

## IMMUNOLOGY

# Hydrogel-guided strategies to stimulate an effective immune response for vaccine-based cancer immunotherapy

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Cancer vaccines have attracted widespread interest in tumor therapy because of the potential to induce an effective antitumor immune response. However, many challenges including weak immunogenicity, off-target effects, and immunosuppressive microenvironments have prevented their broad clinical translation. To overcome these difficulties, effective delivery systems have been designed for cancer vaccines. As carriers in cancer vaccine delivery systems, hydrogels have gained substantial attention because they can encapsulate a variety of antigens/immunomodulators and protect them from degradation. This enables hydrogels to simultaneously reverse immunosuppression and stimulate the immune response. Meanwhile, the controlled release properties of hydrogels allow for precise temporal and spatial release of loads in situ to further enhance the immune response of cancer vaccines. Therefore, this review summarizes the classification of cancer vaccines, highlights the strategies of hydrogel-based cancer vaccines, and provides some insights into the future development of hydrogel-based cancer vaccines.

## INTRODUCTION

Cancer is one of the leading causes of morbidity and mortality worldwide with 19.3 million diagnosed cancer cases and 10 million cancer-related deaths reported worldwide in 2020 (1). Tumor metastasis and recurrence are the major causes of cancer-related deaths, so it is vital to find an effective treatment. Cancer immunotherapy is rapidly attracting widespread interest because it can activate a cancer immune response and ultimately have the potential to achieve the inhibition of tumor growth and prevention of tumor recurrence and metastasis. This can be achieved through a dynamic and complex process known as the cancer-immunity cycle consisting of several main steps: recruiting antigen-presenting cells (APCs), releasing tumor-related antigens, uptaking antigens, processing and presenting antigens, priming and activating T cells, trafficking and circulating T cells to cancer tissues, and recognizing and eradicating cancer cells (Fig. 1) (2, 3). One of the most promising approaches in cancer immunotherapy involves cancer vaccines that typically deliver antigens combined with adjuvants into the body via carriers to induce tumor-specific immune responses and immunological memory, thus triggering the cancer-immunity cycle (3, 4). A diverse selection of antigens has been investigated for cancer vaccines, such as DNA/RNA-coded antigens, peptides, and whole-tumor cell lysates. However, many factors such as the tumor immunosuppressive microenvironment,

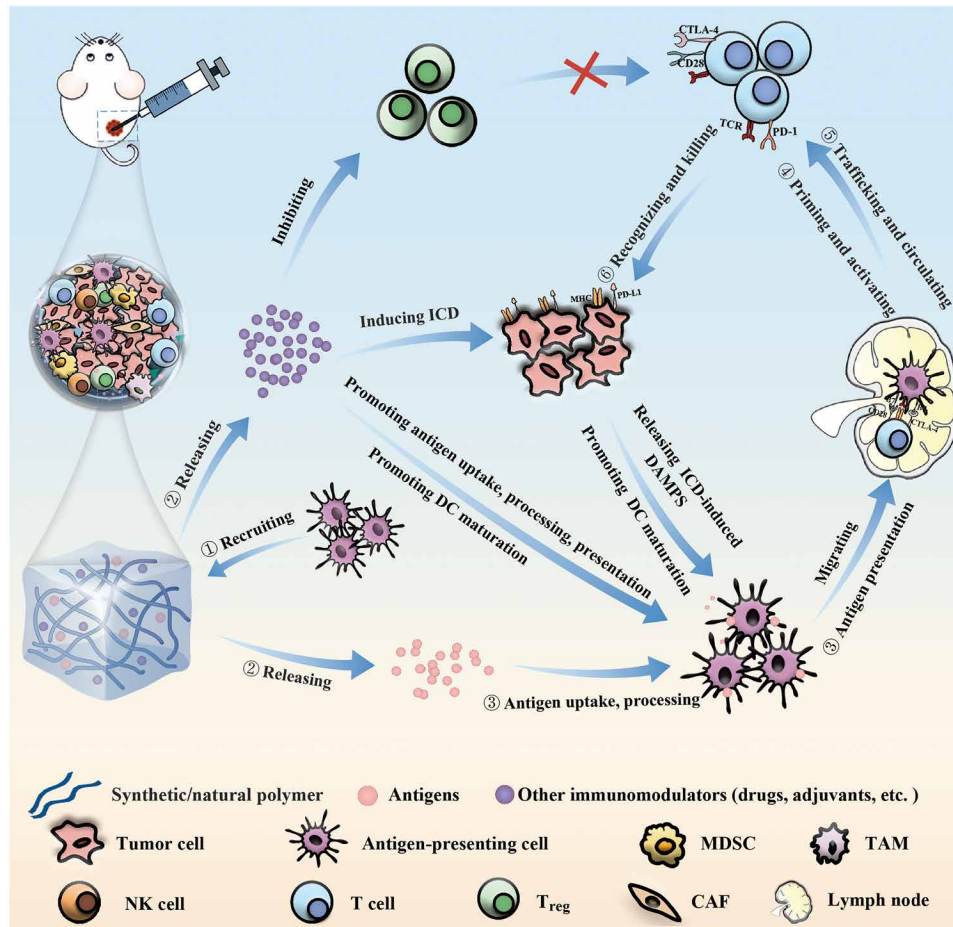
low immunogenicity, and low targeting ability greatly restrict the vaccination efficiency (5).

Over the past few years, many delivery systems have been used for cancer vaccines to overcome above barriers, such as nanoparticles and micelles (5, 6). However, the challenges associated with these carriers, including low antigen encapsulation efficiency, inadequate targeting ability, complex preparation process, and systemic toxicity, may still limit the effectiveness of cancer vaccines (5–7). Hydrogels, as one of the most popular delivery systems, can be described as a three-dimensional (3D) cross-linked network that is capable of holding large amounts of water (8). Notably, the unique properties of hydrogels endow them with great potential in cancer vaccines (8). For instance, hydrogels display excellent hydrophilicity, tunable swelling behavior, and similarities with the extracellular matrix (9, 10). In addition, the 3D porous structure can enhance the hydrogels' encapsulation efficiency (9, 10). Macroporous networks can provide niches to permit trafficking of cells into and out of hydrogels (11). Specifically, the hydrogels can load antigens and multiple immunomodulators, such as adjuvants, cytokines, checkpoint inhibitors, drugs, and some nanoparticles, to synergistically enhance the antitumor efficacy of cancer vaccines (12–14). Moreover, smart hydrogels with responsive polymers or sensitive functional groups provide unique stimuli-responsive properties to achieve precise temporal and spatial controlled release of the loads, ultimately improving the immune stimulation of the cancer vaccines (15). Meanwhile, the hydrogels protect the biologically active payloads to be released from degradation in situ and nonspecific distribution in healthy tissues, thus minimizing undesirable side effects and maximizing immune response (8). In particular, injectable hydrogels formed in situ in tumors can be directly injected into the pathological site through syringes, effectively avoiding the invasive implantation operation with easy manipulation and better patient tolerance, thus attracting much attention in cancer vaccines (8, 16). Notably, many studies have shown that injectable hydrogels have potential as immunoadjuvants (17, 18). Hence, injectable hydrogels have immense promise as the next generation of cancer vaccine delivery systems due to their unique in situ formation, in situ delivery of therapeutic molecules, noninvasive

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**Fig. 1. Schematic illustration of the cancer immunity cycle involving hydrogels, primarily referred to as T cell-mediated cellular immunity.** (1) Recruitment of APCs through loaded immunomodulators or the intrinsic immune properties of hydrogels. (2) Hydrogel release of antigens and immunomodulators. (3) Uptake and presentation of tumor antigens by APCs. (4) Prime and activate T cells in LNs. (5) Circulation and trafficking of T cells. (6) Recognition and eradication of cancer cells by T cells at the tumor site.

administration, and potential as an immunoadjuvant. In this review, we have summarized the development and related strategies for hydrogel-based cancer vaccines, as well as unique perspectives in the field. First, an introduction to the relevant classifications of cancer vaccines is provided in Fig. 2. Next, we discuss the strategies involving hydrogel-based cancer vaccines (Fig. 3). We expect to provide unique insights for future hydrogel-based cancer vaccine design by focusing on the recent relevant strategies that use innovative hydrogel designs to aid immunotherapeutic treatments. Last, the prospects and challenges of hydrogel-based cancer vaccines are discussed with regard to hydrogel loads, delivery strategy, as well as the hydrogel's structure and properties to promote the development of hydrogel-based cancer vaccines.

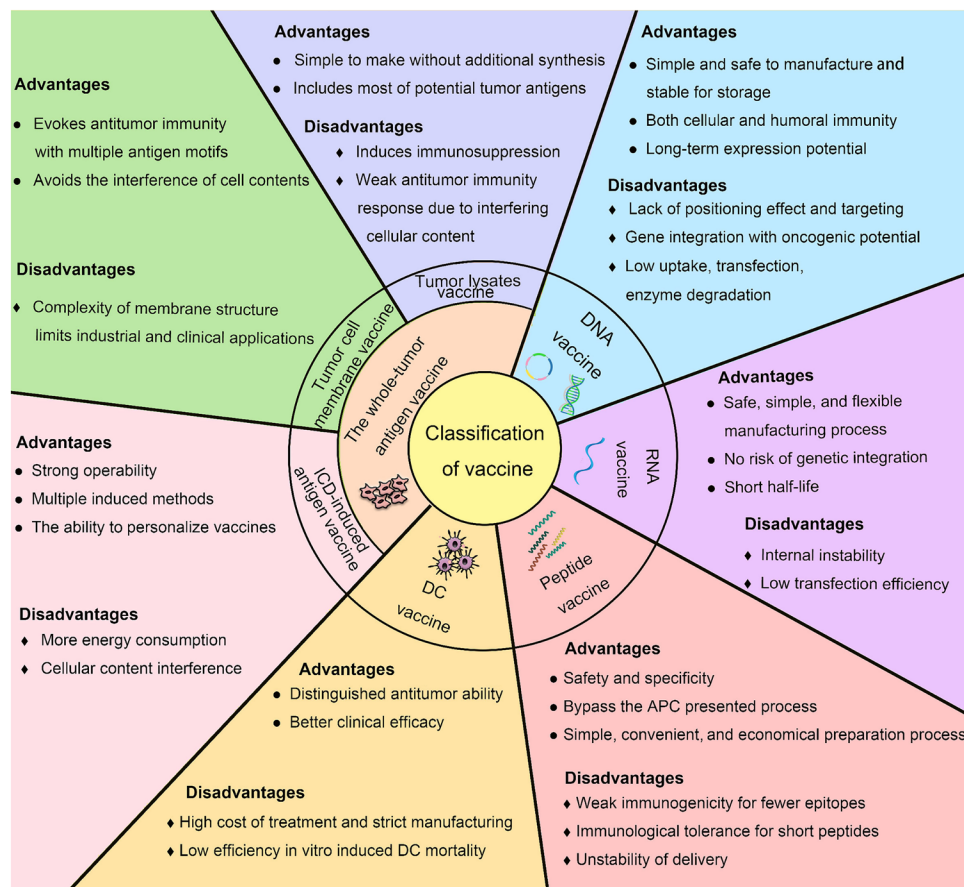
## TYPES OF CANCER VACCINES

Cancer vaccines have made substantial progress as one of the most promising aspects of tumor immunotherapy, especially in the development of personalized cancer vaccine (19). The cancer vaccines play an important role in activating T cell-mediated cellular immunity through antigen presentation. Ideally, the cancer vaccine should deliver

sufficient antigens to the APCs to elicit a robust antitumor immunity. Meanwhile, immunoadjuvants and chemokines can also be delivered to recruit and activate APCs to further promote antigen presentation. The APCs, especially dendritic cells (DCs), will capture and process the antigens and migrate to the lymph node (LN). In the LN, the T cells are activated and differentiated into effector T cells and memory T cells based on the APCs' presented antigens. Subsequently, the effector T cells move to the tumor site through the blood vessels and eradicate the tumor cells with tumor-specific immunity (Fig. 1) (2, 3). In general, the different types of cancer vaccines that have been developed can be categorized on the basis of the type of the delivered antigen, including DNA/RNA vaccines, peptide vaccines, DC vaccines, and whole-tumor cell antigen vaccines. In this section, we will introduce the categories of vaccines (Fig. 2) and summarize the current strategies for vaccine design.

## DNA/RNA cancer vaccines

DNA vaccines attracted great attention in the 1990s since Wolff *et al.* (20) found out that corresponding proteins were expressed after intramuscular injection of plasmid DNA in mice. Subsequently, a large number of studies proved the immune effect of DNA, and it



**Fig. 2. Types of cancer vaccines.** Schematic illustrating six different types of cancer vaccines and their associated advantages and disadvantages.

was widely used in vaccine research (21, 22). DNA vaccines deliver the plasmid DNA encoding tumor antigens or other immunomodulators to elicit or augment the immune response. DNA vaccines are usually administrated intramuscularly or intradermally (22). After administration, DNA vaccines are taken up and expressed by muscle cells/skin cells and the local APCs. Then, the APCs process and present the expressed antigens before ultimately inducing tumor-specific immunity (22). The major advantages of DNA vaccines are the ability to deliver multiple antigens and introduce additional sequences of immunomodulators to the plasmid. However, low cellular uptake and transfection in target cells was observed in DNA vaccines due to the negative charge and large particle size of naked DNA (22, 23). The enzymatic degradation of DNA also limited its development (8, 24). Moreover, low immunogenicity of DNA vaccines attributing to insufficient protein expression levels remains to be resolved (24).

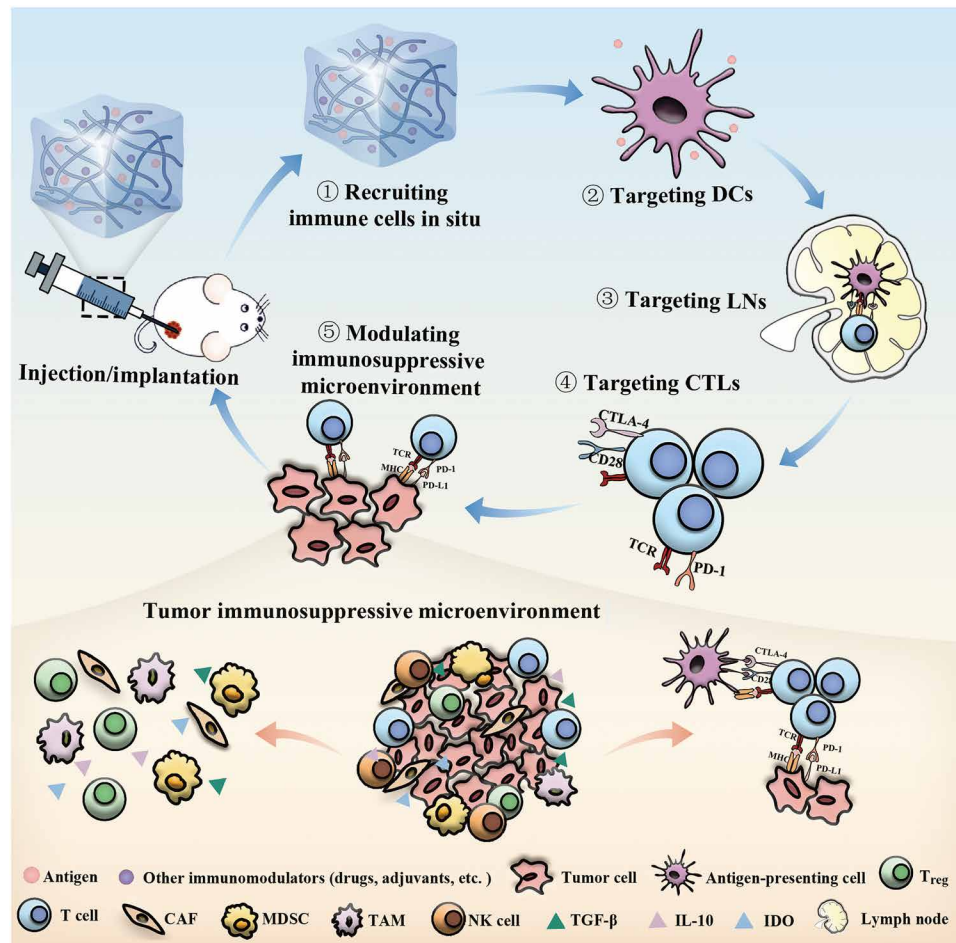
Several strategies have been developed to overcome these barriers, such as plasmid-encoded antigen sequence optimization (24), “gene gun” administration (25), electroporation (26), and appropriate delivery systems (27). In 2010, the first antitumor DNA vaccine demonstrated the safety and efficacy as an adjunctive treatment for oral malignant melanoma in dogs (28), greatly promoting the clinical development of DNA vaccines.

RNA vaccines use the transcription mechanism of host cells to translate antigens through mRNA encoding, thereby stimulating

immune responses (29). As early as the 19th century, the ability to encode antigens using mRNA had been discovered (20). Subsequently, mRNA gained widespread attention as vaccines because of its safety, simplicity, noninfectivity, nonconformity, and flexible manufacturing process (27). Compared to DNA vaccines, mRNA vaccines only cross the plasma membrane for transcription to produce the corresponding antigens, thus avoiding the possibility of integration in the genome (30). Therefore, mRNA vaccines tend to be more efficient and safer than DNA vaccines that need to be delivered to the nucleus.

It must be noted that the mRNA instability and low transfection efficiency are still the major obstacle for mRNA vaccines (29, 31). Because the efficiency and translation half-life of mRNA depend on the sequences of the nucleic acids, optimization of coding sequences can increase its stability, such as 5' cap modifications, optimization of untranslated regions (UTRs), poly-A-tail modifications, codon optimization of open reading frames (ORF), and the nucleoside-modified mRNA (27, 29, 32). In addition, removing double-stranded RNA (dsRNA) contaminations during purification of mRNA is another method to improve the transfection efficiency because dsRNA can block mRNA translation (33). Moreover, a proper mRNA delivery system plays a vital role in protecting the mRNA vaccine from degradation and toxic side effects (29, 31). Recently, an mRNA vaccine called CV9103 for prostate cancer and CV9201 for the small cell lung cancer have been put into a phase 1/2a study (34, 35). Both results showed that the mRNA vaccines were well tolerated





**Fig. 3. Antitumor immune response.** Schematic illustrating different strategies to initiate an antitumor immune response with hydrogels.

and safe when activating the immune response in most of the enrolled patients, indicating the possibility of mRNA vaccines in clinical therapy.

### Peptide cancer vaccines

Peptide cancer vaccines are derived from peptides composed of 8 to 35 amino acids that are expressed on the tumor cells as the antigen source, aimed at eliciting an antitumor T cell immune response (36, 37). The peptide antigen is synthesized chemically based on the short amino acid sequence of tumor-associated antigens, including one or more B or T cell antigen epitopes, which can form a unique vaccine with adjuvants and carriers (37). Similar to DNA vaccines, peptide antigens are easily synthesized, stored, and transported, as well as economical and safe (37).

When peptide vaccines are injected into patients, the peptide directly binds to the major histocompatibility complex (MHC) on the surface of APCs and forms the MHC complex, which is then recognized by the T cell receptors (TCRs) on the T cell surface to generate an antigen-specific cytotoxic T lymphocyte (CTL) cell response (37). However, short peptide (<20 amino acids) vaccines can lead to immunological tolerance rather than initiate an immune response. The injection of specific short peptides generally binds to MHC I on many other cell types, including B and T cells, which can lead to immunological

tolerance and T cell dysfunction due to the absence of costimulatory molecules (38). Moreover, the single epitope carried in the peptide vaccine can limit the strength of the immune response (39).

Therefore, many strategies have been developed to improve the efficacy of peptide vaccines by incorporating a combination of the target peptide, adjuvants, or costimulatory molecules with an appropriate delivery system (38, 39). In addition, a peptide vaccine loaded with multiple epitopes simultaneously would be a good choice to provide the potential for simultaneously inducing both CD4 and CD8 immune responses (40). Recently, a multivalent Wilms tumor 1 (WT1) peptide vaccine has conducted a phase 2 study in acute myeloid leukemia (AML) patients, showing the tolerance, safety, and effectiveness in peptide vaccines (41).

### Whole-tumor antigen vaccines

The whole-tumor antigens provide another way to design cancer vaccines, which contain potential tumor-associated and tumor-specific antigens that can simultaneously evoke a CD4<sup>+</sup> T helper cell (T<sub>H</sub>) activation and CTL response to produce a durable immune response (42). Traditionally, the whole-tumor antigen vaccines stimulate immunity using inactivated autogenous or allogeneic tumor cells or their products (i.e., tumor cell lysates, tumor cell membrane, and tumor-derived extracellular vesicles) obtained ex vivo (42, 43). Interestingly,



autologous whole-tumor cell antigens can also overcome tumor heterogeneity, providing a potential way to prepare personalized tumor vaccines. However, these whole-tumor antigens are composed of many substances, some of which can activate the immune system as antigens, but some of which can exert immunosuppressive effects and induce immune cell apoptosis including Hyaluronic Acid (HA), Fas-L, and transforming growth factor- $\beta$  (TGF- $\beta$ ), thus reducing the efficacy of the cancer vaccine (44). Furthermore, weak immunogenicity of tumor cells may be insufficient to generate an effective antitumor response (42). Therefore, efforts to reverse immunosuppression, improve the immunogenicity, and enhance the safety and efficacy of whole-tumor antigen vaccines should be taken into consideration.

Currently, studies are looking to improve the efficacy with antigen modification, immunoadjuvant and costimulatory molecule assistance, and appropriate delivery system application (43, 45, 46). Among them, macrophage-granulocyte colony-stimulating factor (GM-CSF) is considered to be a costimulatory molecule with excellent auxiliary ability. The GVAX vaccines that contain GM-CSF gene-modified cancer cells have been studied in early-phase clinical trials and have shown remissions of cancers, showing the feasibility of this vaccine approach (45).

In addition, tumor ablation has proven to be a pool of neoantigen source, which has the advantages of overcoming the tumor heterogeneity, reducing immune tolerance, mitigating autoimmunity, and improving the efficacy of T cell-mediated immunity (47). Thus, another effective strategy has been developed, which ablates the tumor in vivo to cause tumor cell death and release the tumor antigens to activate the tumor-specific immune response. When the tumor is ablated, a variety of antigens are released from the tumor site. Subsequently, the antigens are captured, processed, and presented to lymphocytes by APCs, which stimulates lymphocyte proliferation and activation, thus generating the corresponding antitumor immunity. The immunogenic cell death (ICD) induced by tumor cell death also secretes danger-associated molecular pattern (DAMP) proteins, including the translocation of CRT from the endoplasmic reticulum to the cell surface, the release of high-mobility group B1 (HMGB1) from the nucleus, the extracellular secretion of adenosine triphosphate, and the expression of heat shock proteins (HSPs) on the cell surface, which promote antigen phagocytosis, presentation, and DC maturation (48, 49).

Tumor ablation also promotes tumor antigen presentation by providing a proinflammatory microenvironment as immunostimulants (50). The antigens from autologous tumor cells by tumor ablation have stronger immunogenicity than other types of antigen sources. Tumor ablation can be induced by a variety of methods, such as radiofrequency ablation (RFA), microwaves, ultrasound (US), and laser irradiation (51–53). Apart from traditional therapies, photothermal therapy (PTT) and photodynamic therapy (PDT) offer an alternative approach to create an in situ antigen depot, which can generate antitumor immunity and trigger ICD for further synergistic effects with immunotherapies (12, 50, 54).

Recently, many studies have demonstrated the improved efficacy of tumor vaccines using a combinatorial approach. For example, Ito and Evans (53) demonstrated that pre-resectional radiofrequency ablation is able to be a neoadjuvant in situ for tumor vaccines. Yata *et al.* (50) designed a AuNR-DNA hydrogel vaccine based on capturing antigens after laser irradiation. The AuNR-hydrogel can capture the tumor-associated antigens and HSP70 induced by PTT to successfully stimulate the immune response and inhibit tumor growth, thus demonstrating the potential for success in tumor vaccines.

## DC vaccines

DCs, as one of the most effective professional APCs, play a vital role in the innate immune response and adaptive immunity by capturing/processing/presenting antigens (55). Previous studies have demonstrated that DCs can cross-present exogenous antigens and highly express the costimulatory molecule to more efficiently induce an antitumor cytotoxic T cell immune response (55, 56). Therefore, selecting DCs as the carrier of antigens to prepare the DC vaccines can be an effective way to attack tumor cells and inhibit tumor recurrence and metastasis.

Traditionally, DC vaccines refer to the DCs derived from the monocytes in peripheral blood mononuclear cells and are matured in vitro by tumor antigens before being injected back into patients (57). After vaccination, the ex vivo activated DC cells migrate to the draining LN, present antigens to native CD8<sup>+</sup> T cell, and initiate antitumor immunity, demonstrating promising potential in a series of vaccines (57). Notably, only the mature DCs have the ability to elicit the antigen-specific T cell immune response due to the presentation of antigens, expression of costimulatory molecules, and secretion of cytokines (57). The mature DCs can be obtained by a variety of antigens and adjuvant stimulation ex vivo, such as DNA/mRNA, peptide, tumor cell lysates, and Toll-like receptor (TLR) agonists (57). In addition, antigens are more directly targeted to DCs ex vivo, thus avoiding the generation of toxic off-target effects.

On the basis of the specific recognition capability and targeting ability of mature DCs, great progress in DC vaccines has been observed in clinical treatments (58–60). Provenge, a DC cancer vaccine, was first approved by the U.S. Food and Drug Administration (FDA) in 2010, which showed notable primary end point efficacy in a phase 3 study (60). Subsequently, many DC vaccines are undergoing clinical studies, showing the great potential for clinical transformation of DC vaccines (58, 59). However, the preparation process of traditional DC-based vaccines is complex, time-consuming, and expensive, which limits the widespread industrial production and reduces the vigor of DCs (60). Moreover, in vitro induced DCs have poor LN targeting and high mortality before arriving at the LN, which makes it insufficient to activate an effective immune response (61). Meanwhile, the immunosuppressive microenvironment also limits the efficiency of DC vaccines, including the immune checkpoint, myeloid-derived suppressor cells, and immunosuppressive cytokines (55, 62). Therefore, efforts to improve the potency and quality of DC vaccines have included improving the efficiency of DC antigen loading and presentation ability, promoting DC LN homing, and combinations with other therapies such as checkpoint blockades or chemotherapy (55, 57, 61).

## STRATEGIES FOR HYDROGEL-BASED CANCER VACCINE TO INITIATE AN IMMUNE RESPONSE

Cancer vaccines have successfully inhibited tumor recurrence or metastatic disease as a pioneering strategy that evokes the immune response to attack and eliminate tumor cells. Furthermore, cancer vaccines have the additional benefit to potentially induce a long-term immune memory to combat cancer metastasis and recurrence. Despite many advantages of cancer vaccines, there are still many obstacles to stimulating sufficient antitumor immune responses in clinical treatment, such as lack of immunogenicity, lack of costimulation, and immunosuppression in tumor microenvironment (TME) (5, 6). As mentioned previously, cancer vaccines work through a dynamic

and complex process, each of which is indispensable. Therefore, an effective cancer vaccine needs to overcome these barriers and maintain the vital steps to initiate an effective antitumor immune response, including the recruitment of immune cells, the loading, release, processing, and presentation of antigens, the migration of DCs to LNs, the activation of effector T cells, and the recognition of cancer cells (2, 3). With these obstacles in mind, it is highly promising to adopt hydrogels as the delivery system to improve the efficacy of tumor vaccines in a two-pronged manner for the following reasons: (i) co-encapsulation of antigens and other immunomodulators in hydrogels to overcome the initial obstacles of eliciting an immune response using the high encapsulation rate, controlled release, and excellent biological function of hydrogels and (ii) design the appropriate delivery strategy to overcome the obstacles during the immune response process.

In this section, we will discuss the challenges and corresponding strategies for improving the efficacy of cancer vaccines based on the obstacles to the immunologic process, particularly focusing on the application of these strategies in hydrogel-based cancer vaccines (Fig. 3).

### In situ immune cell recruitment

Cancer vaccines can elicit strong immune responses depending on the recruitment of APCs, specifically DCs, which is a prerequisite for the uptake, processing, and presentation of antigens. In addition to APCs, other types of immune cells, such as effector T cells, natural killer (NK) cells, and macrophages, are also involved in cancer immunity, which can be recruited to the tumor tissue to further promote the adaptive and innate antitumor immune response (63, 64). Therefore, designing hydrogels with the ability to recruit immune cells can facilitate the antitumor immunity induced by hydrogel-based cancer vaccines (65, 66). Hydrogels can load multiple antigens and immunomodulators due to their high encapsulation efficiency. Sustained release of loaded cargo from hydrogels can mimic the immune priming that facilitates immune cell recruitment in situ, thus enhancing the potency and breadth of vaccination (67, 68). In addition, hydrogels that exhibit microporous structures or are composed of materials with intrinsic immunomodulating functions could also assist with in situ immune cell recruitment, thus eliciting further downstream immune responses (11). The strategies for in situ immune cell recruitment are summarized in Fig. 4A.

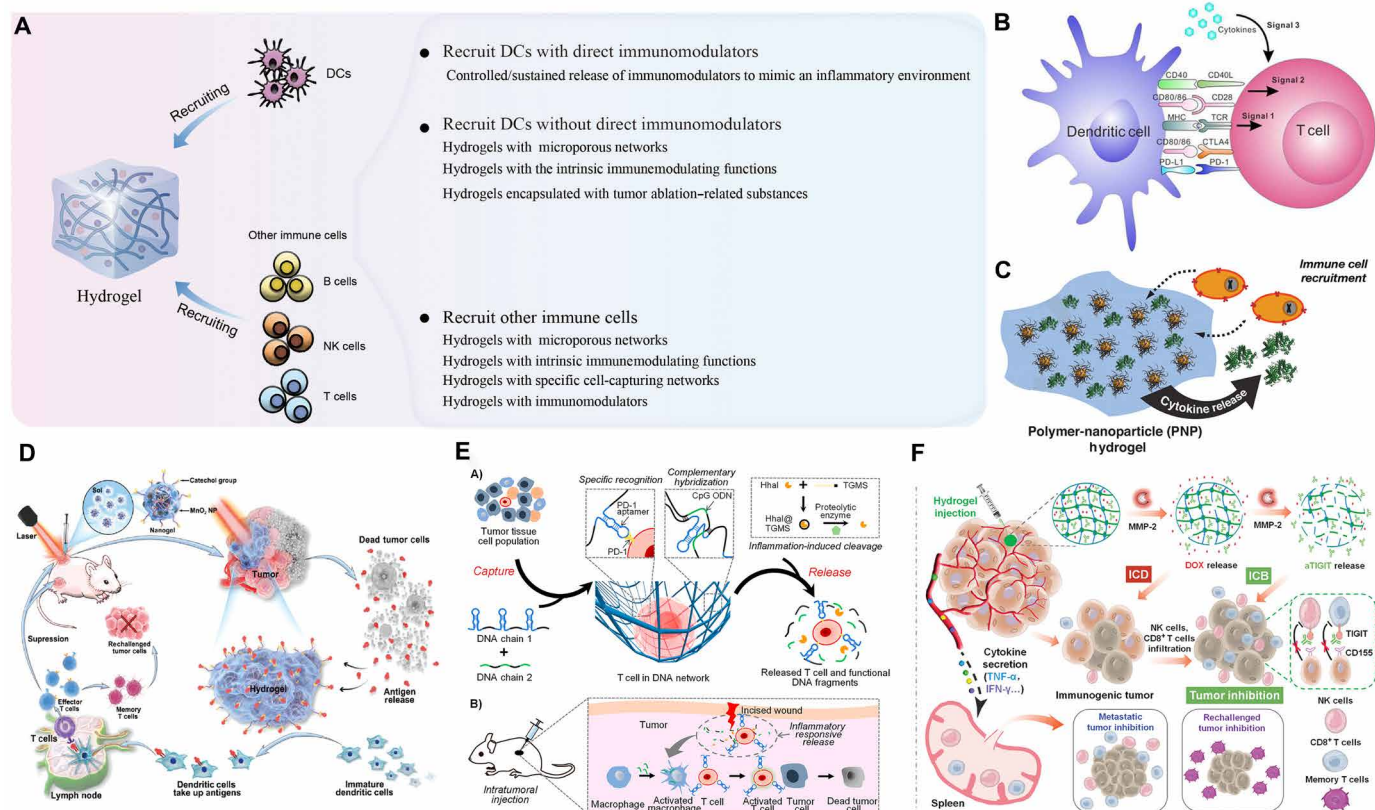
#### In situ DC recruitment

DCs, the most prominent professional APCs, play a critical role in stimulating the antitumor immune response (55). The ability of hydrogels to recruit DCs in situ is closely related to its antitumor immunity efficiency. When hydrogels release the antigens, DC involvement in the immune response is initiated as the following steps (5, 55–57, 69, 70). First, immature DCs capture, process, and present the antigens on the MHC I (antigen cross-presentation) and MHC II molecules. Subsequently, these DCs with peptide-MHC complexes migrate to the LNs. During migration, some immature DCs will gradually mature under inflammatory stimulation, such as Lipopolysaccharide (LPS) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). Mature DCs up-regulate the expression of costimulatory molecules (CD80/CD86 and CD40) and chemokine receptors (CXCR4 and CCR7) and secrete a variety of cytokines that can promote the differentiation and maturation of T cells. Upon arrival at the LNs, mature DCs with peptide-MHC complexes interact with the TCRs on T cells, providing the first signal for T cell activation. Meanwhile, the costimulatory molecules

expressed by DCs (CD80 or CD86 and CD40) bind to corresponding molecules on the surface of T cells (CD28 and CD40L) to provide a second signal to promote the activation and proliferation of T cells. Furthermore, a third cytokine signal [interleukin-2 (IL-2), IL-12, and interferon- $\gamma$  (IFN- $\gamma$ )] is also essential (Fig. 4B). Therefore, efficient antigen uptake, processing, presentation, and DC maturation is the prerequisite for T cell activation, especially for CD8<sup>+</sup> CTLs that play a vital role in the antitumor immune response.

However, due to weak immunogenicity, antigens are oftentimes too difficult to be recognized and are unable to recruit enough DCs to uptake, process, and present to stimulate a downstream response (71). In addition, DC maturation can also be hindered by the lack of promaturation signals. In the absence of sufficient costimulatory signals on the surface of DCs, antigen presentation can result in the generation of immunosuppressive T cells or incompetent T cells (57, 72). Therefore, designing hydrogels with powerful DC recruitment abilities plays an important role in stimulating the downstream immune response. The current strategies for DC recruitment can be divided into two categories, recruitment of DCs with or without direct immunomodulators.

Encapsulated immunoadjuvants in hydrogels, such as Polyinosinic-polycytidylic acid (poly I:C) (TLR3 agonist), monophosphoryl lipid (TLR4 agonist), CpG oligodeoxynucleotide (ODN) (TLR9 agonist), and resiquimod/R848 (TLR7/8 agonist) (73), can enhance immunogenicity and recruit DCs and T cells to the injection site to induce a strong antitumor immunity. Furthermore, the addition of proinflammatory cytokines and chemokines can increase the recruitment of DCs via inflammatory response, such as GM-CSF, TNF- $\alpha$ , and CCL19/21 (74–77). GM-CSF has a substantial impact on recruitment, differentiation, and proliferation of DCs (75). TNF- $\alpha$  mediates the inflammatory signals to exert a profound influence on increasing the immature DCs and inducing the maturation of DCs to migrate to the LN (76). Meanwhile, the duration of the antigenic and immunoadjuvant's stimulation has been demonstrated to be the critical factor for initiating the immune responses (67, 78). Compared to vaccines with a rapid release of antigens (enabled via disulfide linkages), the particulate subunit vaccines with a sustained release of antigens (enabled via thioether linkages) resulted in enhanced antigen presentation, DC maturation, and antitumor immune responses (78). Furthermore, extending antigen release from vaccines also have the potential to prevent tumor recurrence and metastases (68). The exact mechanism is still unclear, but reports suggest that sustained antigen and adjuvant release provides sufficient time for APCs to recognize and process antigens before presenting them (67). Sustained antigen and immunoadjuvant release also helps mimic the “inflammatory infection system” and enrich the recruitment of immune cells, resulting in a stronger downstream immune response (68, 79). As a vaccine carrier, hydrogels loaded with antigens/immunomodulators can be a common strategy to recruit DCs, and it can be further maximized for a longer-lasting in vivo immune response by altering the release kinetics of payloads (9). Moreover, administration of the hydrogel-based vaccines at the tumor site can locally release the loaded cargo, thus further decreasing the loss during the delivery process and promoting the local recruitment of immune cells to avoid systemic toxicity (9). For example, Verbeke, Verbeke and Mooney (66) demonstrated that spatiotemporal controlled release of GM-CSF from the noninflammatory hydrogels significantly enhanced the DC recruitment compared to hydrogels exhibiting burst GM-CSF release. Bencherif *et al.* (80) designed a tumor cell-loaded cryogel sponge as an injectable



**Fig. 4. The strategies for in situ immune cell recruitment.** (A) Schematic of the strategies for in situ immune cell recruitment using hydrogels. (B) Major positive and negative costimulatory interactions between T cells and DCs and the three signals necessary for activation of T cells (70). (C) Schematic of releasing immune factors that enhance immune cell recruitment using the PNP hydrogels. Reprinted with permission from (77). Copyright 2019, American Chemical Society. (D) Schematic illustration of the process of ICD-induced DC recruitment by injectable adhesive hydrogels. Reprinted with permission from (12). Copyright 2021, Wiley-VCH GmbH. (E) T cell-captured network made from DNA: (A) construction of DNA network and cell capture of tumor-infiltrating T cell; (B) in vivo localized immunotherapy via injection of T cell-containing DNA network. Reprinted with permission from (84). Copyright 2021, American Chemical Society. (F) Principles of localized DOX and aTIGIT differential release from enzyme-responsive hydrogel to recruit immune cells by ICD and enhanced immune checkpoint blockade (ICB) therapy. Reprinted with permission from (65). Copyright 2021, Wiley-VCH GmbH.

vaccine platform encapsulated with GM-CSF and CpG ODN. The cryogel was synthesized with Methacrylated alginate (MA-alginate) using a cryogelation technique, which contained large, continuously interconnected macropores throughout the entire cryogel construct, providing high capacity for tumor cell delivery. In addition to releasing the loaded tumor cells, the cryogel coordinated the release of GM-CSF and CpG ODN in a controlled spatiotemporal manner, which significantly promotes DC recruitment, antigen presentation, DC maturation, and cellular infiltration, ultimately evoking a stronger T effector response in a murine melanoma model. Notably, because of the local release of immunomodulators, fewer systemic effects are anticipated compared with systemic delivery. Similarly, Qian's group (13) designed a thermosensitive hydrogel vaccine system composed of GM-CSF and CpG ODN to demonstrate the function of recruiting and activating DCs by local sustained release of antigens and adjuvants. Alternatively, Fenton *et al.* (77) designed a polymer nanoparticle (PNP) hydrogel capable of locally recruiting DCs via the release of DC cytokine, CCL21, providing a different costimulator option (Fig. 4C).

On the contrary, another strategy to recruit immune cells is through indirect immune factors, such as depending on the biological function of the hydrogel itself or adding other substances to induce

immune factors instead of directly adding the immune factors. Hydrogels with a macroporous network provide cellular niches to DCs and other immune cells, resulting in a large number of immune cells migrating and infiltrating into hydrogels, thus initiating immune responses (11). Alternatively, hydrogels composed of materials with intrinsic immunomodulating functions could be another way to recruit DCs (9, 81). Some self-assembled peptide hydrogels have also demonstrated to have the potential as immunoadjuvants (17, 18). Thus, engineering hydrogels with the ability to recruit immune cells via self-biological functions without loading immunomodulators is likely to be a trend for cancer vaccines.

A new type of injectable hybrid hydrogel has been recently explored based on protein-polymer networks, which can act as unique delivery vehicles for antigens/drugs because of their specific microporous structure, biodegradability of the protein, and controllable degradation rate of the polymer (82, 83). Compared to traditional hydrogels, biocompatibility of the microporous networks, especially porous structures with interconnected long pores of the protein-polymer hydrogel, provides a niche to recruit host immune cells without any immunomodulators (82, 83). A hybrid hydrogel (BSA-PCLA) composed of a polymer poly( $\epsilon$ -caprolactone-*co*-lactide)-*b*-poly(ethylene glycol)-*b*-poly( $\epsilon$ -caprolactone-*co*-lactide) triblock copolymer (PCLA)



conjugated with a protein, bovine serum albumin (BSA), was designed to recruit millions of cells via its microporous structure and controlled release property (82). The hydrogel exhibited a 30-fold increase in the number of DCs expressing CD11c MHC II compared with GM-CSF-loaded PCLA (GM-CSF PCLA) and PCLA formulations because of its microporous structure. Subsequently, the hybrid hydrogel further increased the DC recruitment through sustained release of pDNA antigens, thus inducing a robust downstream antigen-specific immune response. Similarly, an injectable protein-polymer-based porous hydrogel network composed of lysozyme and PCLA (Lys-PCLA) bioconjugate has also shown an enhanced ability for DC recruitment (83). This unique microporous type of hydrogel provides a powerful strategy for recruiting DCs to initiate immune responses in hydrogel-based vaccines.

The weak immunogenicity of the tumor site is a fundamental reason for a weak immune response to cancer vaccines (71). Increased immunogenicity can enhance the DC recruitment ability in hydrogels, and the tumor ablation-induced ICD can be a strategy to enhance the antigen immunogenicity and eliminate tumor heterogeneity (47, 48). ICD-induced exposure of DAMPs, including the translocation of CRT, the release of HMGB1, the extracellular secretion of adenosine triphosphate (ATP), and the expression of HSPs on the cell surface, could further promote APC recruitment, antigen internalization, DC maturation, and T cell activation (49). Inspired by this, hydrogels loaded with tumor ablation-related substances (i.e., chemotherapeutics, photothermal materials, and photosensitizers) can be a good strategy to recruit DCs (12, 50, 54). However, the loss of the ICD-induced neoantigen in the tumor would reduce the recruitment of DCs (12). Therefore, designing a hydrogel with the ability to induce ICD to increase immunogenicity and avoid antigen loss to recruit more DCs will promote a strong antitumor-specific immune response. An injectable adhesive hydrogel ( $\text{MnO}_2$ @PND nanogel) as a photothermal-derived antigen reservoir has been developed to enhance the antitumor immune response (12). The nanogel (PND) is prepared by a free radical polymerization between *N*-isoacrylamide (NIPAM) and dopamine methylacrylamide (DMA) and then loaded with  $\text{MnO}_2$  nanoparticles to form  $\text{MnO}_2$ @PND nanogels via an in situ mineralization procedure (Fig. 4D).  $\text{MnO}_2$  nanoparticles endow the nanogels with ICD-induced massive antigen release capacity under near-infrared irradiation, and the catechol groups in the DMA endow the nanogels with an antigen adsorption property to capture the released antigens. Subsequently, the release of these antigens will recruit more DCs to stimulate an intensive and lasting  $\text{CD8}^+$ -mediated antitumor response, thus providing an innovative strategy for hydrogel-based vaccines without directly delivering immune factors.

#### **In situ recruitment of other immune cells**

The ultimate goal for all of the above strategies based on DC recruitment is to recruit large numbers of T cells to the tumor site and eradicate the tumor. Therefore, hydrogels that can directly recruit T cells is also a promising alternative. Yao *et al.* (84) designed a T cell-capturing DNA network to capture infiltrating T cells with DNA aptamers, which provides the possibility for hydrogels to recruit T cells in situ (Fig. 4E). Notably, T cell-induced antitumor responses are not autonomous and highly depend on the innate immune system (including NK cells, macrophages, and DCs), which can launch the adaptive immune response to kill tumor cells through antibody-dependent cellular phagocytosis (ADCP) and antibody-dependent cellular cytotoxicity (ADCC) (63). Therefore, NK cells, macrophages, and other immune cells also play an important role in immunity.

Similar to DCs, NK cells are also involved in both innate immunity and antitumor immunity (64). At the tumor site, activated NK cells can directly kill tumor cells by releasing perforin and granzymes or indirectly kill tumor cells by secreting cytokines (IFN- $\gamma$ , TNF- $\alpha$ , GM-CSF, and IL-3) (64). Furthermore, through the production of chemokine (C-C motif) ligand 5 (CCL5)/chemokine (C motif) ligand 1 (XCL1), NK cells can promote the infiltration of DCs into the tumor, resulting in the maturation of DCs and differentiation of T cells (85). Therefore, active recruitment of NK cells is beneficial toward inducing both a strong innate immunity and an adaptive antitumor immunity.

Recently, Ji *et al.* (14) reported an implantable hydrogel loaded with resiquimod (R848) and anti-OX40 antibody (aOX40) for sequential activation of innate and adaptive immunity for colorectal cancer postoperative immunotherapy. With controlled simultaneous release of R848, the hydrogel has demonstrated that it can recruit large numbers of NK cells and DCs to the tumor site over the first several days and secrete a high level of IFN- $\gamma$ , indicating the launch of innate immunity. Following the first few days, an increased number of infiltrating T cells has also been observed at the tumor site, suggesting the activation of an adaptive antitumor immune response. Last, the hydrogel successfully induced the long-term immune memory, providing a strong strategy for hydrogel-based immunity.

However, the activity of NK cells can be restrained by the engagement of inhibitory cell surface receptors that contain the intracytoplasmic immunoreceptor tyrosine-based inhibition motifs (ITIMs) (86). Therefore, a matrix metalloproteinase 2 (MMP-2)-degradable hydrogel with doxorubicin (DOX) and blockade of TIGIT [T cell immunoglobulin (Ig) and ITIM domain] has been studied. Anti-TIGIT monoclonal antibody (aTIGIT), a coinhibitory receptor expressed by both NK and T cells, can reverse the exhaustion of NK cells and T cells (65). Therefore, upon release of DOX and aTIGIT in response to MMP-2, the hydrogels would recruit and activate a large number of infiltrating NK cells and effector T cells by DOX-induced ICD and aTIGIT-induced reversal of NK and T cell exhaustion (Fig. 4F).

As mentioned previously, besides encapsulating specific immunomodulators to recruit specific immune cells, hydrogels with macroporous networks or composed of materials with intrinsic immunomodulating functions permit all immune cells into and out of hydrogels. This provides an alternative strategy for recruiting immune cells (i.e., NK cells, T cells, and B cells) to evoke immunity; however, this structure-dependent recruitment ability is nonspecific and could enhance the entry of immunosuppressive cells.

#### **Active targeted delivery to the immune system**

In addition to passive in situ recruitment of immune cells by cytokines or antigens, active targeting of immune cells is another strategy to activate T cells including encapsulation or decoration with corresponding ligands of targeted immune cells and loading nanoparticles with targeting properties (Fig. 5A).

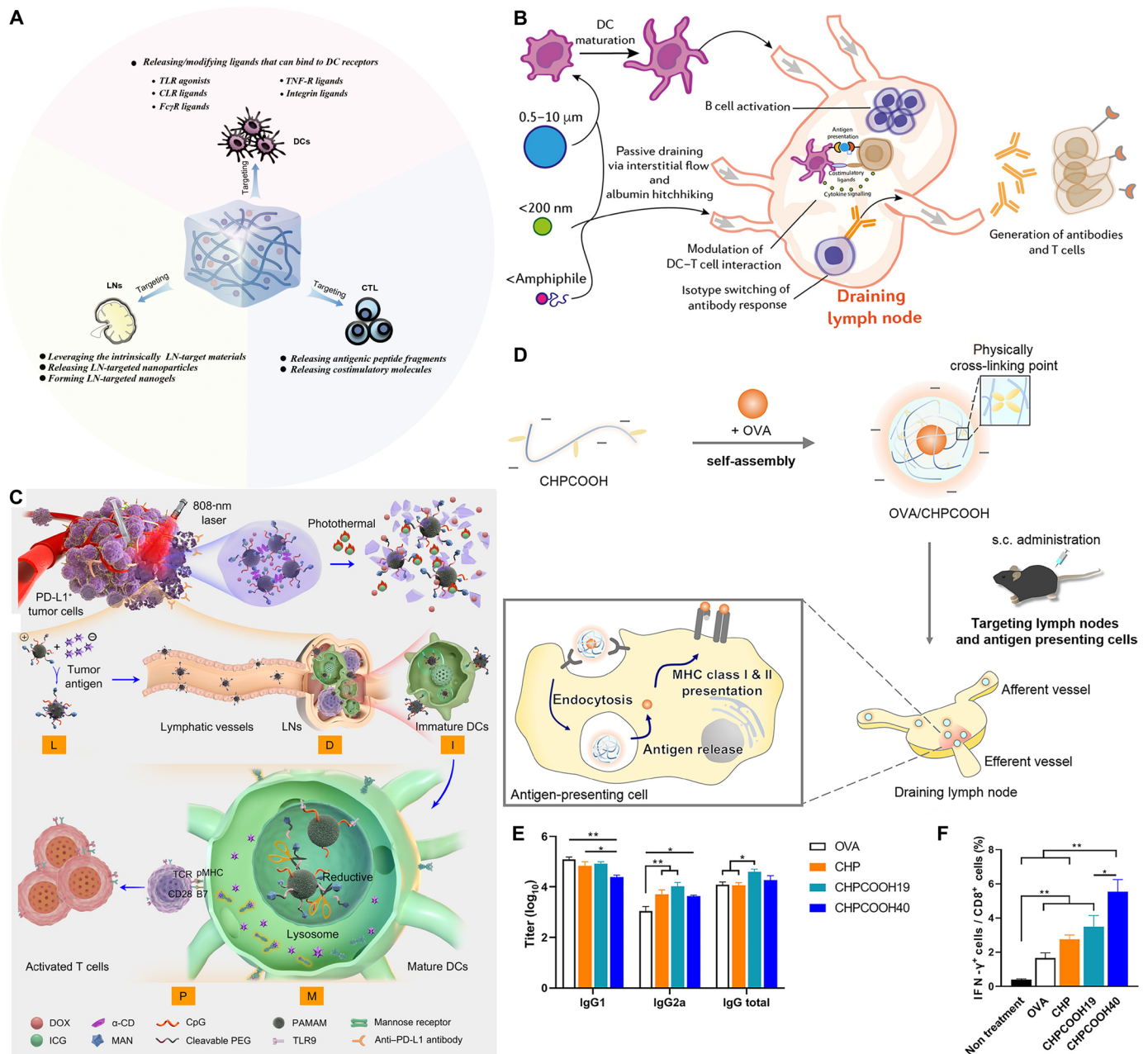
#### **Strategies to target the LN and DCs**

The key for cancer vaccines is to induce cytotoxic T cell antitumor immunity, so the strategy to activate a  $\text{CD8}^+$  T cell response is a major objective. As mentioned above, DCs not only are capable of cross-presentation to process exogenous antigens using the MHC I pathway in the cytosol to stimulate  $\text{CD8}^+$  T cells but also can present antigens using the MHC II pathway to stimulate the  $\text{CD4}^+$   $\text{T}_\text{H}$  cells (56). DCs can also induce the differentiation of  $\text{T}_\text{H}$  cells into different phenotypes to perform different functions, such as  $\text{T}_\text{H}1$ ,  $\text{T}_\text{H}2$ ,

and  $T_H17$  (69). Thus, active targeting of DCs to promote antigen internalization, DC uptake, and DC maturation can be another effective approach for cancer therapeutic vaccines.

DC surface molecules and their receptors as potential targets have attracted extensive attention in the design of actively targeted injectable vaccines (87). To have the ability to target DCs, hydrogels can be decorated or encapsulated with ligands that bind to DC surface

receptors, such as TLRs, TNF receptor (TNF-R) family molecules (i.e., CD40), C-type lectin binding receptors (CLRs) (i.e., mannose receptors, CD205, and DC-SIGN), and integrin receptors (i.e., CD11c-CD18) (69, 87–89). TLRs, one of the pathogen pattern recognition receptors (PPRs), can activate DCs by recognizing different pathogens, making DCs express a variety of costimulatory and cytokine signals, and activating T cell immunity (88). Thus, TLR



**Fig. 5. The strategies for active targeted delivery to the immune system.** (A) Schematic illustration of the strategies for active targeted delivery to the immune system using hydrogels. (B) Schematic illustration of the strategies for the LN-targeted delivery. Reprinted with permission from (101). Copyright 2020, American Chemical Society. (C) Schematic illustration of the integrated regimen and the release process of CpG-P-SS-M and simplified mechanisms of CpG-P-ss-M-mediated LN and DC targeting for cancer immunotherapy (54). Copyright 2020, the Authors. (D) Illustration of the CHPCOOH nanogel vaccine system for targeting LNs. s.c., subcutaneous. (E and F) Immune activation using CHPCOOH nanogel vaccines. (E) Total serum antibody titers following immunization of ovalbumin (OVA) alone, OVA/CHP, or OVA/CHPCOOH. (F) Ratios of OVA-specific activated CTL to total CD8<sup>+</sup> cells (\* $P$  < 0.05 and \*\* $P$  < 0.01). Reprinted with permission from (102). Copyright 2020, American Chemical Society.

agonists have been widely applied as vaccine adjuvants (88, 90). CLR plays a vital role in antigen presentation. CD205 seems to have more potential in mediating cross-presentation than mannose receptors and DC-SIGN (91), but all of them are capable of enhancing the internalization of vaccines by ligand receptor-mediated binding. Many CLRs share a primary structural homology in their carbohydrate recognition domain that binds to specific or nonspecific sugar residues (87). Strategies involving targeting CLRs fall into two categories: binding with the natural ligands or specific antibodies. Glycoconjugates terminated with mannose, fucose, or *n*-acetylglucosamine are natural ligands that have been widely used in DC-targeting strategies (89). Coen and colleagues (92) synthesized a mannosylated nanogel via core-crosslinking of amphiphilic non-water-soluble block copolymers composed of an acetylated mannosylated block and a pentafluorophenyl (PFP)-activated ester block. Because of the glycosyl binding to the mannose receptors on the DC surface, mannosylated nanogels greatly promoted DC recognition and internalization. Alternatively, CD40 agonist antibodies can improve T cell priming by targeting DCs (93). A slow-releasing injectable hydrogel depot loaded with immunostimulatory CD40 agonist showed great efficacy in a syngeneic dual-flank model of B16F10 melanoma (93). Using hydrogels as the carrier reduced the CD40 agonist-induced weight loss, hepatotoxicity, and cytokine storm.

Aside from the above potential DC targets, the Fcγ receptors (FcγRs) expressed on DCs can be another target, including the activating receptors FcγRI, FcγRIIa/c, and FcγRIIIa/b and the inhibitory receptor FcγRIIb, which binds to the Fc domain of IgG (87). The FcγRs are involved in endocytosis and/or phagocytosis of immune complexes, presentation of antigen-derived peptides on MHC molecules, and modulation of the adaptive cellular immune responses (94). This approach loads the corresponding antibodies or antibody-antigen conjugates to improve DC targeting and antigen uptake and evoke an immune response (95).

Ultimately, decorating the carrier surface with relevant receptors or loading relevant antibodies provides a classically effective strategy to target DCs, promote DC maturation, and enhance T cell activation (Fig. 5A). However, the expression of most receptors is not restricted to DCs. Notably, the effective strategies aimed at targeting DCs will require a costimulation; otherwise, immune tolerance would be induced.

LN, especially the tumor-draining lymph nodes (TDLNs), are composed of many different immune cells (i.e., APCs, T cells, NK cells, and B cells), which are an important site to promote antigen presentation and immune cell activation (96, 97). Differentiated T cells also come into play by traveling to the tumor site from the LNs, where they are initially activated (96, 97). Moreover, TDLN, located downstream of the tumor, is the first tissue to metastasize in cancer (98). Therefore, designing an LN-targeting cancer vaccine carrier is another important strategy to initiate immune responses and inhibit the tumor recurrence and metastasis.

First, leveraging materials that exhibit intrinsically high LN accumulation has attracted much interest in targeting LNs (97). For instance, serum albumin can barely disseminate into systemic circulation because of its large size, thus exhibiting nearly 100% direct drainage to LNs (99). Moreover, some studies have shown that vaccines targeting the LNs depend on the size of the vehicle, as well as other characteristics, such as the surface charge, shape, and composition (Fig. 5B). Because of the size of capillaries and lymphatic capillaries, the size of carrier vehicles around 10 to 200 nm can passively drain

to LNs. Neutral or negative charge on the carrier vehicle and hydrophilic materials can also improve the ability to target LNs (97, 100, 101). Although the implanted and injectable macroscopic hydrogels by themselves do not have the ability of targeting LNs because of their large size and hydrophilicity, they can modulate the ability of forming a reservoir for immune cell recruitment and continuous drainage to the LNs as mentioned before (9, 10, 65, 66).

In addition, loading LN-targeted nanoparticles in hydrogels or preparing nanohydrogels (nanogels) is another strategy to endow hydrogels with the ability to target LNs. Qin and colleagues (54) have developed an  $\alpha$ -cyclodextrin-based gel system containing DOX, the photothermal agent indocyanine green (ICG), and LN-targeted and DC-targeted CPG-SS-M nanoparticles. The CPG-SS-M nanoparticles were obtained by the mannopyranoside- and CPG-modified reductive-responsive polyamidoamine (PAMAM) dendrimer. Upon irradiation, the thermal-responsive hydrogels released the loaded cargo. ICG-induced tumor ablation and the DOX-induced ICD produced a lot of antigens, resulting in the rapid recruitment of immune cells by forming an antigen repository. The CPG-SS-M nanoparticles could adsorb large amounts of antigens drained to the LNs because of their neutral charge and small size. Meanwhile, the CPG-SS-M nanoparticles targeted the DCs with the mannopyranoside modifications. Last, the hydrogel-based LN-targeted cancer vaccine successfully stimulated a robust antitumor-specific immune response (Fig. 5C).

Alternatively, an anionic carboxyl group-modified cholesterol-bearing pullulan (CHPCOOH) nanogel vaccine was synthesized for cancer therapy (102). Miura *et al.* demonstrated that the CHPCOOH nanogels had the ability to deliver antigens to the LNs and APCs because of their small size, spherical shape, and negative charge (Fig. 5, D to F). Specifically, the negative charge can not only promote the vehicle transfer to LNs from the extracellular matrix of the interstitium but also suppress nonspecific interactions with cells and act as a ligand for scavenger receptors (SRs) that are expressed on APCs (100). The nanogel ultimately activated the T<sub>H</sub>1 immune pathway and showed a strong CTL response, suggesting a promising therapeutic cancer vaccine.

Interestingly, recent studies have shown that a specific subset of LN macrophages may also play a major role as APCs in tumor vaccination, which also have the cross-presentation ability (103). Muraoka *et al.* (104) developed a nanogel-based immunologically stealth vaccine to target draining LN via small size and uncharged surface. The nanogel was preferentially swallowed by medullary macrophages and evaded capturing by other macrophages and DCs via immunological stealth. This study provides an alternative strategy to design LN-target cancer vaccines. In addition to targeting LNs and DCs to enhance antitumor immunity, the strategies to target NK cells, B cells, and T cells can also be applied to cancer vaccines. In general, the strategies to target other immune cells are based on the interaction between corresponding ligands and receptors. Notably, the strategies for targeting T cells will be described in detail below.

### Strategies to target CTLs

CTLs play a critical role in cancer vaccine-induced antitumor immunity by attacking and mediating cytotoxic effects on tumor cells. A vaccine with the ability to target CTLs can be a good way to enhance antitumor immunity. However, the induction of CTLs is the result of multiple signal interactions on DCs, including peptide-MHC I complex and TCRs, costimulatory signals (CD80/CD86 with CD28, CD40 with CD40L, etc.), and cytokine signals (Fig. 4B) (70). The lack of the costimulatory signals in T cell activation might



promote the development of regulatory T cells that is related to immunosuppression (72). Therefore, designing hydrogels loaded with multiple signal molecules to specifically activate CTLs is necessary to enhance the CTL-induced immune response and alleviate immunosuppression.

For the first signal, loading antigenic peptide fragments that can bind MHC I or peptide–MHC I complexes in hydrogels is efficient in inducing proper CTL immune response. The second and third signals can be provided by encapsulating costimulatory molecules (CD80/86) and adjuvants such as alum or TLR agonists (lipid A, CpG, and poly I:C). Adjuvants can induce costimulatory molecules (i.e., CD40, CD80, and CD86) on the surface of DC and also promote T cell maturation by stimulating the release of cytokines (i.e., IFN- $\alpha$ , IFN- $\gamma$ , and IL-12) by DCs (2, 5, 46, 90, 105).

Song *et al.* (90) demonstrated that an injectable polypeptide hydrogel encapsulating tumor cell lysate (TCL) and TLR3 agonist could enhance potent CTL responses against melanoma. Highton *et al.* (105) designed a chitosan hydrogel vaccine containing the model protein antigen, ovalbumin, and adjuvant, Quil-A (QA), to generate long-lasting memory CD8<sup>+</sup> T cells via sustained release of ovalbumin and QA. Notably, simultaneous delivery of multiple signals greatly increases the antitumor immune response. Kapadia *et al.* (46) designed a redox-sensitive hydrogel composed of PRINT NPs (particle replication in nonwetting template nanoparticles) co-conjugated with MHC I epitope (SIINFEKL) and a TLR-9 agonist. The hydrogel released the loaded cargo by breaking the redox-sensitive linkers via the intracellular reducing environment. SIINFEKL induced cross-presentation of SIINFEKL via MHC I protein molecules, providing the first signal for T cell activation. The TLR9 agonist induced the DC maturation and up-regulated CD80 and CD40, providing the costimulatory molecules for the second signal. Both of the above facets could also induce IFN- $\gamma$  as the third signal. Ultimately, this hydrogel-based vaccine elicits potent CTLs, resulting in strong antigen-specific T cells and cytotoxic activity.

### Modulating the immunosuppressive microenvironment

The TME, composed of tumor cells, immune cells, matrix cells, extracellular matrix (ECM), and various soluble molecules, plays an important role in the development of the tumor (106). In the TME, a variety of immunosuppressive immune cells infiltrate into the tumor under the attraction of chemokines and cytokines, resulting in a high level of immunosuppression. The unique characteristics of the TME (i.e., low pH and hypoxia), the interaction between immune cells and tumor cells, the interaction between immune cells and immune cells, and the secretion of immunosuppressive factors are all contributing factors for maintaining an immunosuppressive microenvironment and inducing immune tolerance, thus greatly limiting the effectiveness of cancer vaccine-induced immunotherapy (62). Therefore, modulating the immunosuppressive microenvironment has gained much attention for enhancing the efficacy of cancer vaccines. Strategies that address this problem are primarily divided into the following two types: alternating the TME and blocking the immunosuppressive environment (Fig. 6A).

#### Alternating the TME

The abnormal metabolism of tumor cells and the chaotic vascular system in the TME can lead to nutrient depletion and hypoxia, resulting in unique physicochemical properties for an environment that is weakly acidic and hypoxic. These physicochemical properties are involved in the induction of immunosuppression and the immune escape process, ultimately leading to tumor growth and metastasis

(107). Therefore, alternating the physical and chemical properties of the TME to reverse immunosuppressive conditions will greatly promote the development of tumor vaccines.

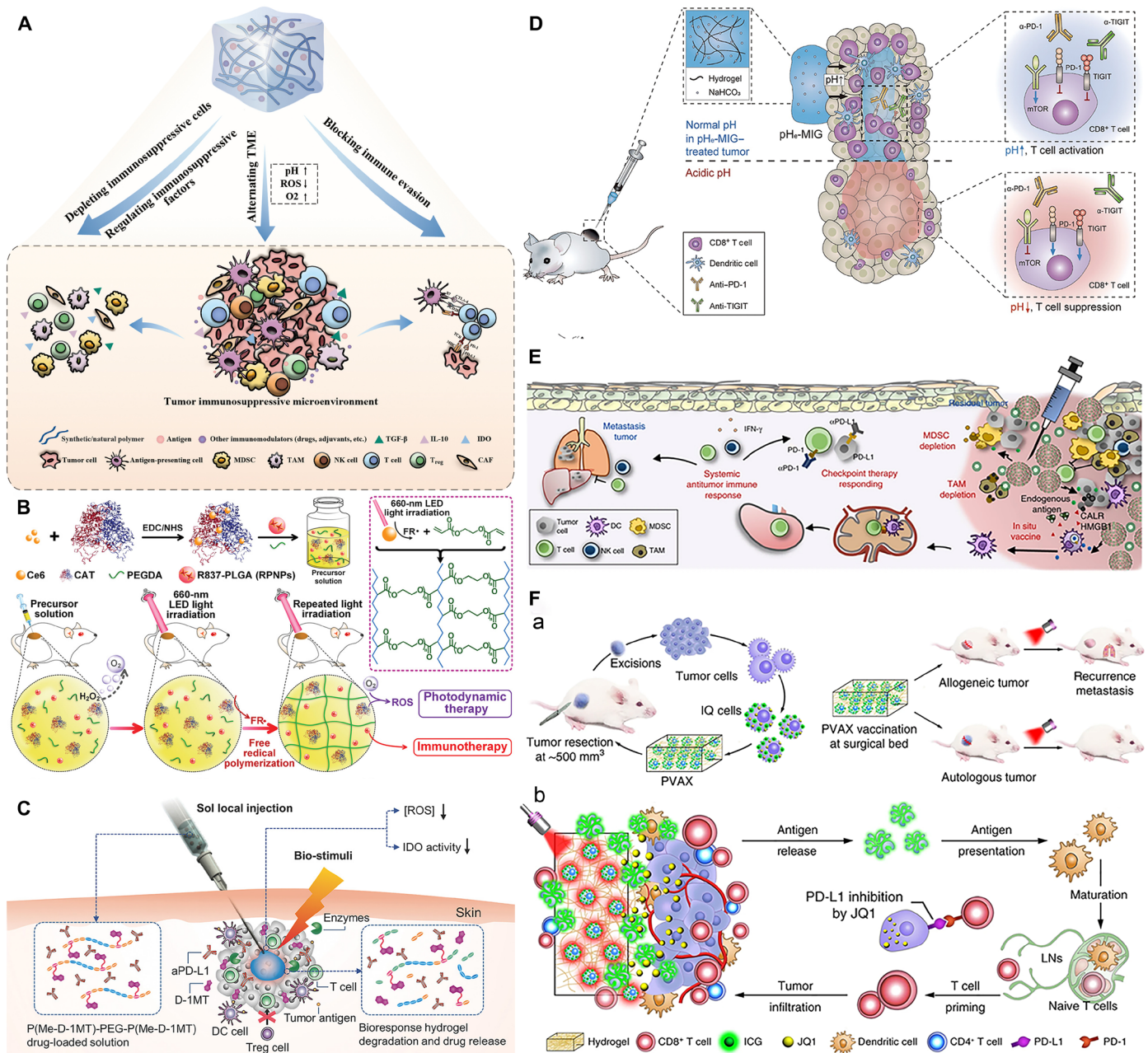
Tumor hypoxia is frequently induced by the large amount of oxygen consumed in tumor growth and the insufficient supply of internal new blood vessels, resulting in increased interstitial fluid pressure (108). Hypoxia has been considered as the signal of abnormal metabolism in the tumor and has a notable influence on the tumor growth, proliferation, angiogenesis, invasion, and metastasis (108, 109). Moreover, hypoxia is closely associated with immune evasion and suppressing CTL-mediated tumor cell responses (108, 109).

A number of studies have attempted to use carriers to produce oxygen and increase the local oxygen levels at the tumor site to relieve immunosuppression and enhance the therapeutic efficacy (108, 110, 111). PDT, an effective strategy for tumor therapy, uses a photosensitizer to generate reactive oxygen species that attack the tumor, which is also limited by the hypoxic microenvironment (112). Therefore, the combined treatment of modulating the hypoxic microenvironment and PDT can not only improve the efficacy of PDT but also reduce the immunosuppression, enhance the antitumor immunity, and inhibit tumor recurrence and metastasis. Once the hydrogels are loaded with a substance that supplies oxygen, long-term oxygen supply can be achieved to alleviate hypoxia and modulate the immune response.

On the basis of this, Zhou *et al.* (110) designed a prolonged oxygen-generating phototherapy hydrogel (POP-Gel) loaded with photosensitizers, calcium superoxide (CaO<sub>2</sub>), and catalase (CAT) that can generate oxygen for up to 5 days. First, POP-Gel showed the great potential as a powerful internal antigen repository through PDT-induced tumor ablation. Notably, POP-Gel observed a more robust antitumor immune response compared to the phototherapy hydrogel (P-Gel) by improving immunosuppression with a sufficient oxygen supply. Last, POP-Gel significantly stimulated an antitumor immune response and inhibited tumor proliferation and metastasis under the dual strategy of PDT and hypoxia alleviation. Meng *et al.* (111) manufactured a light-triggered in situ gelation system containing photosensitizer-modified CAT and an immunoadjuvant together with poly(ethylene glycol) double acrylate (PEGDA) as the polymeric matrix (Fig. 6B). CAT catalyzed tumor endogenous H<sub>2</sub>O<sub>2</sub> to produce O<sub>2</sub> and alleviate hypoxia. Similar to the previous example, the hydrogel system promoted the efficacy of PDT to induce tumor antigens, modulated immunosuppression, and evoked a robust antitumor response in combination with an immunoadjuvant.

Tumor hypoxia can also promote the level of reactive oxygen species (ROS) produced by the metabolic processes of the mitochondria or peroxisome (113). ROS plays multiple roles in cancer as a signal molecule affecting various physiological processes such as mutagenesis of DNA, inhibition or activation of proteins, apoptosis, and inflammation (113). Although locally high concentrations of ROS are used to kill tumor cells in tumor PDT therapy, high levels of ROS throughout the microenvironment suppress immune responses via regulatory T cell-mediated tumor immunosuppression (114). Furthermore, elevated ROS is associated with regulating PD-1 expression and the polarization of macrophages (114, 115). Therefore, regulating ROS can promote the polarization of the immunosuppressive M2 macrophages to M1 macrophages and down-regulate PD-1 to avoid immune invasion.

Notably, the ROS-responsive release hydrogels can not only release the loaded cargo to stimulate the immune response but also



**Fig. 6. The strategies for improving the tumor immunosuppressive microenvironment. (A)** Schematics of the strategies for improving the immunosuppressive TME using hydrogels. **(B)** Schematics of the light-triggered formation of Ce6-CAT/PEGDA hydrogel in tumor of mice. Reprinted with permission from (111). Copyright 2019, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. **(C)** Schematics of biostimuli-triggered aPD-L1 and D-1MT localized delivery based on the injectable thermosensitive hydrogel for tumor microenvironmental regulation and immunotherapy. Reprinted with permission from (15). Copyright 2018, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. **(D)** Schematic overview by which the pH-MIG normalizes the acidic TME and potentiates immune checkpoint inhibitor therapies. Reprinted with permission from (120). Copyright 1969, Elsevier. **(E)** Schematic illustration of injectable immunomodulatory multidomain nanogels for depletion of MDSCs and TAMs to enhance antitumor immunity. Reprinted with permission from (124). Copyright 2019, the Author(s). **(F)** Schematic of a personalized cancer vaccine for blocking the immunosuppressive microenvironment using JQ1 to inhibit the recurrence and metastasis in postoperative breast cancer. Reprinted with permission from (136). Copyright 2018, the Author(s).

consume the ROS to modulate the immunosuppressive microenvironment. Yu *et al.* (15) synthesized a ROS-responsive hydrogel composed of functional triblock copolymers comprising a central PEG block flanked by two polypeptide blocks containing ROS-responsive L-methionine (Me) and D-1MT [designated as

P(Me-D-1MT)-PEG-P(Me-D-1MT)]. When injected into the tumor, the hydrogel underwent sol-gel transformation and released indoleamine 2,3-dioxygenase (IDO) inhibitors and anti-PD-L1, as well as modulated the ROS level in the intratumoral microenvironment. The consumption of ROS by poly(L-methionine) combined

with the release of IDO inhibitors and anti-PD-L1 resulted in enhancing antitumor efficacy, improving survival of T cells, and reducing immunosuppressive properties (Fig. 6C). Alternatively, Chen *et al.* (116) used an albumin-based complex mixing anti-PD-1 (aPD1) and anti-CD47 (aCD47) with ROS-responsive linkers, which can reverse the immunosuppressive microenvironment and promote effective antitumor immune responses.

Acidic extracellular pH is another unique characteristic of the TME, primarily due to the accumulation of lactic acid produced by glycolysis of tumor cells and CO<sub>2</sub> produced by respiration, which can be considered as a by-product of tumor hypoxia (117, 118). The acidic tumor environment plays an important role in avoiding immune surveillance and inducing immunosuppression by blunting T and NK cell activation, as well as recruiting more immunosuppressive myeloid-derived suppressor cells (MDSCs) (119). Mechanistically, acidity in the TME is shown to up-regulate coinhibitory immune checkpoint receptors and block mechanistic targets of rapamycin (mTOR) signaling pathways in memory CD8<sup>+</sup> T cells, which leads to T cell dysfunction and tumor escape (118–120).

Lactic acid, one of the main contributors to the acidic microenvironment, has been demonstrated to directly induce and recruit immunosuppression-related cells and molecules, ultimately promoting tumor development (121). For example, lactic acid can promote the polarization of tumor-associated macrophages to the M2 type that promote the tumor growth (121). In addition, lactic acid prevents DCs from differentiating and makes them tolerant (121). Moreover, the low pH and high extracellular lactic acid prevents the transport of intracellular lactic acid via monocarboxylic acid transporter, resulting in inhibiting T cell activation, proliferation, and cytotoxicity (119). Therefore, altering the acidic pH in the TME, particularly lactic acid, is urgently needed to overcome tumor immunosuppression.

Delivery of a monocarboxylate transporter inhibitor and relative drugs to restrain the lactic acid efflux to remodel the tumor acid metabolism has attracted much attention in improving T cell immunotherapy (122). Alternatively, hydrogels can also enhance the antitumor response by physically or chemically encapsulating pH regulators and monocarboxylate transporter inhibitors. For example, Jin *et al.* (120) was able to use thermosensitive hydrogels (PHe-MIG) loaded with NaHCO<sub>3</sub> to modulate the acidic pH microenvironment (Fig. 6D). Normalization of the acidic TME through the release of NaHCO<sub>3</sub> enhanced the cytotoxic immune cell infiltration and improved the efficacy of anti-PD-1 and anti-TIGIT blockade therapies. Similarly, Huo *et al.* (123) introduced CaCO<sub>3</sub> into a DC vaccine to increase the pH of the TME and promote the polarization of M2-type macrophages to M1-type macrophages, thus reversing the immunosuppressive microenvironment and relieving the immunosuppressive effect on T cells. The resulting hydrogel vaccine evoked a strong antitumor immunity through the continuous stimulation of released tumor-associated antigens and the reversal of the immunosuppressive microenvironment, showing the great potential in suppressing tumor recurrence and metastasis. Cancer vaccines that change the pH to modulate the immunosuppressive microenvironment will provide a unique strategy to improve immunotherapy.

#### **Blocking the immunosuppressive microenvironment**

Immunosuppressive cells, including MDSCs, M2 tumor-associated macrophages (TAMs), regulatory T cells (T<sub>regs</sub>), and cancer-associated fibroblasts (CAFs), play a dominant role in the TME (62, 107). These immunosuppressive cells and immunosuppressive factors contribute to an immunosuppressive microenvironment that restricts

the function of infiltrating APCs and T cells, resulting in immune tolerance, immune escape, and weak antitumor T cell immunity. Thus, directly blocking the immunosuppressive microenvironment is another effective method to improve antitumor responses compared to altering the physical and chemical properties of the TME mentioned previously.

Depletion of these immunosuppressive cells via hydrogel-encapsulated immunomodulatory drug-induced death [i.e., gemcitabine, clodronate, and 6-thioguanine (62)] provides the possibility to directly remodel the tumor immunosuppressive microenvironment. For instance, Song *et al.* (124) developed an injectable immunomodulatory multidomain nanogel (iGel), which is formed by electrostatic interactions with negatively charged nonconcentric multi-nanodomain vesicles (MNDVs) loaded with immunomodulatory drugs [gemcitabine and hydrophobic imiquimod (R837)] and positively charged clodronate-cationic nanoliposomes (Fig. 6E). iGel demonstrated the ability to reshape the tumor immunosuppressive microenvironment via the spatiotemporal release of gemcitabine to deplete MDSCs, the clodronate-cationic nanoliposomes to deplete TAMs, and immunostimulants to recruit DCs. Similarly, Phuengkham *et al.* (125) also demonstrated a local peritumoral implantation of the synthetic immune niche (iCD) based on blocking the immunosuppressive microenvironment, which contained gemcitabine and nanoadjuvants carrying poly(I:C) with tumor lysate-based antigens. Through sustained release of gemcitabine, the iCD can significantly improve the limitations on T cell function, resulting in a strong antitumor immune response, assisted by the activation of tumor lysate and adjuvants. This study demonstrated that the cancer vaccine can prevent primary tumor recurrence and metastasis in an advanced-stage primary 4T1 breast tumor model.

In addition, many immunosuppressive factors are also involved in immunosuppression (i.e., TGF- $\beta$ , IL-10, IDO, and adenosine), due to their direct and indirect involvement in antigen presentation, activation, and proliferation of T cells (62, 107). Using hydrogels to deliver and release inhibitors of these factors may reprogram the immunosuppressive condition. IDO, an immunosuppressive enzyme, is involved in catalyzing the tryptophan degradation through the kynurenine pathway (126). Overexpressed IDO in tumors can activate suppressive T<sub>regs</sub> and prevent T cell activation due to the lack of tryptophan and higher concentration of kynurenine metabolites (127). Recent studies have shown that combining IDO inhibitors with the other strategies for blocking the immunosuppressive microenvironment could be synergistic and more effective (15, 128, 129). As mentioned previously, Yu *et al.* (15) reprogrammed the microenvironment by consuming ROS at the tumor site. An IDO inhibitor was also encapsulated in the hydrogel to provide synergistic effects for blocking the T<sub>reg</sub> activation. Similarly, adenosine, hydrolyzed from ATP, is involved in tumor immune evasion by binding with A2A adenosine receptors (A2AR) on the surface of T cells, known as the adenosine-A2AR negative feedback pathway (130). Inhibition of adenosine generation and signaling pathways have been shown to effectively block the adenosine-A2AR pathway (131). Recently, Zhao *et al.* (132) introduced adenosine deaminase (ADA) into hydrogels, which enabled the catalytic transformation of adenosine (immunosuppressor) into inosine (an immunopotentiator), thus reversing tumor immunosuppression and enhancing antitumor immune responses.

Furthermore, immune checkpoints (PD-1 and CTLA-4) expressed in tumor cells can cause T cell dysfunction, resulting in immune evasion (Fig. 4B) (133). The immune checkpoint blockade therapy (i.e., anti-PD-1/PD-L1 and anti-CTLA-4) can prevent the termination



of the immune response by blocking the immune checkpoint expression and arousing the exhausted T cells, which has been widely used in tumor immunotherapy (133–135). Thus, hydrogels loaded with the inhibitors of immune checkpoints can reverse the immunosuppressive microenvironment and enhance antitumor immunity in cancer vaccines. For example, Wang *et al.* (136) presented a personalized cancer vaccine (PVAX), which effectively inhibits the recurrence and metastasis in postoperative breast cancer. JQ1, a BRD4 inhibitor, and ICG, a photothermal molecule, were co-loaded in tumor cells and then encapsulated in a tumor-penetrable peptide hydrogel, named PVAX (Fig. 6F). Upon laser irradiation, PVAX can induce the release of JQ1 and tumor antigens by ICG-mediated photothermal ablation. The continuous release of antigens from PVAX can promote the maturation of DCs and the infiltration of CD8<sup>+</sup> T cells. Notably, JQ1 down-regulates the expression of PD-L1 in tumor tissues and tumor cells to block the PD-1/PD-L1 immune checkpoint pathway, avoiding T cell dysfunction, ultimately enhancing the antitumor immune response of PVAX. Chao *et al.* reported an alginate hydrogel composed of catalase, CpG, and anti-CTLA-4 antibody can significantly elicit the antitumor efficacy by remodeling CTLA-4-mediated immunosuppression in CD8<sup>+</sup> exhausted T cells (137).

In conclusion, the tumor immunosuppressive microenvironment is the result of a complex and dynamic combination of many factors. Altering a single factor to remodel the immunosuppressive TME has limited efficacy. Simultaneously blocking multiple immunosuppressive pathways in the TME could be the effective approach to improve the efficacy of immunotherapy, especially in cancer vaccines. For hydrogel-based vaccines, the large loading capacity and controlled release properties can be used to encapsulate a large number of immunomodulators, antigens, and adjuvants to overcome the immunosuppressive TME. Moreover, it is necessary to pay attention to the interaction between the loads and the order and speed of release, which might have a notable influence on the efficacy of hydrogel-based cancer vaccines.

## CONCLUSIONS AND OUTLOOK

In this review, combining the advantages of cancer vaccines and hydrogel delivery, we briefly introduced the recent developments in hydrogel-based cancer vaccines. On the basis of the difficulties associated with inducing an effective antitumor immune response using hydrogel-based vaccines, we have proposed corresponding solutions based on the critical steps in the cancer vaccine-induced immune response, which are typically achieved by encapsulation or modification of immunostimulants in the hydrogel and controlled in vivo release to stimulate the immune response.

In general, the key challenges for the hydrogel-based cancer vaccine to generate a robust immune response are as follows. (i) Selection of the loads in hydrogels, including antigens and multiple immunomodulators: Appropriate antigens and immunoadjuvants are the basis of vaccine-initiated antitumor immunity. Immunomodulators can be used to overcome the barriers in immunity and activate the immune response. (ii) The strategy for hydrogel delivery: Antitumor immunity is a complex, continuous process, involved in a variety of cells, cytokines, tissues, and pathways. Thus, it is important to develop strategies that target one or more of the key steps/cells in the immune response, such as DCs, T cells, antigen presentation, and T cell activation. Notably, targeting multiple immune pathways/cells may elicit a more robust antitumor immunity than targeting a

single pathway. (iii) Selection and design of the hydrogel system: The design of hydrogel-based cancer vaccines should take the physical and mechanical properties, safety, and patient compliance into consideration, which directly determines the reliability of the delivery strategy. The hydrogel design can be affected by the polymer, cross-linking agent, cross-linking type, density, structure, and other factors (138–140). Therefore, selecting appropriate materials and formulations of hydrogels could further control the syringeability, optimize encapsulation efficiency, and exhibit appropriate release kinetics (138–140). Notably, the release rate of antigens/immunomodulators is a key variable for regulating the immune response with hydrogels. The release rate of payloads is mainly determined by the loading mechanism, gel swelling/degradation, and chemical properties, which can be tuned by adjusting several parameters in hydrogels, such as hydrogel porosity and pore size, cross-linking type and density, expansion, gel-sol transition, permeation property, and mechanical strength (141).

Recently, with the rapid development of hydrogels, biomedical hydrogels have made substantial clinical progress and many have been put into the market for different applications, including antibacterial coatings (142) and scaffold tissue engineering (143), showing the remarkable clinical and translational ability of hydrogels as the delivery system. However, hydrogel-based cancer vaccines have not been clinically approved yet, perhaps due to the technical difficulties in preparation and storage, implantation and injection at specific sites, and the balance between structure and function. Although hydrogel-based cancer vaccines have demonstrated that they can promote DC recruitment, alleviate immunosuppression, and evoke a robust antitumor-specific immune response, there are still many obstacles to overcome as previously mentioned. Recently, the emergence of injectable self-healing hydrogels and shape memory hydrogels has shown rapid development (144). They have greatly improved the mechanical properties and stability of hydrogels in response to the influence of external destructive factors, which might be a direction for designing future hydrogels. More innovative appropriate strategies and preparation methods will still be required to further promote the development of hydrogel-based cancer vaccines.

In addition to hydrogel-based cancer vaccines, numerous nanovaccines have been explored to induce a strong immune response. Several functional nanomaterials, such as liposomes, inorganic nanoparticles, and polymers, have greatly promoted the development of nanovaccines (5, 145). Similar to hydrogel-based cancer vaccines, nanovaccines can load multiple components and protect them from degradation. The loads can be on-demand released from nanovaccines, enhancing the duration of antigen presentation and APC processing and eliciting a downstream immune response. Many nanovaccine carriers have immunogenicity, which can be used as immunoadjuvants to further enhance immune response. Moreover, the targeting ability can be further enhanced by surface modification and size adjusting. Notably, different from hydrogel-based vaccines, nanovaccines have the advantage of nano-sized range, which make it easier to be internalized by APCs (5). Meanwhile, the adjustable size of nanovaccines could improve vaccine effectiveness by increasing the targeting and retention of LNs based on size effects (146). However, the main drawback of nanovaccines is the high clearance rate by the reticuloendothelial system, immune cells, and renal filtration. This clearance rate leads to low localization at target sites, resulting in a weaker immune response (147). Therefore, nanovaccines often require repeated injections to achieve optimal treatment, which greatly reduces patient comfort and compliance. Hydrogels, as a reservoir

for cancer vaccines, is directly localized at the target site by local administration or implantation, followed by localized release, which greatly decreases the toxicity and the required dose for an effective immune response (10). Currently, nanocomposite hydrogels have emerged, integrating the advantages of hydrogel-based vaccines and nanovaccines, by improving the physical and mechanical properties, exhibiting durable controlled release locally, improving LN drainage and retention, extending immune response time, and enhancing efficacy of the vaccine (148, 149). Nanocomposite hydrogels are also predicted to become increasingly more used in applications outside of cancer vaccines, due to their unique advantages as mentioned.

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