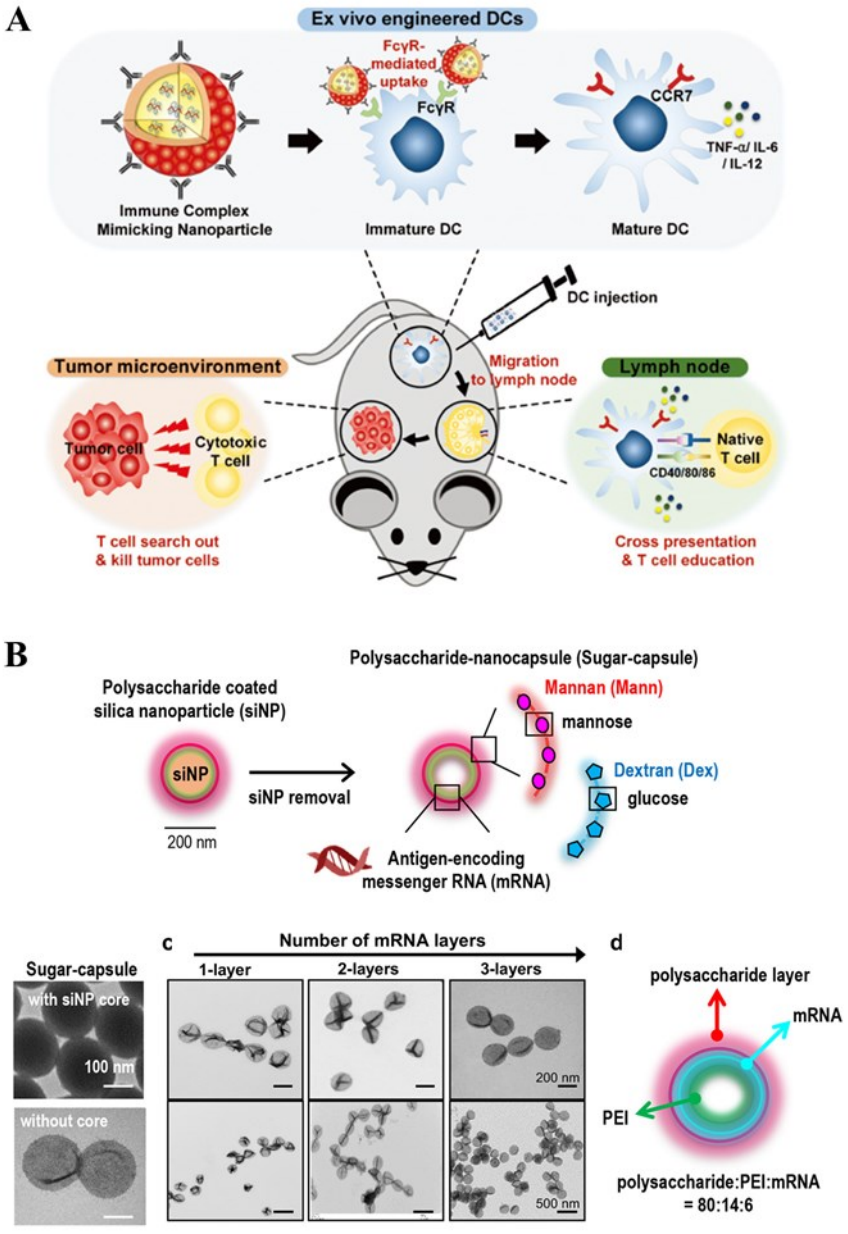

Figure. 4 (A) Illustration of an ex vivo engineered DCs-based cancer immunotherapeutic strategy. PLGA(IC/CpG) could target DCs and lead to DC maturation. The matured DCs were then injected to mice to promote migration to lymph nodes as well as T cell priming[114]. (B) Schematic illustration of synthesis of mRNA-loaded sugar-capsules. TEM images of sugar-capsules before (top) and after (bottom) removal of a core silica nanoparticle; TEM images of sugar-capsules with multilayered mRNA loading at high (top) and low (bottom) magnification; Illustration of an mRNA-sugar-capsules with the weight ratio of components[43].

However, tumors cells tend to escape from immune attacks through various immune evasion mechanisms, and one of the most important mechanisms is immunosuppressive microenvironment (e.g., acidity, high concentration of ROS, hypoxia, infiltration of tumor-associated macrophages and overexpression of a variety of immune checkpoints). Presentation of antigens alone is unable to overcome immunosuppressive tumor microenvironment which plays a negative role in immunotherapy. Reversal of immunosuppressive microenvironment provides new opportunities for immunotherapy. To realize tumor remission more efficiently, efforts have shifted to leveraging multiple modalities with rational design[120]. Therefore, it is necessary to combine cancer vaccines that increase immunogenicity with those strategies, such as immune checkpoint blockade and re-education of immune-suppressive macrophages, to further promote tumor remission and inhibit metastasis and relapse. Zhang and co-workers[121] encapsulated adjuvant CpG into biodegradable PLGA nanoparticles by double emulsion procedure, which were then coated with immunogenic membrane derived from melanoma cells. The nanovaccine (CpG-CCNPs) enabled the delivery of a variety of autologous antigens, inducing immunogenicity and multiantigenic immune responses (**Figure. 5A**). Encapsulated in the membrane coated vaccine, CpG adjuvant was much more readily to be internalized by bone marrow-derived DCs, leading to increased secretion of representative proinflammatory cytokines. Additionally, the immunogenic vaccine formulation could elicit DCs maturation and antigen-specific immune responses when administered in vivo, and prevent recurrence in prophylactic study. With the increased immunogenicity, ICB (CTLA4 and PD1 antibodies) could inhibit tumor growth more efficiently and half of tumors were still below the experimental endpoint threshold on day 48 postchallenge. Cruz and co-workers[122] utilized biodegradable PLGA nanoparticles to deliver adjuvants pIC, R848 and MIP3α, individually or in combinations, along with long peptide antigens for the increase of immunogenicity and the activation of immune responses. The adjuvant effects were related to myeloid population alterations and lymphocytes. Besides, the tumor-associated macrophages (M2 phenotype) were also re-educated to tumor-suppressive ones (M1 phenotype), providing a more favorable microenvironment for immune responses and finally promoting tumor eradication.

Zhang and co-workers[123] recently synthesized a type of immunogenic vaccine (Fe_3O_4/T-MPs-CpG/Lipo) for the delivery of tumor-derived antigenic microparticles (T-MPs) to induce abundant cytotoxic T lymphocytes infiltration and transform "cold tumor" into immunogenic "hot tumor" (**Figure. 5B**). Meanwhile, the released nano-Fe_3O_4 could reverse M2 phenotype macrophages to M1 phenotype ones, facilitating host immune responses. The subsequent combination with PD-L1 blockade further accelerated immune responses, inhibiting tumor progression (~83%) and extending average survival time to 3 months. In comparison, free anti-PD-L1 antibody was much less efficient in tumor inhibition, indicating the great necessity of pretreatment with the vaccine to improve immunogenicity.

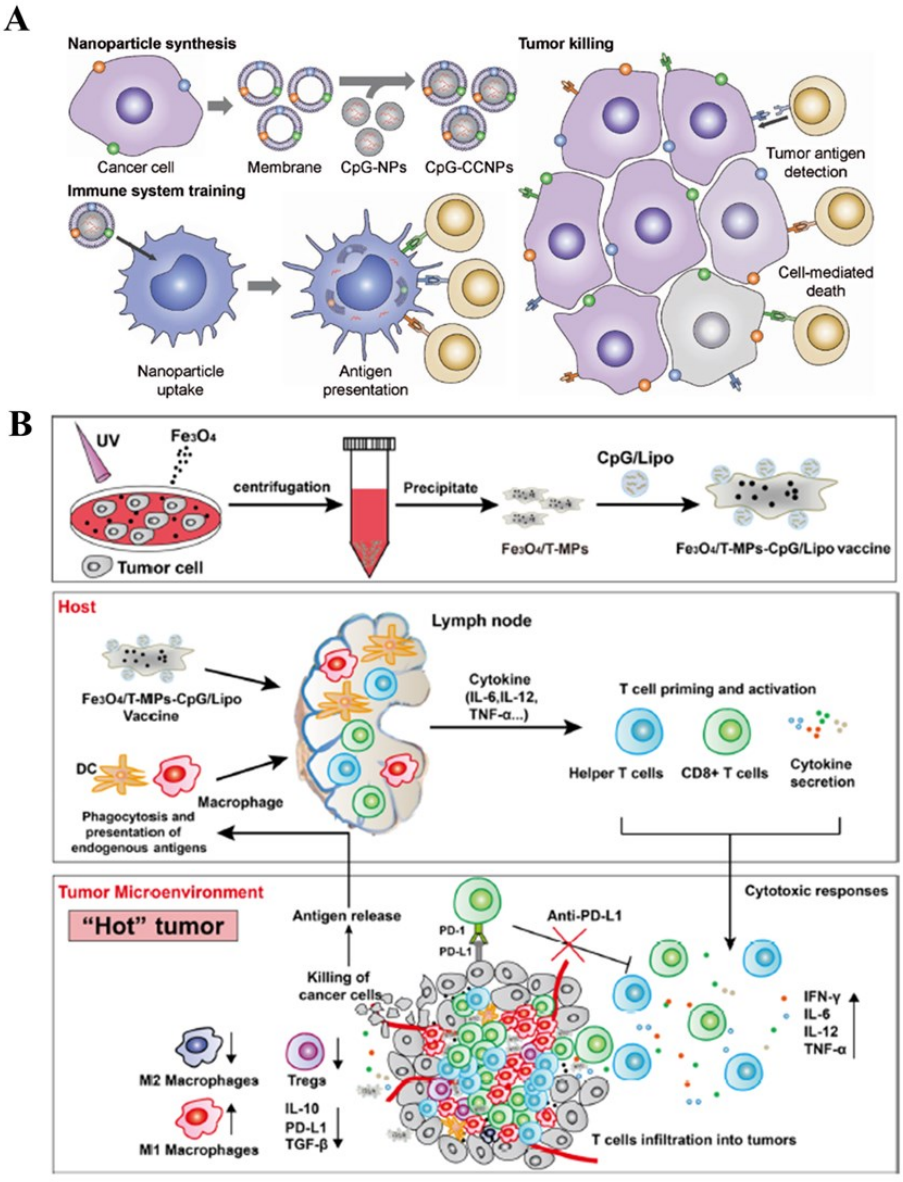

Figure. 5 (A) Schematic of CpG-CCNPs for anticancer vaccination. Membrane derived from cancer cells (purple), along with the associated tumor antigens. Adjuvant CpG in the core would further improve the efficacy of vaccine[121]. (B) Nano-Fe_3O_4-carried tumor-derived antigenic microparticles with surface decoration of CpG-loaded liposomes to yield an anticancer vaccine (Fe_3O_4/T-MPs-CpG/Lipo), promoting APC (including DCs and macrophages) maturation, activating tumor-specific T cells, increasing pro-inflammatory cytokines production, and remodeling the tumor microenvironment to boost antitumor responses to immunotherapy[123].

3.2.2 Chemotherapeutic nanomedicines to improve immunogenicity

Systematic distribution of therapeutic drugs could bring about severe side effects and trigger tumor resistance. Nanotechnology is an efficient and facile strategy to deal with these challenges. With efficient toxic agents delivered to tumor regions by nanomedicines, chemotherapy-induced ICD could be amplified, increasing immunogenicity more efficiently and evoking stronger immune responses. In addition, some in-depth innovations have been made in the development of nanomedicines for chemotherapy, for instance, (1) co-delivering multiple therapeutics to avoid drug resistance; (2) introducing targeting and responsive moieties for improved tumor accumulation, cellular uptake and controlled drug release; (3) designing nanoplatforms with both therapeutic and diagnostic functions to monitor pharmacokinetics and accumulation of drugs as well as tumor progression, offering vital insights in heterogeneity of tumors. Despite the robust and beneficial immune responses achieved by ICD-inducing chemotherapy, initial immune responses are always accompanied by tumor growth[124]. This could be ascribed to the secretion of immunosuppressive cytokines, upregulation of immune check points and recruitment of tumor-associated T cells and macrophages, which impair tumor remission. Thus, it is reasonable to apply immunotherapies to combat tumor immune evasion mechanisms following the increase of immunogenicity. On one hand, immunotherapy is able to compensate the shortcomings of chemotherapy; on the other hand, the increased immunogenicity induced by chemotherapeutic agents could in return make tumors more susceptible to immunotherapy. So far combination of chemotherapy and immunotherapy has become an attractive approach in clinic.

Residual tumor cells tend to adaptively overexpress PD-L1 to interact with PD-1 on T cells, escaping immune surveillance after chemotherapy cessation[125]. To overcome this immune evasion mechanism, PD-L1 blockade therapies were applied to inhibit tumor growth durably by unleashing the function of tumor-infiltrating T cells. However, PD-L1 blockade couldn't work on immune-deserted tumors due to the lack of immunogenicity. Given that certain chemotherapies are able to induce ICD and make a more immunogenic microenvironment, it is highly reasonable to combine ICD-

inducing chemotherapy with PD-1/PD-L1 blockade for synergistic tumor inhibition Wang and co-workers[126] reported an injectable fibrin hydrogel for the local delivery of cyclophosphamide (CTX) and PD-L1 antibody to increase immunogenicity and implement ICB, respectively (**Figure. 6A**). In situ hydrogel loaded with CTX and PD-L1 antibody was formed from fibrinogen via thrombin-triggered polymerization. Firstly, CTX would spread out and induce immunogenic tumor cell death, increasing the tumor immunogenicity by downregulating the levels of $CD4^+CD25^+$ T_{reg} and promoting lymphocytic infiltration. PD-L1 antibodies would be released after CTX owning to their greater molecular weight. More importantly, the efficacy of PD-L1 blockade would be maximized by the ICD induced immunogenic microenvironment where a large amount of T cells could be activated to fight against tumor cells. In mice models, the hydrogel formulation exhibited promising inhibition of postsurgery recurrence and metastasis due to the activation of systemic immune and memory T cells. Yang and co-workers[127] synthesized backbone-degradable polymer-epirubicin complex to induce ICD and increase immunogenicity, followed by the treatment with multivalent polymer-peptide based PD-L1 antagonist which can overcome adaptive PD-L1 enrichment after chemotherapy (**Figure. 6B**). The PD-L1 antagonist could bias the recycling of PD-L1 to lysosome degradation through surface receptor crosslinking rather than transient PD-L1 blockade, leading to the prolonged elimination of immune check points. The increased immunogenicity induced by chemotherapy coordinated with PD-L1 degradation could greatly propagate durable antitumor immunity. Indoleamine 2,3-dioxygenase (IDO) is another typically checkpoint that overexpressed in a variety of tumor cells and tumor-draining lymph nodes after chemotherapy. It can catalyze the metabolism of amino acid $_L$-tryptophan to $_L$-kynurenine to inhibit the clonal expansion of T cells and promote T cell apoptosis, resulting in the inactivation of immune-support T_{eff} and the proliferation of immune-suppressive T_{reg}[128]. Since IDO could deplete immune-support T cells infiltrated in tumor, it is reasonable to combine IDO inhibition with ICD-inducing chemotherapy to accelerate the accumulation of T_{eff} and the depletion of T_{reg}. Wang and co-workers[129] combined chemotherapy with small interfering RNA targeting IDO (siIDO1) to amplify the outcome of ICD (**Figure. 6C**). Cationic lipid-assisted nanoparticles (CLANs) were applied for contemporaneous delivery of oxaliplatin (OXA) and siIDO1 to promote DCs maturation, tumor-infiltrating T lymphocytes recruitment and T_{reg} depletion. The nanomedicine could not only eradicate orthotopic pancreatic tumors, but also offer a robust immunological memory effect, protecting patients from tumor rechallenge. In contrast, siIDO1 alone exhibited much lower efficacy than CLANs loaded with both OXA and siIDO1 due to the insufficient infiltration of T cells, demonstrating the significant role of immunogenicity in immunotherapy.

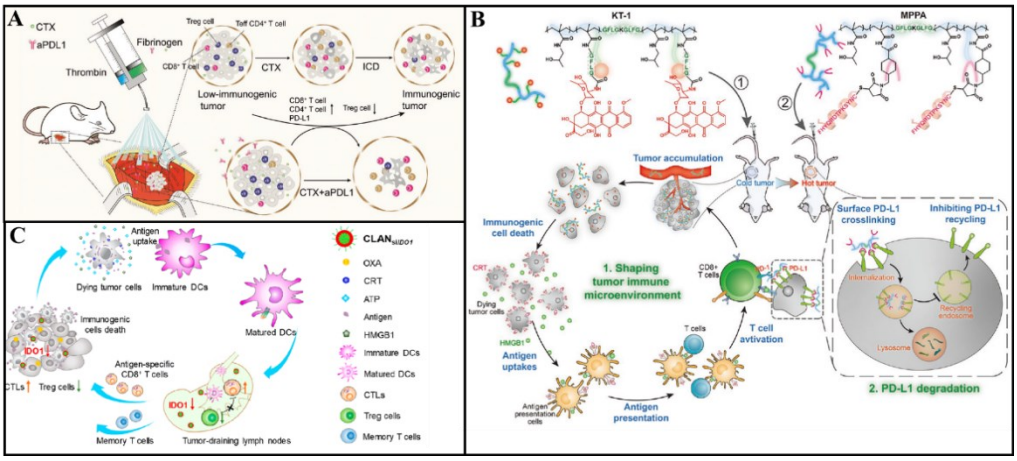

Figure. 6 (A) Schematic of combination chemoimmunotherapy using a fibrin scaffold to deliver CTX and aPDL1 into the resection site. CTX could enhance tumor immunogenicity and maximize the efficacy of PDL1 blockade[126]. (B) Schematic illustration of polymer-enhanced combination of immunogenic chemotherapy and PD-L1 degradation. The backbone-degradable polymer-epirubicin complex induces ICD and the multivalent polymer-peptide based PD-L1 antagonist overcomes adaptive PD-L1 enrichment after chemotherapy [127]. (C) $CLAN_{siIDO1}$-mediated IDO1 inhibition in tumor-draining lymph nodes (TDLNs) and tumor tissues synergizes with immunogenic chemotherapy. Cationic lipid-assisted nanoparticles (CLANs) were applied for contemporaneous delivery of oxaliplatin (OXA) and siIDO1 to promote DCs maturation, tumor-infiltrating T lymphocytes recruitment and T_{reg} depletion[129].

In addition to integrating different therapeutics into one formulation, designing nanoplatforms with specific functions, for example, tumor microenvironment responsive and targeting ability, is another way to efficiently induce immunogenicity through the enhanced accumulation in tumor regions, priory uptake by tumor cells and programmed drug release. Tumor tissues are distinct from normal tissues from moderate acidity, high levels of ROS, GSH and MMP, which enables the innovation of smart nanoplatforms to recognize and respond to these features for enhanced accumulation. Zhang and co-workers[130] coated pH responsive chitosan-based nanogel (**Figure. 7A**) with red blood cell membrane (RBCm) for the codelivery of PTX (loaded in nanogel with the help of HP-β-CD) and IL-2 (loaded on RBCm) to tumors (**Figure. 7B**). Triggered by the moderate acidic microenvironment, the nanogel would swell quickly to release PTX, maximizing the accumulation of ICD inducers at tumor sites to induce amplified tumor immunogenicity. Once losing the support of nanogel core, RBCm would be disintegrated, facilitating the release of IL-2 for the activation of CTLs and NK cells to synergize with the increased immunogenicity. Compared with individual therapeutic drug, two cytotoxic drugs would be more efficient in enhancing immunogenicity, because the combination drugs might minimize the resistant selection

for cancer cell clones. Moreover, when combined with immunotherapy, different drugs could stimulate distinct anti-tumor immune populations to accelerate antitumor process[131]. Thomas and co-workers[132] reported a redox and esterase responsive nanoparticle (pPTX/pCD-pSNO) from the co-assembly of polymerized paclitaxel (pPTX) and polymerized β-cyclodextrin with nitric oxide incorporation (pCD-pSNO). After cellular uptake, the nanoparticles would respond to intracellular chemical environment, leading to the accurate release of PTX at tumor sites and in situ formation of NO. PTX-induced immunogenicity could be amplified by the responsive feature of nanoparticles and NO which has chemosensitizing effects through preventing drug efflux[133, 134]. The further combination of increased immunogenicity with CTLA-4 blockade allows pPTX/pCD-pSNO to elicit robust anti-tumor effects and prolong animal survival. As is known to all, prolonged circulation in blood and efficient cellular uptake are both significant in antitumor therapies. Negative charged nanomedicines show superiority in blood circulation while are not suitable for cellular uptake due to the repulsion between nanomedicines and negatively charged cell membrane. The dilemma also exits in particle size. Nanomedicines with size around 100 nm are beneficial to prolonged circulation but can barely penetrate to deep parts of tumors. Given all these, it is necessary to design charge reversal and/or size switchable nanomedicines to overcome the dilemma to target tumor cells. Yang and co-workers[135] recently developed a type of dual responsive nanomedicine with size shrinkage and charge reversal functions to promote penetration and endocytosis for more efficient accumulation of chemotherapeutic agents and IDO inhibitors at tumor sites, greatly improving tumor immunogenicity and the efficacy of ICB (**Figure. 7C**). When nanodrug was delivered to tumor regions, the weak acidity would trigger the cleavage of pH-responsive bond, leading to the removal of PEG shell and charge reversal from negative to positive due to the exposure of PEI, which would synergistically accelerate cellular uptake. Meanwhile, the PEG removal would lead to a smaller particle size, enhancing tumor penetration. After endocytosis, the nanomedicine would respond to enriched GSH in cytoplasm and disassemble to release chemotherapeutic drugs and IDO inhibitor. Both the prompted penetration and endocytosis of nanomedicine would improve tumor immunogenicity owing to the efficient accumulation of chemotherapeutic drugs in tumor regions, resulting in potent antitumor immune responses when synergized with IDO blockade.

Despite the advances in chemoimmunotherapy, therapeutic schemes are frequently impeded ascribed to the lack of connection between pharmacokinetics and pharmacodynamics of drugs in vivo, hindering the comprehensive understanding of immunogenicity. Therefore, it is of great significance to develop a visible strategy to monitor and further tailor the behaviors of therapeutic drugs. Wang and co-workers[136] loaded stromal-cell-derived factor-1α (SDF-1α) and DOX on Ag_2Se quantum dots (QDs) with the help of heparin and mPEG-DSPE for the monitoring of drugs in vivo (**Figure. 7D**). Mediated by the chemotaxis of SDF-1α, Ag_2Se QDs tended to

accumulate in tumor regions and release DOX when stimulated by moderate acidity in tumor microenvironment, eliciting ICD and immunogenicity. NK cells labeled with Ag_2S QDs were subsequently intravenously injected for immunotherapy. The dual-tunnel near-infrared (NIR) II fluorescence imaging enabled simultaneous monitoring of each injection behaviors in vivo and further optimization of administration regimens, in return facilitating tumor inhibition.

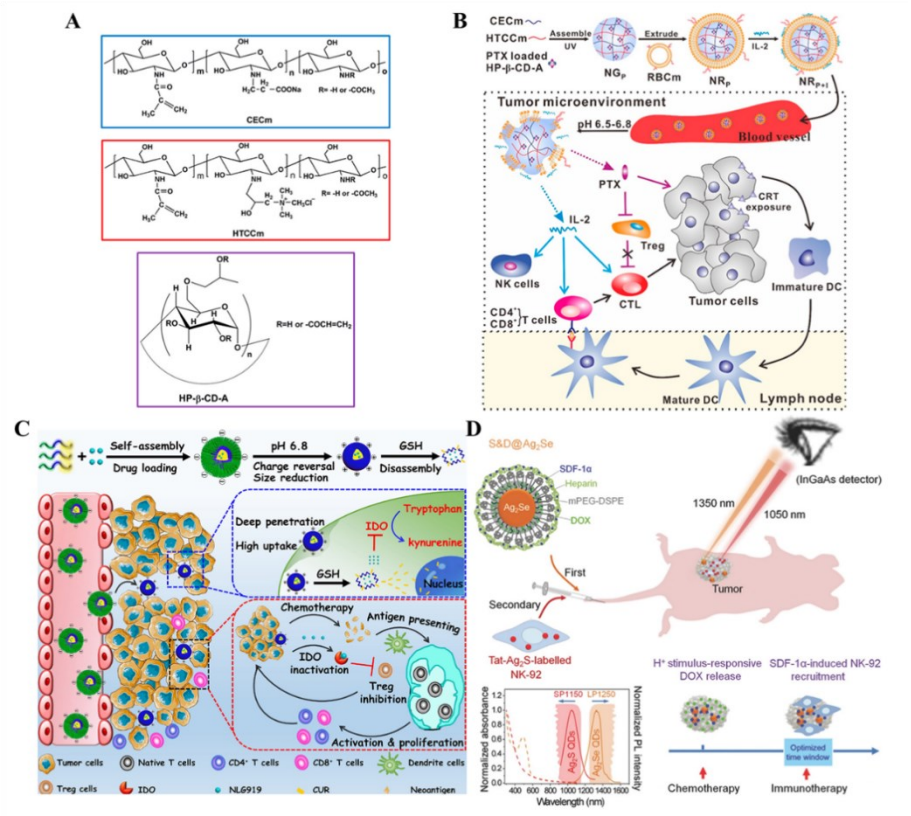

Figure. 7 (A) Preparation of pH responsive chitosan-based nanogel coated with red blood cell membrane and (B) schematic illustration of chemo-immunotherapy. Triggered by the moderate acidity in TME, the nanogel would swell quickly to release PTX, inducing amplified tumor immunogenicity. Then, RBCm would be disintegrated, facilitating the release of IL-2 for the activation of CTLs and NK cells[130]. (C) Illustration of size-shrinkable and charge-reversal system for tumor chemo-immunotherapy in vivo. The weak acidity would trigger the cleavage of pH-responsive bond, leading to the removal of PEG shell and charge reversal from negative to positive. PEG removal would lead to a smaller particle size, enhancing tumor penetration. After endocytosis, the nanomedicine would respond to enriched GSH in cytoplasm and disassemble to release chemotherapeutic drugs and IDO inhibitor[135]. (D) Ag2S QDs and Ag_2S QDs labeled NKs were used for multiplexed NIR-II fluorescence imaging and programming the chemotherapy and immunotherapy[136].

3.2.3 Radiotherapeutic nanomedicines to improve immunogenicity

Ionizing radiation could produce intracellular ROS to interact with DNA, resulting in the generation of toxic adducts and single/double-strand breaks. DNA damages induced by radiation could further inhibit cell cycle and lead to necrosis, apoptosis and autophagy of tumor cells, eliciting immunogenicity and stimulating diversification of T cell repertoire[120, 137]. Though tumor cells are more vulnerable to radiotherapy owing to the defects in DNA repair mechanism, it is inevitable that nonmalignant tissues around tumor would also undergo these DNA damages, resulting in mucositis and pneumonitis in the short term while collagen deposition, neoangiogenesis, contracture and second malignancies in the long term. Thus, it is necessary to apply strategies, such as delivering radiosensitizers by nanomedicine to tumor cells and relieving hypoxia which hinders the generation of ROS, to make tumors more sensitive to radiation and induce higher immunogenicity, avoiding the usage of high dose of radiation. In addition, low dose of radiation can not only decrease severe side effects, but also promote vascular normalization, facilitating tumor exclusion[138]. And nanoplatforms play a significant role in the relief of hypoxia and specific delivery of these radiosensitizers to tumor tissues to induce immunogenicity and subsequent stimulation of tumor-directed immune responses both inside and outside irradiation sites. However, antitumor immunity elicited by individual radiation is rarely sufficient to achieve systemic tumor inhibition due to a variety of immune evasion mechanisms, for example, overexpression of immune checkpoints and infiltration of immunosuppressive macrophages. Despite all these challenges, the combination of radiotherapy with immunotherapy for increased immunogenicity and reversal of immune evasion, respectively, has attracted increasing interest and has made progression in clinic, offering more opportunities for cancer treatment.

As a commonly observed pathophysiological feature, hypoxia is the result of imbalance between oxygen consumption and oxygen supply within tumors that have poor blood flow and aberrant new blood vessels. Hypoxia would impede antitumor efficacy and lead to tumor resistance to a variety of therapies[139-141]. Meanwhile, oxygen plays an important role in inhibiting the repreparation of DNA damages induced by radiotherapy[142]. Thus, it is highly reasonable to promote oxygenation status inside tumors to further enhance immunogenicity and the efficacy of radiotherapy. Taking the high level of hydrogen peroxide (H_2O_2) in tumor microenvironment into consideration, it is a good alternative to decompose endogenous H_2O_2 for the stable generation of oxygen in situ. Various catalysts have been used for the decomposition of H_2O_2 including MnO_2 nanostructures and catalase enzyme. In addition, immunosuppressive microenvironment is one of the major reasons that attenuate immune responses elicited by the increase of tumor immunogenicity. Thus, further reversal of immunesuppressive microenvironment would be of great importance when combined with radiotherapy. Liu and co-workers[143] developed catalase (CAT) loaded liposomes (CAT@liposome) to

trigger oxygen generation from H_2O_2 to improve antitumor efficacy in combination with increased immunogenicity induced by radiotherapy (**Figure. 8A**). To relieve hypoxia more efficiently, exogenous H_2O_2 was also delivered to tumor regions 4 h after CAT@liposome injection. The subsequent release of H_2O_2 from H_2O_2@liposome would improve long lasting oxygen generation with the help of CAT@liposome to remarkably enhance tumor immunogenicity elicited by radiotherapy and reverse immune-suppressive M2-type macrophages into immune-support M1-type ones that are in favor of antitumor immunities. Compared with free anti-CTLA-4 antibodies, CTLA-4 checkpoint blockade showed better outcomes following the radiation and hypoxia relief due to the prompted CTLs infiltration. These results demonstrated the significance of tumor immunogenicity which could be induced by radiation and amplified by hypoxia relief. The authors later fabricated core-shell PLGA nanoparticles with water-soluble CAT in the core and hydrophobic Toll-like-receptor-7 agonist (R837) in the shell (**Figure. 8B**)[144]. During radiation, the synthesized PLGA-R837@CAT nanoparticles could not only relieve hypoxia by CAT-triggered H_2O_2 decomposition to amplify radiation-led immunogenicity, but also strengthen antitumor immune responses by modulating immunosuppressive microenvironment with the help of R837 adjuvant. In combination with CTLA-4 blockade subsequently, the synergistic strategy efficiently inhibited tumor growth and metastases via stimulation of robust immune responses and abscopal effect, as well as offered immunological memory effect to fight against tumor relapse.

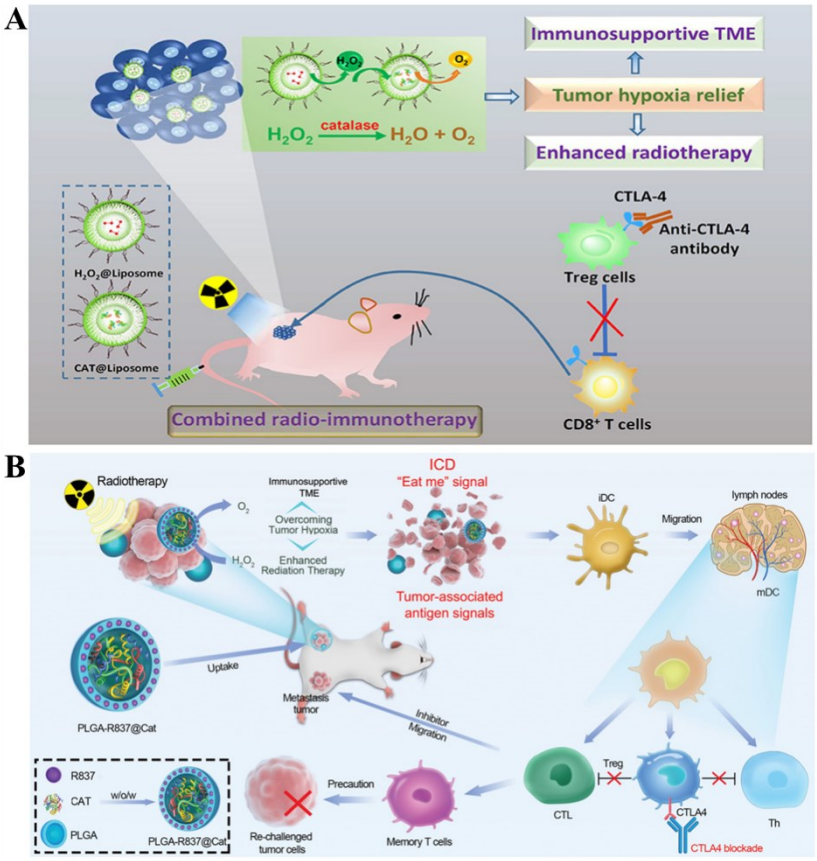

Figure. 8 (A) A schematic diagram showing the liposome compositions and oxygen generation process, which synergized with radiotherapy to relieve hypoxia. CTLA-4 blockade would further improve the therapeutic efficacy[143]. (B) The schematic illustration for mechanism of antitumor immune responses induced by PLGA-R837@Cat-based radiotherapy in combination with checkpoint-blockade to inhibit cancer metastases and recurrence[144].

Given that high dose of radiation might cause severe side effects and low dose radiation is able to promote vascular normalization and facilitate tumor exclusion, several strategies have been developed to minimize radiation dose while maintain the efficacy of radiotherapy at the same time. It has been highlighted that the application of materials containing high-Z metal are able to enhance radiotherapeutic outcomes due to their excellent radiation energy absorption and conversion capabilities[145], avoiding the usage of high dose radiation. Lin and co-workers[146] synthesized a type of nanoscale metal-organic framework (nMOF) containing hafnium (Hf, a kind of X-ray scintillator) and photosensitizers, with an IDO inhibitor loaded into the pores (**Figure. 9A**). The resulting nMOF complex can produce •OH radicals and excite photosensitizers to generate $^1O_2$ during low dose radiation (the latter is known as radiodynamic therapy). Meanwhile, the integration of Hf allowed more absorption of radiation into tumors,

eliciting stronger immunogenicity to invoke T cell immune responses. The incorporation of IDO inhibitor could further enhance immune responses in breast and colorectal cancer models, rejecting both irradiated and non-irradiated distal tumors. To further improve radiation sensitivity of tumor cells and magnify •OH formation during radiation, Bu and co-workers[46] incorporated Fe^{3+} into Hf-containing nMOFs to induce in situ Fenton reaction, leading to prolonged ROS stress in tumor cells and highly increased tumor immunogenicity during radiotherapy (**Figure. 9B**). The elevated ROS levels could promote G2/M phase in cell cycle to make cancer cells more radiosensitive to X-ray and meanwhile inhibit DNA damage reparation induced by radiation, enhancing immunogenicity and inducing potent immune responses. Additionally, electrons generated after radiation could accelerate the reduction of Fe^{3+}, and the resulting Fe^{2+} would further increase •OH formation in Fenton process, amplifying immunogenicity and antitumor efficacy.

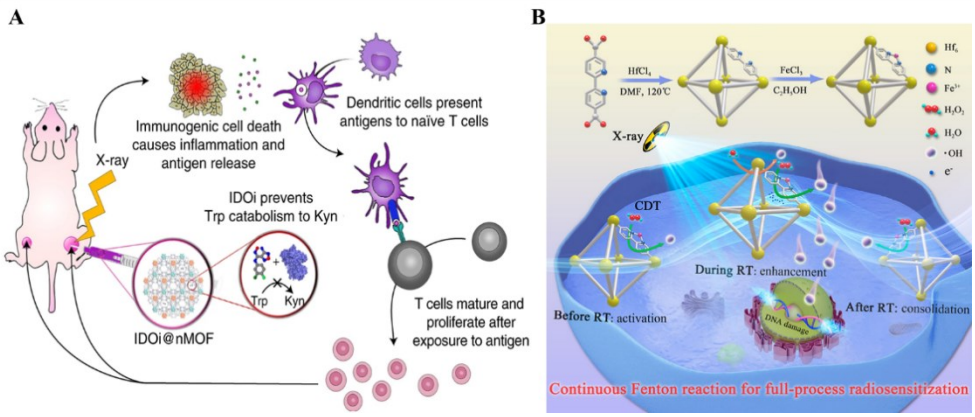

Figure. 9 (A) nMOFs enable synergistic radiotherapy–radiodynamic therapy and immunotherapy using extremely low dose of X-rays. The integration of Hf allowed more absorption of radiation into tumors, eliciting stronger immunogenicity to invoke T cell immune responses. The incorporation of IDO inhibitor prevented Trp catabolism to Kyn, further facilitating immune responses[146]. (B) Schematic view of nMOFs synthesis and full-process radiosensitization. The electrons generated after radiation could accelerate the reduction of Fe^{3+}, and the resulting Fe^{2+} would further increase •OH formation in Fenton process, amplifying immunogenicity and antitumor efficacy[46].

3.2.4 Photo-activated therapeutic nanomedicines to improve immunogenicity

With the presence of photosensitizers, PDT could generate cytotoxic ROS (type I PDT: singlet oxygen; type II PDT: superoxide anion radicals and hydrogen peroxide and hydroxyl) under irradiation[147], inducing ICD and increasing tumor immunogenicity to evoke immune responses. Photosensitizers usually contain

hydrophobic aromatic repeating units, which could cause loss of photoactivity due to their undesired physicochemical properties such as poor solubility and aggregation in biological media[148]. Nanoplatforms are usually applied for the efficient and in some cases targeted delivery of these photosensitizers to tumor regions for improved accumulation and minimized side effects resulted from systematic distribution, inducing strong tumor immunogenicity to activate immune responses. However, hypoxia in tumor microenvironment and the short lifetime of ROS and would impair the generation and accumulation of ROS, respectively. Therefore, it is reasonable to relieve hypoxia and generate ROS in situ at endoplasmic reticulum (ER) where calreticulin exposure occurs to dictate tumor immunogenicity and further improve immunogenicity. In addition, tumors tend to escape immune attacks via a variety of immune evasion mechanisms, thus, it is a good choice to synergize PDT with immunotherapy to fight against immune escape. Moreover, the increased immunogenicity induced by PDT could in return make tumor cells more susceptible to immunotherapy, improving the antitumor efficacy of combined therapy.

As is reported, CRT locates in endoplasmic reticulum (ER) which plays a significant role in protein synthesis and processing, calcium homeostasis, as well as maintaining intracellular signal transduction. ER stress could activate intracellular signaling pathways to mediate ICD and immunogenicity[149]. Despite the high toxicity to tumor cells, ROS have very short half-life (10 ~ 320 ns for $^1O_2$) and could only diffuse within 10 ~ 55 nm, resulting in the reduction of ROS accumulation in ER and ER stress. In view of this, Chen and co-workers[150] synthesized reduction-sensitive nanoparticles loaded with photosensitizer that could efficiently target ER (Ds-sP/TCPP-T^{ER} in **Figure. 10A**). Once internalized by tumor cells, Ds-sP/TCPP-T^{ER} would respond to escalated GSH and disassemble. The released photosensitizer modified with targeting moiety N-tosylethylenediamine would target and accumulate in ER for the in-situ generation of ROS under NIR laser irradiation, inducing ER stress, amplifying immunogenicity and activating immune cells to realize the augmented immunotherapy effect. Tang and co-workers[151] reported two new type 1 photosensitizers with selective accumulation in ER and efficient ROS generation ability. The ROS based ER stress also has high potential as a precursor of the immunostimulatory effect for immunotherapy. Wong and co-workers[152] synthesized a rhodamine-decorated iridium(III) complex via variating cyclometallating ligand to enhance ROS generation capacity and ER localization ability. The complex showed outstanding ROS generation efficacy (1.6-fold higher than that of common photosensitizer) and highly specific ER targeting ability, resulting in disruption of ER function and remarkable tumor growth inhibition.

However, the collateral damage to normal cells could lead to the release of self-antigens, inducing immune tolerance or suppression. And oxidative modification of danger signals could also improve immune tolerance and immunosuppressive cytokines release[153]. Thus, it is highly reasonable to combine PDT with immunotherapy for the

reversal of immunosuppressive microenvironment. Lin and co-workers[103] reported a type of biocompatible core-shell nanoparticles (ZnP@pyro) with Zn and ZnP in the core and a lipid-pyropheophorbide conjugate in the shell for efficient PDT (**Figure. 10B**). ZnP@pyro could kill cancer cells under irradiation via inducing tumor cells necrosis and/or apoptosis and destroying tumor vasculature, leading to increased immunogenicity. Meanwhile, the immunogenic PDT based on ZnP@pyro could sensitize tumor cells to PD-L1 antibody blockade, eradicating primary 4T1 breast tumor and at the same time eliciting abscopal effects to prevent metastasis to the lung. Pu and co-workers[154] fabricated a kind of semiconducting pro-nano-stimulant with a photoactivable immunotherapeutic action. The fabricated biomaterial could convert light into ROS for PDT. The released IDO inhibitor could further modulate immune-suppressive tumor microenvironment to potentiate immunotherapy.

Taking advantage of the distinct microenvironment, tumor microenvironment responsive nanomedicines have been developed to further improve specific accumulation of these nano-photosensitizers to tumor cells, which could not only increase immunogenicity and PDT efficacy, but also decrease side effects resulted from reduced distribution in normal tissues. Wang and co-workers[155] synthesized redox-activable porphyrin-phospholipid conjugate and prepared nanoparticles (IND@RAL) through their assembly accompanied with the remote loading of IDO inhibitor in the interior lumen (**Figure. 10C**). The fabricated IND@RAL shut down its fluorescence and photoactivity in blood stream while realized exponential activation of fluorescent signal (over 100 fold) and photoactivity (over 100 fold) in response to GSH after endocytosis by tumor cells. Upon laser irradiation, PDT based on IND@RAL could not only induce ICD through mitochondria dysfunction and cell apoptosis, but also reverse immunosuppressive microenvironment via the simultaneous release of IDO inhibitors from interior lumen. The microenvironment responsive feature accelerated the accumulation of photosensitizers in tumor cells, leading to the efficient increase of immunogenicity and enhanced sensitivity of tumor cells to IDO blockade. In mice models, IND@RAL could efficiently inhibit primary and distal tumors as well as prevent metastasis of 4T1 breast cancer.

To take full advantage of the differences in physiochemical properties between tumor and normal tissues, multiple responsive nanomedicines have been designed for more accurate location and activation of drugs in tumor cells to improve immunogenicity and decrease side effects. Yu and co-workers[156] developed a type of Boolean logic prodrug nanomedicine (BLPNs) for targeted codelivery of prodrugs and immune modulators into tumor cells for the first time (**Figure. 10D**). A variety of stimuli-activatable BLPNs was prepared via adjusting the input responsive combinations including extracellular MMP 2/9, moderate acidity and intracellular GSH. Two polymeric prodrugs of photosensitizer (PPa) and IDO inhibitor (NLG919) assembled into nanomedicines with three or less responsive moieties. Selective and

tunable control over BLPNs disassembly and prodrug activation was studied via illuminating the connectivity of orthogonal stimuli-vulnerable spacers when exploiting the endogenous signals in tumor microenvironment. It has been confirmed that combination group of MMP-2/9-activable E-YES gate, acidity-responsive A-AND gate, and GSH-activable R-AND gate displayed the best antitumor efficacy via synergistically eliciting immunogenicity and overcoming IDO-based immune evasion. In this case, the nanomedicine containing two prodrugs with PEG shell possessed good colloidal stability and could prevent premature drug leakage in blood circulation. Upon reaching tumor regions, the accumulated MMP2/9 would lead to peptide degradation, which resulted in the removal of PEG shell, improving uptake by tumor cell. Meanwhile, the residual nanomedicine would undergo charge reversal owing to the protonation of tertiary amine, further accelerating endocytosis by tumor cells and activating photosensitizers due to the elimination of homo-FRET effect. After laser irradiation, activated photosensitizers would generate ROS to induce ICD, greatly increasing tumor immunogenicity and evoking tumor specific immune responses. At the same time, intracellular GSH would trigger the stimulation of IDO inhibitor prodrug to reverse immunosuppressive microenvironment, further amplifying immune responses with the help of increased immunogenicity.

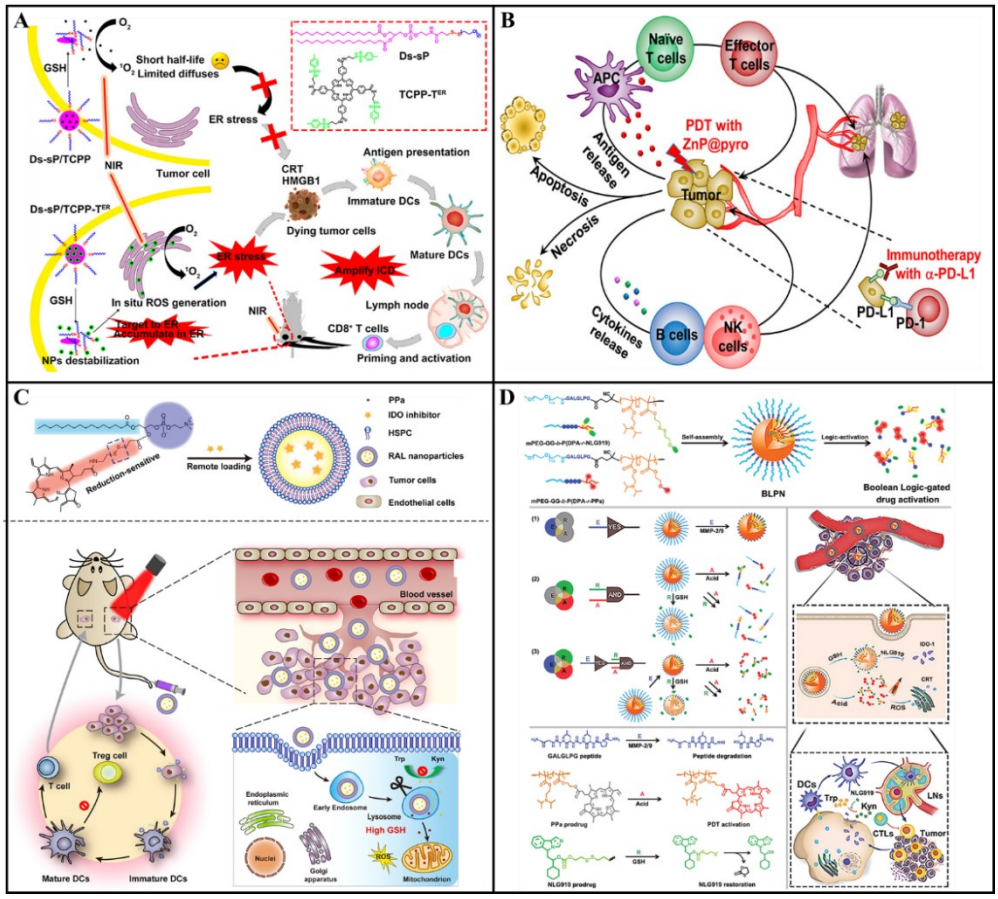

Figure. 10 (A) Ds-sP/TCPP-TER can accumulate in the ER and generate ROS under near-infrared (NIR) laser irradiation, resulting in ER stress that amplifies ICD[150]. (B)

Immunogenic ZnP@pyro PDT sensitizes tumors to PD-L1 blockade immunotherapy for the treatment of metastatic tumors[103]. (C) Schematic illustration of combined PDT and immunotherapy by IND@RAL for cancer therapy. IND@RAL shut down its fluorescence and photoactivity in blood stream while realized exponential activation of fluorescent signal and photoactivity in response to GSH after endocytosis by tumor cells[155]. (D) Schematic illustration of the stimuli-activatable BLPNs for cancer immunotherapy. A variety of stimuli-activatable BLPNs was prepared via adjusting the input responsive combinations including extracellular MMP 2/9, moderate acidity and intracellular GSH[156].

However, hypoxia and photosensitizer aggregation remain to be challenges for PDT. Tang and co-workers[157] first proposed the concept of aggregation-induced emission (AIE), highlighting that molecules undergo AIE don't emit fluorescence in bulk solution while emit strong fluorescence in aggregation. So far, AIE has been widely applied in PDT to avoid the inactivation of photosensitizers resulted from self-quenching. Photosensitizers undergo AIE are able to generate abundant ROS in aggregation, eliciting strong immunogenicity to stimulate immune responses. Though direct oxygen delivery to tumor regions could relieve hypoxia (similar to strategies reviewed in part 3.2.3 "radiotherapy and immunotherapy"), intracellular redox-sensitive transcription factors could also be activated to upregulate antiapoptotic pathways. B-cell lymphoma 2 (Bcl-2), one of the most typical PDT resistance-related proteins, could yield accelerated intracellular GSH, leading to extra consumption of ROS. Thus, Bcl-2 inhibitors are able to consume intracellular GSH for enhanced tumor cell apoptosis. In view of these, Liu and co-workers[158] prepared hybrid nanospheres by coordination-driven co-assembly of Fe^{3+}, Bcl-2 inhibitor sabutoclax and AIE photosensitizer (**Figure. 11A**). Once uptaken by tumor cells, AIE photosensitizers would generate ROS under laser irradiation, leading to increased tumor immunogenicity. Meanwhile, Fe^{3+} would increase intracellular oxygen concentration via Fenton reaction and Bcl-2 inhibitor would fight against PDT resistance, which could amplify antitumor efficacy for further increased immunogenicity. Insufficient light penetration into tissues is another challenge that impedes the application of PDT on deep tumors, because laser intensity would attenuate sharply with the increase of depth. Upconversion nanoparticles (UCNPs) are confirmed to be able to convert NIR light that can penetrate deep in tissues into high-energy visible light by which most photosensitizers are efficiently excited[148]. Thus, the incorporation of UCNPs would endow PDT the ability to treat deep seated tumors. Ling and co-workers[159] developed pH sensitive photodynamic nanoagents (PPNs) from the self-assembly of photosensitizer grafted polymeric ligands and UCNPs (**Figure. 11B**). In physiological conditions, photosensitizers were self-quenched due to their aggregation in PPNs. When triggered by moderate acidity in tumor microenvironment, PPNs would undergo

charge reversal for enhanced tumor cell internalization and disassemble into well-dispersed positively charged UCNPs that were grafted with photosensitizers, enabling deep-tissue penetration. Upon NIR irradiation, UCNPs would convert NIR light into visible light to activate photosensitizers and then generate a large amount of ROS even in deep tumor tissues, efficiently enhancing immunogenicity and improving immune responses.

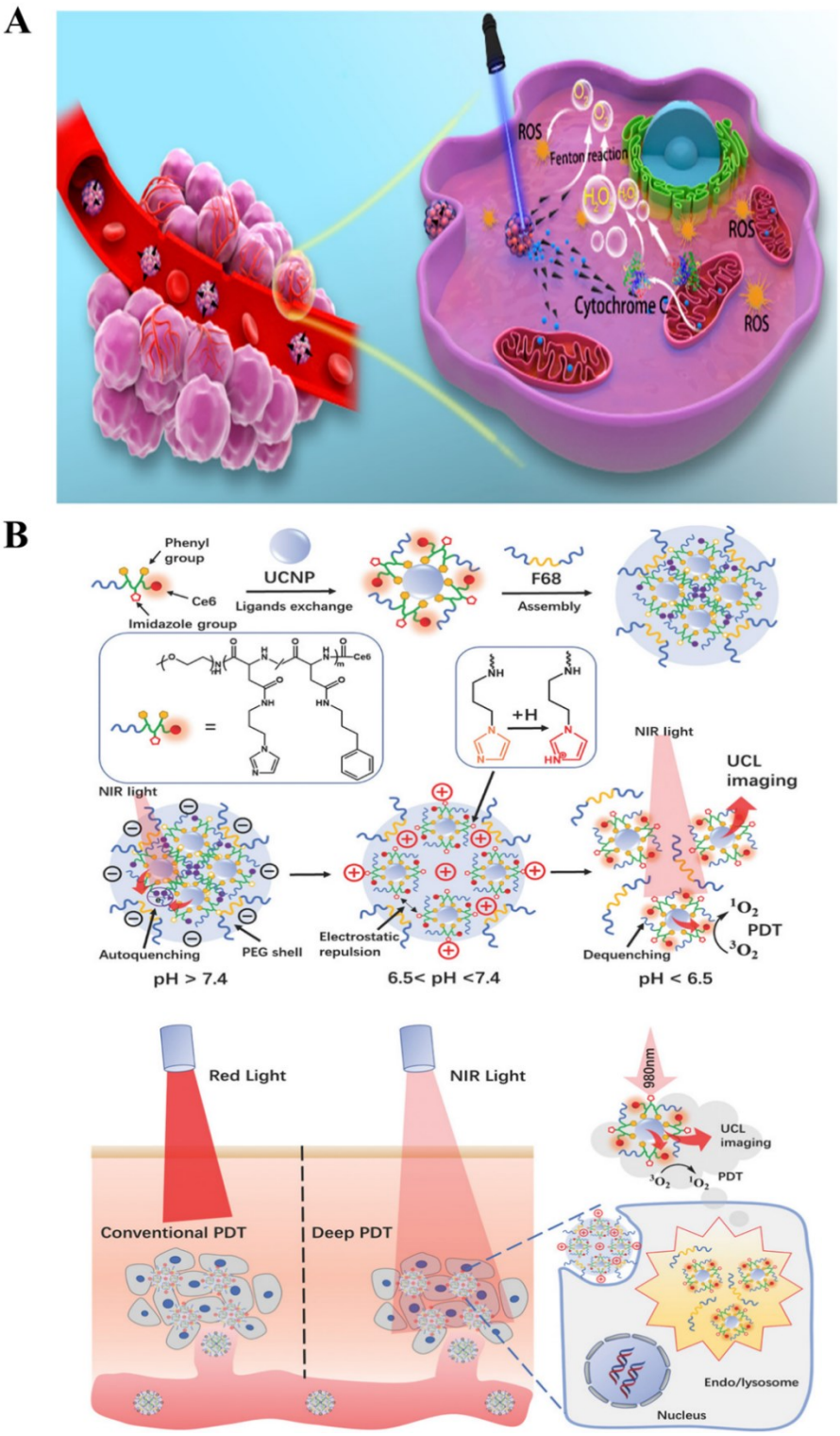

Figure. 11 (A) Chemical structure of TPEDCC, sabutoclax, and the formation of hybrid nanospheres as well as schematic representation of the hybrid nanospheres taken up by tumor cells, Fe^{3+}-activated Fenton reaction to increase intracellular O_2 concentration[158]. (B) Design and mechanism associated with tumor-pH activation of PPNs. Schematic illustration of pH responsive ligand-assisted assembly of UCNPs. Schematic representation of tumor-pH-responsive deep tissue PDT[159].

Photo thermal therapy (PTT) is another promising strategy for the efficient thermal ablation of localized solid tumors by hyperthermia. In addition to cause tumor cell death, PTT could also lead to the release of tumor antigens in situ under certain conditions[93], which could promote tumor immunogenicity, leading to escalated therapeutic responses. Fernandes and co-workers[160] successfully fabricated Prussian blue nanoparticles (PBNP-PTT) for the administration of PTT (**Figure. 12A**). The fabricated PBNP-PTT accorded with the "more is better" paradigm, which means that higher doses of PBNP-PTT generate more heat, thus leading to more cell death. In vivo analysis of ICD markers (ATP, HMGB1 and CRT) elicited by PTT indicated that PBNP-PTT triggered an optimal temperature window (63.3 ~ 66.4 ^{o}C) wherein ICD markers were highly expressed, which increased tumor immunogenicity and evoked immunity.

However, the efficacy of PTT could be greatly restrained by the immune-suppressive microenvironment. To improve the PTT efficacy as much as possible, it is highly reasonable to remodel tumor environment. Chen and co-workers[161] developed red blood cell membrane coated camouflage 2D MoSe2 nanosheets for PTT to stimulate cytotoxic T lymphocytes (**Figure. 12B**). The nanomedicine was endowed with increased hemocompatibility and stability in blood through the inhibition of macrophage phagocytosis. The RBC-$MoSe_2$ induced PTT showed potent antitumor efficacy via triggering the release of multiple tumor-associated antigens for the stimulation of cytotoxic T lymphocytes. In combination with PD-1 blockade, both primary tumor and distant tumor could be inhibited. Furthermore, the tumor-associated macrophages were reeducated to tumor-suppressive M1 phenotype to escape from immune evasion. Insufficient penetration of nanoparticles to deep parts of tumors, which causes incomplete ablation of solid tumors, usually hinders the efficacy of PTT and leads to recurrence. Though particles with small size could facilitate tumor penetration, they are bound to undergo renal clearance, which shortens the lifetime of nanoparticles. Thus, it is necessary to come up with certain strategies to overcome the dilemma. Ma and co-workers[162] fabricated a prodrug nanoplatform which could respond to tumor microenvironment for the efficient delivery of PEGylated IDO inhibitor and photosensitizer. The core-shell nanostructure would transform into small complexes (< 40 nm) once reaching tumor microenvironment. The small dual-drug complexes were able to undergo caveolae-mediated endocytosis, facilitating cellular uptake, and then kill tumor cells directly to trigger immune responses, as well as modulate IDO-mediated immunosuppression. Moreover, the combination with PD-L1 blockade could further promote immunotherapy effect, as well as inhibit the progress of abscopal tumors.

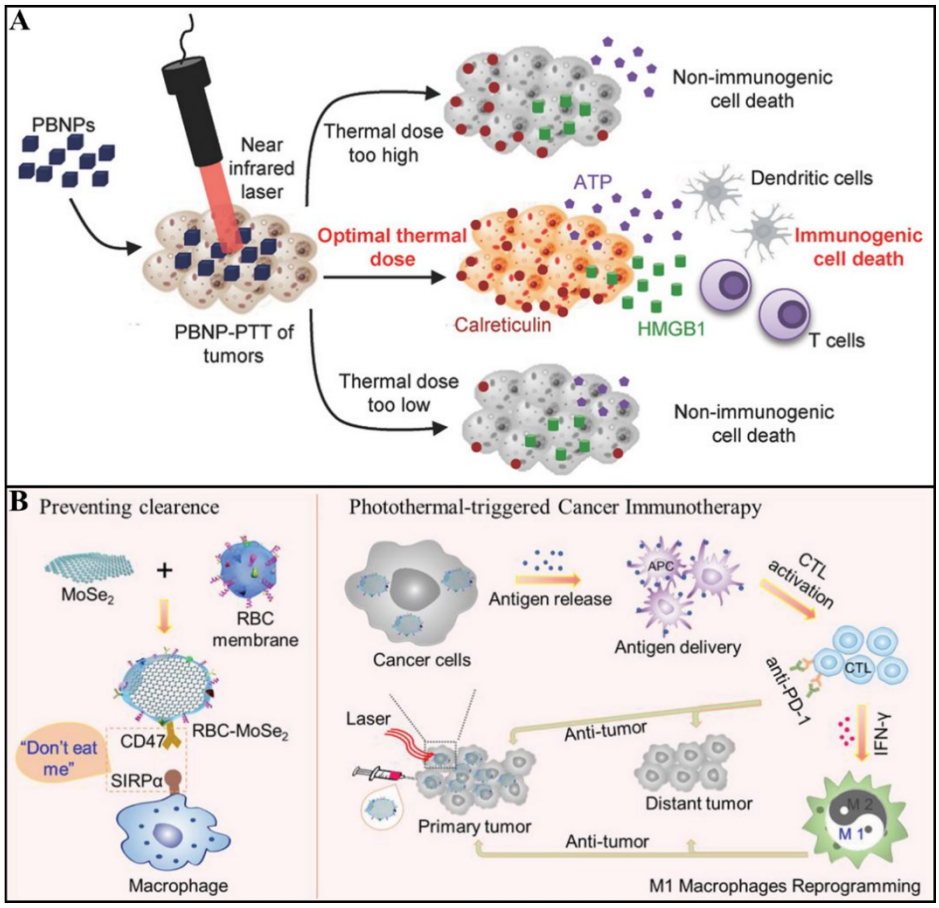

Figure. 12 (A) Prussian blue nanoparticle-based photothermal therapy (PBNP-PTT) generates a thermal window wherein ICD markers, such as ATP and HMGB1, were highly expressed to induce DCs maturation and T cell priming for more efficient PTT[160]. (B) Rational design and synthesis of RBC membrane-coated $MoSe_2$ nanosheet to prevent macrophages phagocytizing during circulation and schematic illustration of RBC–$MoSe_2$ nanosheet for efficient photothermal-triggered cancer immunotherapy. Antigen release would lead CTL activation, secretion of IFN γ as well as M1 macrophages reprogramming. In combination with PD-1 blockade, both primary tumor and distant tumor could be inhibited[161].

3.2.5 Other therapeutic nanomedicines and nanomedicine-combinations to improve immunogenicity

Besides the above-mentioned nanomedicine-based therapies, other therapies in combination with nanotechnology could also be used to induce ICD and increase immunogenicity for the stimulation of immune responses, such as chemodynamic therapy (CDT) and sonodynamic therapy (SDT). And compared to laser triggered therapy, sonodynamic therapy performs better in deep tissue penetration owing to the good penetrating ability of ultrasound. Park and co-workers[163] fabricated sonosensitizer Au-TiO_2 nanocomposites (Au-TiO_2 NCs) that were able to generate a

large quantity of ROS under ultrasound, inducing strong immunogenicity and completely suppressing tumor growth. Shen and co-workers[98] designed an IL-12 gene delivery system to efficiently transfects both tumor-associated macrophages (TAMs) and tumor cells, making them the factory for IL-12 generation. The potent pro-inflammatory chemokine IL-12 could promote T-helper 1 differentiation, facilitate T cell associated killing of cancer cells as well as inhibit tumor angiogenesis. The key design is that the esterase in TAM can catalyze the hydrolysis of the cationic polymer, leading to charge reversal and efficient DNA release.

However, mono-modality therapy might be not sufficient to elicit strong ICD due to various immune evasion mechanisms. To overcome these limitations, dual or more tumor therapeutic modalities have been combined to strengthen the ICD, inducing a more immunogenic tumor microenvironment and evoking more robust host immune responses. The combination of chemotherapy and photodynamic therapy is one of the mostly used strategy to induce immunogenicity synergistically. Li and co-workers[164] developed a type of tumor microenvironment responsive nanomedicine integrating PEGylated photosensitizer and oxaliplatin (OXA) prodrug for tumor specific accumulation, as well as controlled activation and deep penetration of drugs into tumors upon stimulation of acidic and enzymatic tumor microenvironment, greatly increasing immunogenicity for tumor cell ablation (**Figure. 13A**). In physiological conditions, the nanomedicine with PEG shell and negative surface charge exhibited superior colloidal stability. Upon arrival at tumor sites, PEG shell would be stripped by MMP-2, and the nanomedicine would undergo charge reversal with surface charge changing from negative to positive due to the cleavage of pH sensitive bond at tumoral moderate acidity. After internalized by tumor cells, OXA prodrug would be activated by the high level of intracellular GSH for chemotherapy. Under laser irradiation, the generation of ROS along with the instantaneous release of OXA would trigger enhanced tumor immunogenicity via immunogenic cell killing. Meanwhile, PDT could combat drug resistance caused by chemotherapy, and the multi-responsive feature enabled more chemotherapeutic drugs and photosensitizers accumulation at tumor sites, both facilitating the occurrence of ICD and elicitation of tumor immunogenicity to make tumor cells more susceptible to ICB. The subsequent combination with CD47 blockade propagated the host antitumor immunity of ICD by blocking the overexpressed CD47 (“don’t eat me signal”) on tumor cells, which facilitate phagocytosis and antigen presenting function of macrophages. The therapeutic combination could not only suppress orthotopic and abscopal tumors, but also inhibit metastasis and recurrence. Besides polymeric nanomedicines, porous MOFs are another type of nanoplatforms that have been wildly researched due to their good stability, biocompatibility and porosity for efficient loading of drugs. Yan and co-workers[165] designed core-shell nanoplatforms (TPZ/UCSs), where UCNPs cores were coated with MOFs incorporating porphyrin and hypoxia activated tirapazamine (TPZ) prodrug (**Figure. 13B**). The combination of hypoxia triggered chemotherapy and NIR activated PDT

synergistically improved tumor immunogenicity and generated infiltration of CTLs in tumor regions, promoting the subsequent PD-L1 blockade to combat the immune evasion mechanisms.

Both PDT and PTT are triggered by laser and thus usually combined to induce amplified immunogenicity for robust immune responses. The cooperation of PDT and PDT appears to be a breakthrough in surmounting respective shortcomings and achieving synergistic effects with improved therapeutic outcomes. For instance, due to the increased blood flow rate owing to PTT caused temperature rise, the enhanced oxygen supply in the tumor tissue would prompt PDT effect, which in return ablates the heat-resistant tumor cells in PTT. You and co-workers[149] proposed a type of double ER-targeting strategy for PDT and PTT induced immunotherapy. The prepared nanoplatform (FAL-Hb lipo) was composed of indocyanine green (ICG) conjugated hollow gold nanoparticles (modified with ER-targeting peptide pardaxin (FAL)) and hemoglobin (Hb) liposome with oxygen-delivering capability. ER-targeting contributed to the robust ER stress and subsequent calreticulin exposure on tumor cell surface under NIR irradiation, eliciting immunogenicity and further promoting $CD8^+$ T cell proliferation and cytotoxic cytokines secretion for immunotherapy. Besides PDT and PTT, Dong and co-workers[166] introduced a third therapeutic modality to elicit stronger immunogenicity. They fabricated a multifunctional nanoplatform for synergistic PDT, PTT and chemotherapy prompted immunotherapy. The nanoplatform exhibited excellent photothermal conversion ability and PDT efficacy. The release of docetaxel (DTX) synergized with PTT and PDT could greatly enhance tumor immunogenicity and CTLs infiltration to promote PD-L1 blockade, suppress myeloid-derived suppressor cells and polarize M2 phenotype macrophages to M1 macrophages.

Other combined strategies have also been developed to induce robust immunogenicity for the activation of immune system. Xu and co-workers[167] fabricated selenium-containing nanoparticles that could deliver DOX into tumor sites (**Figure. 13C**). On one hand, radiation could facilitate DOX release and further improve tumor immunogenicity along with chemotherapy. On the other hand, the radiation could oxidize diselenide bond into seleninic acid to enhance NK cell function, accelerating radio-chemotherapy induced immune responses. Zhao and co-workers[168] fabricated heterojunction structured, high Z element-containing $WO_{2.9}$-WSe_2-PEG nanoparticles for synergistic radiotherapy, PTT and immunotherapy. The nanomedicines accumulated in tumor regions could induce hyperthermia under NIR irradiation and make tumor cells more sensitive to radiation, as well as generate non-oxygen-dependent ROS from accelerated H_2O_2 in tumor microenvironment. More importantly, both nanomedicine-mediated PTT and radiotherapy could sensitize tumors to ICB via improving tumor immunogenicity. When combined with PD-L1 blockade, the nanomedicine could efficiently eliminate orthotopic tumors and prevent metastasis as well as tumor recurrence.

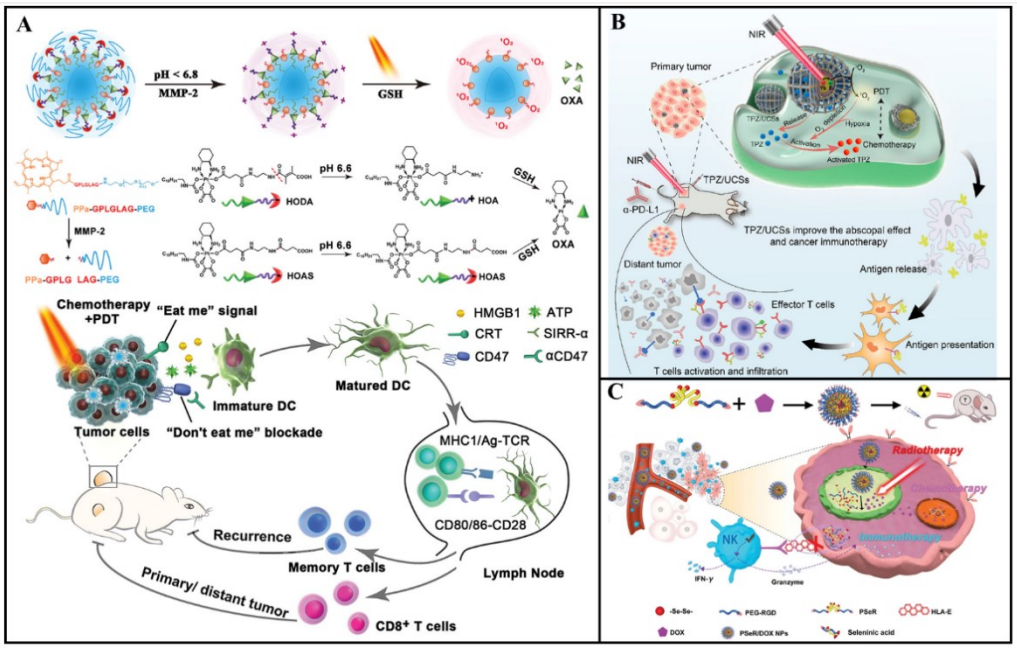

Figure. 13 (A) Schematic design of the acidity and MMP-2 dual-responsive prodrug vesicles and simplified mechanism of MPV-HOAD-mediated chemo-immunotherapy and CD47 blockade to inhibit tumor growth, recurrence, and distant metastasis[164]. (B) Schematic illustration of the structure of TPZ/UCSs and their application to tumor treatment through a combination of NIR light-triggered PDT and hypoxia-activated chemotherapy with immunotherapy[165]. (C) Schematic illustration of utilizing selenium-containing nanoparticles to implement combined treatment of chemotherapy, radiotherapy, and immunotherapy to improve tumor immunogenicity and generate infiltration of CTLs in tumor regions [167].

In all combination therapies, different release kinetics or PK of each therapeutics must be considered because different drugs may have different targets and different therapeutic mechanisms, which greatly affect the process of immunotherapy. Nanodrugs correspond to the different drugs and their different targets and release kinetics are highly required to achieve successful combination therapies. By carefully designing nanomedicines with hierarchical structure and/or tumor microenvironments responsiveness, scientists have developed a lot of inspiring cases. For example, Gu and co-workers[169] developed ROS sensitive protein complex with aPD1 in the core and aCD47 in the shell. First, the enriched ROS in tumor microenvironment would trigger the sustainable release of aCD47 from the shell to activate the recognition of cancers by the innate immune system as well as boost T cell responses. Then, ROS would subsequently trigger the release of aPD1 to exert the PD1 blockade and effectively increase alloreactive T cells to attack the cancer cells. The hierarchically release of drugs would benefit different immune cascades and result in better therapeutic outcomes. In some cases, two or more drugs may have similar therapeutic effects, so it is necessary to synchronize their release kinetics and PK. Liu and co-workers[170]

fabricated hollow MnO_2 nanoshells with Ce6 and DOX loading and post modification by PEG. In drug release curves, Ce6 and DOX have similar release kinetics, which would benefit the combination of PDT and chemotherapy.

Table 2. Various nanomedicines reported to improve immunogenicity to potentiate tumor immunotherapy

Immunogenicity inducing strategy	Delivery platform	therapeutic drugs	Therapeutic strategy	Ref.
Delivery of exogeneous immunogenic antigens	hydrogels	OVA	Nanovaccine	[171]
	Tumosomes	lipid-based adjuvants	Nanovaccine	[112]
	PLGA NPs	CpG and OVA-OVA antibody ICs	Nanovaccine	[114]
	hollow sugar-capsules	mRNA	Nanovaccine	[43]
	Tumor cell membranes coated CPG-PLGA	Melanoma cell membranes, anti-CTLA4 and anti-PD1	Nanovaccine & ICB	[121]
	PLGA nanoparticles	pIC, R848 and MIP3α, individually or in combinations, along with long peptide antigens	Nanovaccine & re-education of immunosuppr-essive macrophages	[122]
	Fe_3O_4/T-MPs-CpG/Lipo	CpG and PD-L1 blockade	Nanovaccine & re-education of immunosuppr-essive macrophages & ICB	[123]

	polymer-epirubicin complex	epirubicin and multivalent polymer-peptide based PD-L1 antagonist	Chemotherapy & ICB	[127]
	pPTX/pCD-pSNO	pPTX/pCD-pSNO and anti-CTLA-4	Chemotherapy & ICB	[133]
	PFC@PLGA-RBCM NPs	PFC and RBCM	Radiotherapy & hypoxia relief	[172]
	Core-shell PLGA nanoparticles	CAT, Toll-like-receptor-7 agonist (R837)and anti-CTLA-4	Radiotherapy & hypoxia relief & ICB	[144]
Inducing immunogenic cell death (ICD) to release endogenous immunogenic agents	ZnP@pyro	Pyro and anti-PD-L1	PDT & ICB	[103]
	IND@RAL	Porphyrin and IDO inhibitor	PDT & IDO inhibition	[155]
	RBCM-camouflage 2D $MoSe_2$ nanosheets	Red blood cell membrane, 2D $MoSe_2$ nanosheets and anti-PD-L1	PTT & ICB	[161]
	MPV-HOAD vesicles	PEGylated photosensitizer, oxaliplatin (OXA) prodrug and anti-CD47	Chemotherapy & PDT & ICB	[164]
	FAL-Hb lipo	ICG, gold nanoparticles and hemoglobin	PDT & PTT & hypoxia relief	[149]
	$WO_{2.9}$-WSe_2-PEG nanoparticles	$WO_{2.9}$-WSe_2 and anti-PD-L1	PTT & radiotherapy & ICB	[168]

4. Summary and perspective

Immunotherapy is greatly revolutionizing the knowledge and clinical treatment of cancer while most tumors barely respond to immunotherapy due to their lack in immunogenicity. Thus, it is highly reasonable to increase tumor immunogenicity for better therapy outcomes. Nowadays nanotechnology could enable specific and controlled delivery of therapeutic agents to tumor sites and even immune cells to elicit potent immunogenicity and amplify the efficacy of immunotherapy. This review provided a general illustration of the significance of immunogenicity for tumor immunotherapy, and further systematically summarized the strategies (delivering exogeneous immunogenic antigens like cancer vaccines, and inducing immunogenic cell death (ICD) to release endogenous immunogenic agents like chemotherapy, radiotherapy, PDT and their combination therapies) to increase tumor immunogenicity and induce immune responses for efficient antitumor therapy. The nanomedicine strategies integrating latest nanotechnologies with immunogenicity-inducing treatments were highlighted and comprehensively introduced, which exhibited many advantages. Nanomedicines could promote preferential accumulation in solid tumors or immune cells to decrease the side effect and injection dose/frequency of drugs. Compared with individual therapeutic modality, properly selected dual or more therapeutic drugs combination would synergize with each other and result in improved therapy effect. Nanomedicines enable the integration of dual or more therapeutic modalities and elicit tumor immunogenicity more efficiently. The modification of PEG and certain targeting moieties endows nanomedicines with prolonged circulation in blood and further enhanced accumulation in specific tissues/cells. Moreover, given the sophisticated nature of in vivo environment, different or even contradictory properties are required in different stages of drug delivery. Smart nanomedicines with size changeable or charge reversal properties are constructed to overcome the dilemma to induce robust tumor immunogenicity and strong immune responses. In addition, smart nanomedicines help take full advantage of the distinct tumor microenvironment and realize the hierarchical release of drugs on demand. Recent researches pay more attentions to deal with challenges, such as tumor heterogeneity, hypoxia, low drug penetration in solid tumors and immunosuppressive tumor microenvironment, to improve antitumor efficacy maximumly. Nonetheless, some scientific and technical issues related to tumor immunogenecity/immunotherapy and the correlative nanomedicines still remain to be addressed.

(1) Highlighting fundamental researches on tumor immunity. Despite the progress scientists have made in identifying mutated or highly inflamed tumors, our deep understanding of the way to counteract nonimmunogenic tumors efficiently is limited. To achieve more efficient therapeutic outcomes, it is obvious that we have to account for other progress in the immune cycle and make better understanding of the relationship between tumor immunity and immune evasion. Besides, multiple immune pathways remain to be identified to prompt immune cascade and prohibit immune evasion.

(2) Developing improved nanomedicines and delivery technologies, optimizing long-term survival with multi-agent immunotherapy combination regimens. Single treatment modality tends to be insufficient in tumor inhibition, and two or more modalities are applied to improve the tumor immunogenicity and antitumor efficacy due to the sophistication of tumor microenvironment. However, the random combination of different strategies may just lead to complexity of treatment instead of "1+1>2" effect. In order to induce immunogenicity and elicit immune responses more efficiently, it is important to figure out the exact mechanisms and related immune effects of different treatments to design precise delivery systems according to diverse tumor conditions for a synergistical therapy rather than the simple superposition of two or more therapies.

(3) For the potential clinical translation, the long-term stability and biosafety of nanomedicines should be assured and the behavior of nanomedicines in blood should be figured out. Despite the declared good biocompatibility and negligible toxicity of various nanomedicines, comprehensive studies should be carried out not only on mice models, but also on mammals like pigs, monkeys that are more similar to human. Nowadays, only a few kinds of nanomedicines gain success in clinic trials, and the increases in therapeutic efficacy and overall survival are very narrow. More fundamental researches should be carried out to reveal how would the nanomedicines behave after their injection into blood, how could nanomedicines truly accumulate in tumor regions and so on.

(4) Maximizing personalized approaches for better therapeutic outcomes. The nature of tumor is bound to affect the efficacy of immunotherapy owing to their considerable differences in their mutational load between different tumor types. A commonly used therapeutic schedule may not benefit all patients due to the differences among personals. Personalized approaches have aroused great concerns and have the opportunity to screen minimum therapeutic combinations to address all stages of cancer immune cascade. Thus, more efforts are required to develop personalized approaches that ordinary persons can afford.

In a word, this review provided a panoramic display of tumor immunogenicity for enhanced tumor immunotherapy. In spite of the intractable challenges remained, we believe that pretreatment or simultaneous treatment with certain therapeutics to enhance immunogenicity would benefit tumor immunotherapy dramatically and nanotechnology would bring great breakthroughs with more efforts paid in this area.

Declaration of Competing Interest

The authors declare no conflict of interest.

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