Chapter 3

New Strategies to Improve Therapeutic Vaccines

Chunsong Yu and Haipeng Liu

Additional information is available at the end of the chapter

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Abstract

Vaccination represents a viable and attractive strategy for therapeutic treatment of cancers by the power of a patient's own immune system. Major advances in cellular and molecular immunology have led to the approval of the first therapeutic cancer vaccine by FDA. However, the development of cancer vaccines remains infant. Maximizing the therapeutic efficacy while minimizing side effects of the therapeutic cancer vaccine remains key challenges to this field. In this review, we summarized the recently developed strategies to induce anti-tumor responses *in vivo* to improve the outcomes of cancer vaccines, with an emphasis on the guiding principles that are critical for rational design of effective and safe vaccines against cancers.

Keywords: immunotherapy, cancer vaccine, anti-tumor immunity, APCs, effector T cells

1. Introduction

Recent understanding in cancer immunology and the development of cancer immunotherapy have remarkably advanced the clinical treatment of cancer, leading to US Food and Drug Administration approvals of cell-based immunotherapies (Provenge, Kymriah, and Yescarta), and immune checkpoint inhibitors (Ipilimumab, Nivolumab, Atezolizumab, Avelumab), among others. Regardless of the progress, in most immunotherapies for cancer patients, the response is often of low frequency and moderate avidity, and does not result in objective clinical responses [1, 2]. For example, while immune checkpoint blockade therapies of various cancers yield impressive clinical outcomes, these therapies do not alter the frequency of tumor-specific T cells. Additionally, although dendritic cells (DCs) pulsed with tumor associated antigens can result in the expansion of antigen-specific T cells, the level of responses is often too low to mediate long-lasting tumor destruction [3]. This situation can be remedied with

therapeutic cancer vaccines which are designed to induce or augment the magnitude and quality of antitumor immune responses.

Currently many diverse therapeutic vaccine strategies are under development or being evaluated in clinical trials. Based on their content, they may be classified into different categories, including cell-based vaccines, subunit vaccines, and genetic vaccines. Each of these vaccine platforms targets specific immune pathways and has strengths and weaknesses detailed in our next discussion. One of the major goals for these vaccine strategies is to break the tumor-related immunosuppression. This challenge can be partially addressed by the development of new vaccine strategies, or optimization of current vaccines including the choice of antigen, the immunological adjuvants, formulations for delivery, vaccine efficacy, safety and toxicity considerations. Additionally, preclinical studies have clearly demonstrated that vaccines alone might not be sufficiently potent to overcome the complex immunosuppression within the tumor microenvironment [4]. Therefore, vaccines in combination with other immunotherapies might provide synergistic mechanisms to amplify the therapeutic outcomes. For example, the preclinical success of vaccines combined with immune checkpoint blockade have highlighted the potential to move beyond current paradigms of cancer vaccines [5, 6]. Here we summarize recent strategies to improve therapeutic vaccines for cancer.

2. Immunological background

The immune system is comprised of a network of lymphoid organs, tissues and different types of cells including lymphocytes, dendritic cells and nature killer cells. The immune system plays a crucial role in protecting the body against microbial pathogens and also in restraining the development of cancer [7–9]. Engineering the immune system to provide protective immunological memory (a procedure called vaccination) has been one of the most successful and cost effective medical interventions to date, saving millions of lives every year via pediatric and adult immunizations [9]. The process that immune system responds to foreign pathogens, allergies, self-damaged cells, and graft is called an immune response, which can be generally classified into innate response and adaptive response.

Innate response or nonspecific immune response, recognizes invading pathogens via PAMPs (pathogen associated molecular patterns) that are evolutionarily conserved molecular motifs expressed by a variety of microbes [10, 11]. PAMPs are mainly detected and recognized by innate immune cells through Toll-like receptors (TLRs) and other pattern recognition receptors (PRRs) [10]. Recognition of PAMPs by immune cells including phagocytic cells (macrophages and neutrophil) and antigen presenting cells (APCs) triggers a cascade of signaling pathways and activates these immune cells, promoting phagocytosis of pathogens and providing the first line of defense against many common pathogens. Innate response causes rapid inflammation at the site of infection which results in redness, swelling, heat, and pain. Innate response also plays a crucial role in the initiation of adaptive immune responses [10, 11].

Adaptive response, on the other hand, is referred to as a specific immune response. During adaptive response, highly specialized lymphocytes including T cells and B cells are activated by APCs engulfing and processing pathogens or antigenic molecules associated with pathogens.

Once activated, these lymphocytes undergo proliferation and differentiation into effector cells which can eliminate pathogens or inhibit their proliferation and growth. In addition to specificity, another feature that differentiates adaptive response from innate response is immunological memory which is developed after initial adaptive response to a specific pathogen and can recall specific immune response to the same pathogen in future encounters. Adaptive immune responses are tightly linked to innate immune responses [12]. For example, the TLR stimulus promote maturation of dendritic cells (DCs), the most efficient APCs and trigger the upregulation of costimulatory molecules on DCs for efficient antigen presentation.

Although it appears that adaptive response is more advanced and sophisticated than innate one, their roles in immunomodulation are inseparable and they complement each other in eliciting effective immune response to pathogens. Innate response is generally prerequisite to the activation of adaptive response which in return can enhance innate immunity by effector molecules such as cytokines and antibodies [10–12].

Cancer is one of the leading causes of death worldwide, accounting for more than 8 million death each year [13]. While traditionally cancer is treated with surgery, radiation, or chemotherapy, immunotherapy which harnesses the power of patients' own immune system has come of age over the last decades as a new treatment modality to fight against cancer, with cancer vaccine emerging as a novel approach to cancer treatment [14–18]. Unlike the traditional vaccine by which antibody responses are needed to prevent the disease from developing, therapeutic cancer vaccines heavily rely on cytotoxic T cell responses that are designed for patients with established diseases [14]. The initiation and maintenance of anti-tumor immune responses is a multi-step, complex process that involves the coordinated action of immune cells and molecular signals within the immune system [14]. For example, the induction of systemic antitumor immunity involves the priming of both $CD4^+$ and $CD8^+$ T cells specific for tumor-associated antigens. The process is initiated with antigen uptake by professional APCs especially DCs. In the presence of appropriate immune signals (e.g., TLR ligands), DCs are activated and migrate to LNs, where they present antigen fragments in the context of major histocompatibility complex (MHC) to effector T cells. In the draining LNs, $CD4^+$ and $CD8^+$ T cells recognize peptides presented via MHC class II and MHC class I on DC surface, respectively. And if DCs are properly activated, these T-cells proliferate and differentiate into effector cells that can directly kill cancer cells ($CD8^+$ "killer" T-cells) or secrete cytokines that help other cells ($CD4^+$ "helper" T-cells) [19–21]. Effector T cells traffic to tumor site, recognize tumor cells by T cell receptor (TCR), and secrete cytotoxins such as perforin and granzymes which trigger tumor cell apoptosis. An effective cancer vaccine aims to target these essential steps and reinforce tumor-specific T cells immunity to combat tumors. Adaptive immunity-dominated anti-tumor activities are illustrated in **Figure 1**.

Most vaccines in use today were developed by techniques that were pioneered more than 100 years ago and do not provide protection in many diseases. For example, although highly effective for combating acute infections such as polio, measles and diphtheria, traditional vaccination technologies have failed to elicit immune responses that provide protection against chronic infections (e.g. HIV, malaria) and have not succeeded in therapeutic settings, which are designed to harness the patient's immune system to treat an existing disease (e.g. HIV or cancer). Traditional vaccine approaches induced transient anti-tumor immunity that failed to

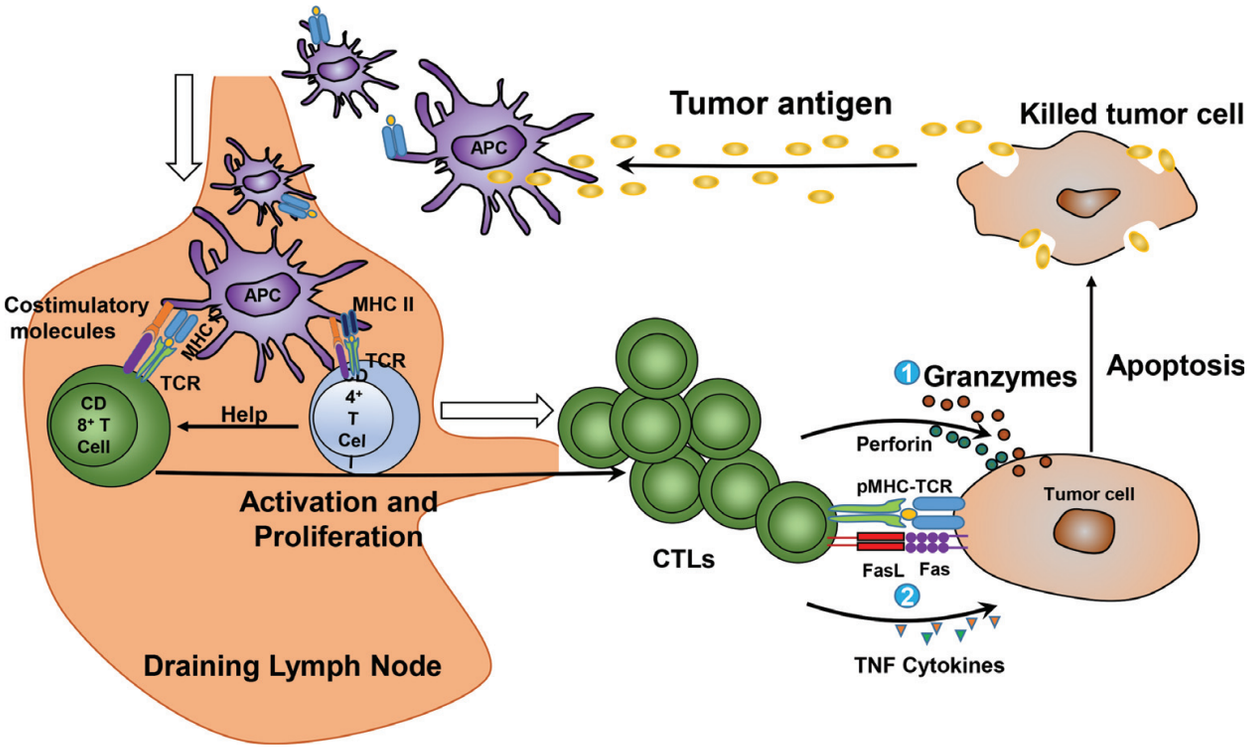

Figure 1. Immune activation of tumor-specific CTLs and the mechanisms of action of CTLs killing tumor cells. APCs acquire tumor antigen, migrate to the draining LNs, and present antigen to T cells in the context of peptide/MHC complex. Activated CTLs traffic to tumor site, trigger the programmed death of tumor cells through the perforin-granzymes pathway or FasL-Fas/TNF-TNFR death receptor pathway.

control tumor growth, primarily due to tolerance mechanisms induced by tumor cells [22]. To shield themselves from immune attack, tumor cells are able to evade the immune detection, recognition and subsequent immune attack through a variety of mechanisms [23]. First, most tumor antigens recognized by cytotoxic $CD8^+$ T cells are encoded from "self". Self-antigens expressed by solid tumors are intrinsically nonimmunogenic and do not efficiently stimulate naïve T cells. As a disease of mutations, the genetic instability or changes in cancer cells may potentially promote the generation of tumor antigen variants that are theoretically recognized as "non-self" by the immune system [24]. Thus, cancer vaccines that introduce neoantigens or tumor cell variants are promising in the induction of effective anti-tumor immune responses. Second, survived tumor cells have acquired the ability to resist immune recognition by expressing low level or defective MHC molecules, leading to insufficient antigen presentation [23]. Third, the upregulation of immune checkpoint ligand programmed death-ligand 1 (PD-L1) on tumor cells also leads to inactivation of effector T cells [23]. Accordingly, inhibitors of immune checkpoints, which target the PD-L1/PD-1 pathways, might reinforce the potency of immune response induced by cancer vaccines. In addition, tumor cells can produce suppressive cytokines including VEGF (vascular endothelial growth factor), TGF-β (transforming growth factor-β) and IL-10 (interleukin-10) to develop an immunosuppressive microenvironment, which further inhibits the activation and functions of tumor-specific effector cells [23]. These suppressive cytokines in turn recruit regulatory immune cells, especially regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) [23]. Typically, Tregs and MDSCs function as major effectors of immunosuppression to inhibit host-protective anti-tumor

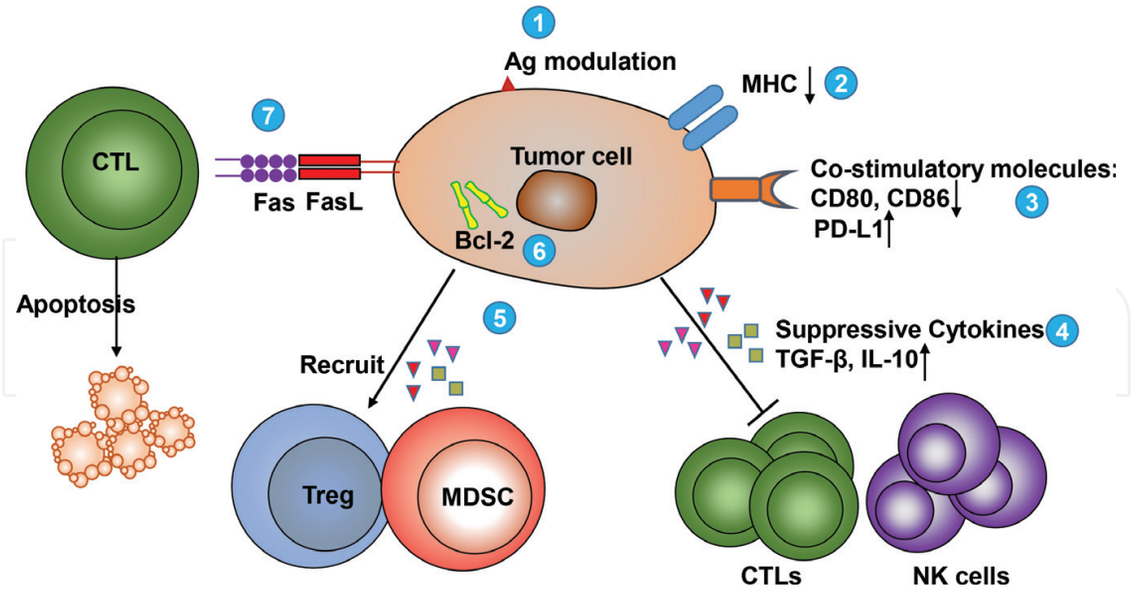

Figure 2. Mechanisms associated with immune escape of tumor cells. Fundamental Ag (antigen) modification leads to compromised immunogenicity of tumor cells (1); downregulation of MHC molecules on tumor cells also reduces the chance of tumor antigen presentation (2); abnormal expression of co-stimulatory molecules CD80, CD86 and PD-L1 leads to the inactivation or anergy of effector T cells (3); suppressive cytokines e.g., TGF-βand IL-10 produced by tumor cells inhibit the proliferation of effector CTLs and NK cells (4) but stimulate regulatory cells (Treg) and MDSC to expand, creating an immunosuppressive microenvironment (5); intracellular overexpression of anti-apoptotic molecules Bcl-2 prevents tumor cells from immune response-induced apoptosis (6); FasL expressed on tumor cells in turn induces the programmed death of CTLs through death receptor pathway (7).

immune response by secreting suppressive cytokines IL-10 and TGF- β, and by expressing high level of co-inhibitory molecules cytotoxic T-lymphocytes-associated protein 4 (CTLA-4) and PD-1 [23]. Administration of molecular adjuvants such as TLR agonists, which promote the production of proinflammatory cytokines, could be an attractive approach to neutralize the impact of suppressive cytokines modulated by tumor cells. Finally, to escape immune destruction, tumor cells cunningly overexpress anti-apoptotic proteins, such as B-cell lymphoma 2 (Bcl-2), which regulate cell death and protect themselves from immune response-induced apoptosis [25]. In parallel, FasL expressed on tumor cells binds to Fas on CTLs and directly causes the apoptosis of CTLs [26]. Collectively, as demonstrated in **Figure 2**, a combination of these underlying mechanisms ultimately contribute to the immune escape of tumor cells, which have posed challenging and complicated hurdles for the development of cancer vaccines. To improve the therapeutic efficacy of cancer vaccines and break the tolerance in tumors, the orchestration of therapeutic strategies that induce long-lasting antitumor immunity and overcome immune escape is the key for a successful treatment.

3. Cell-based cancer vaccines

Dendritic cells (DCs) are professional APCs that play a pivotal role in the regulation of cell-mediated immunity, and thus are key targets in cancer vaccine design [16–18]. The promising results from clinical trials recently have led to the approval of the first DC-based therapeutic

cancer vaccine by FDA [3]. There are generally two approaches to target DCs: *in situ* delivery of antigens via ligands that are specific for endocytic receptors expressed at the surface of DCs and ex-vivo generated antigen-loaded DCs. Though the latter approach requires laborious and expensive manipulation, immunotherapy based on *ex-vivo* tumor antigen loaded DCs bypasses the intrinsic dysfunctions of endogenous DCs in cancer patients, enabling the efficient priming of both CD4 and CD8 T cells. One of most successful examples of *ex vivo* DC-based vaccines is the use of sipuleucel-T for treating metastatic prostate cancer [27]. The FDA-approved sipuleucel-T cellular immunotherapy is comprised of autologous peripheral blood mononuclear cells (PBMCs) that are *ex vivo* pulsed with prostatic acid phosphatase (PAP) and activated with granulocyte-macrophage colony stimulating factor (GM-CSF). With sipuleucel-T treatment, the risk of death of patients was reduced by 22% in contrast with that of patients who received the placebo treatment. As a result, overall survival among male patients with metastatic castration-resistant prostate cancer was prolonged via the administration of sipuleucel-T therapy [27]. Despite the fact that DC-based vaccines can induce T cell responses, objective clinical responses are low and DC-based vaccinations have not met their expectation as an effective modality in treating other cancers [3]. Several factors might be limiting the efficacy of current DC vaccines: the types and sources of DC, the route of injection, and the migration to LNs. It has been estimated that less than 5% of the injected DCs reach the LNs [28], the anatomic sites where the immune responses are orchestrated. To overcome the insufficient migration of DCs, intranodal (IN) administration of DCs has been explored. In several clinical studies [29, 30], IN administration of mature DCs appeared to be safe, and resulted in superior T-cell sensitization.

Another challenge associated with DC vaccine is the insufficient antigen-presentation by DCs. Recent research suggests that high affinity [31, 32] and prolonged peptide–MHC presentation [33–35] of targeted epitopes are required for effective tumor eradication and tumor stroma destruction by specific T cells, presumably through the persistent T cell stimulation. However, DC pulsed with tumor associated peptides exhibits low T cell affinity and short half-lives of peptide–MHC complexes due to the clonal deletion of high affinity T cells and dissociation of peptide from MHC, respectively. In the later scenario, peptide degradation and rapid MHC turnover, leading to weak and transient T cell stimulation [36]. In addition, matured DCs loss their ability for antigen uptake and processing. This has posed a major barrier to the development of effective DC-based vaccines in clinic. Attempt to improve and stabilize MHC epitopes on DC surface has encompassed the use of altered peptide ligands (APLs) [37, 38], which incorporates mutated amino-acids in MHC anchor residues, and genetic modifications, which reprogram dendritic cells to express tumor antigens [39, 40].

Whole tumor cell vaccine is another cell-based vaccination approach currently in preclinical development and clinical trials. In this approach, tumor cells are modified to prevent replication and administered to patients to induce antitumor immune responses. The efficacy of whole-tumor cells vaccine has been investigated for more than 20 years [41]. One of the key advantages of using whole tumor cells as vaccine is that the cells provide a source of all potential antigens including neoantigens, eliminating the need for antigen identification. GVAX, by which tumor cells are genetically modified to overexpress granulocyte macrophage colony stimulating factor (GM-CSF), irradiated and adoptively transferred back to the patient,

is an early example of tumor cell vaccine. A meta-analysis about 1800 patients revealed that patients treated with whole tumor vaccines showed a more robust objective response—8.1% than those immunized with formulated tumor antigens—3.6% [42]. Prostate GVAX vaccine is an excellent example of whole tumor vaccines. In a clinical trial, administration of Prostate GVAX vaccines in patients with metastatic HRPC (hormone-refractory prostate cancer) exhibited improved survival of most patients, compared with the treatment of taxane chemotherapy alone [43–45]. Despite the promise, whole tumor cell vaccination typically requires substantial *ex vivo* genetic modification, leading to high cost, long processes, and stability, reproducibility and regulatory concerns. Additionally, immunization with whole-tumor cells has not resulted in significant long-term benefits in both preclinical models and in clinical trials. To address these issues, recently, injectable tumor cell-loaded cryogel sponges which deliver antigen-carrying tumor cells along with GM-CSF and TLR agonist was developed [46]. This biomaterials-based vaccination eliminates genetic modification, yet still delivers key DC activating factors. Immunization with cryogels in mice elicited local infiltration of DCs, which subsequently induced potent, durable T-cell responses in a melanoma model.

Apart from manipulating DCs and tumor cells to activate effector T cells, T cell-based immunotherapy provides a straightforward method to augment tumor-specific T cell immunity. One outstanding example of this therapy is adoptive T cell therapy, which involves the *ex vivo* manipulation and proliferation of antigen-specific T cells. Using this technique, two CAR (chimeric antigen receptors) T cell therapies have recently been approved by FDA. The approved therapies are targeted CD19, which is a common marker of lymphoma cells, to treat relapsed and refractory diffuse large B-cell lymphoma (DLBCL) and acute lymphoblastic leukemia (ALL), respectively [47]. The CAR T cell therapy features the structural modification on autologous T cells to target virtually any tumor antigens. In general, T cells are engineered with the CAR structure which consists of a target element, scFv (single-chain variable fragments), and co-stimulatory domain and essential activation signaling domain (**Figure 3**). In the most recent approved CAR T therapy, patients with relapsed and refractory ALL were infused with autologous T cells transduced with a CD19-directed CAR, and 90% of them succeeded in complete remission [48]. Although adoptive T cell therapy has achieved remarkable efficacy in leukemia, it is less successful when this therapy is applied to solid tumor partially due to immunosuppression and rapid dysfunction of transferred effector T cells. To overcome these obstacles, a recent study demonstrated T cell surface coupling of nanoparticles loaded with IL-15 and IL-21 which fuel the T cells and boost the cell-based therapy [49]. Further study from the same group demonstrated that targeting TGF-β inhibitors to adoptive T cells via immunoliposomes greatly enhanced tumor-specific T cell immunity and significant B16F10 tumor regression in comparison to free adoptive T cells. This study suggested a complementary factor to maximize the efficacy of adoptive T cell therapy in cancer treatment [50].

Although cell-based therapy is a promising and effective strategy for cancer treatment, there are still several drawbacks related to this type of therapy. For example, *ex vivo* manipulation on DCs or T cells is labor intensive and expensive, plus the safety concerns about CARs in clinical trials [51]. A promising strategy to simultaneously overcome the cost and safety limitation is to create effective CAR T cells *in vivo* without T cell isolation. Recently, nanoparticles carrying genetic materials was delivered to T cells in mice. This approach avoided the tedious and

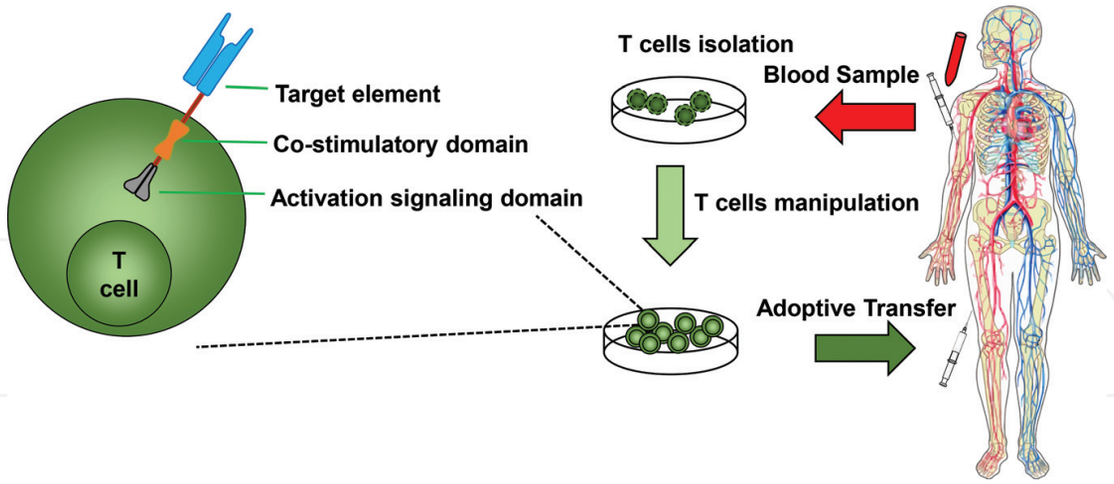

Figure 3. The general idea of CAR T cell therapy. T cells are isolated from patients' blood and subsequently engineered with a special CAR; genetically modified T cells are then expanded *ex vivo* and adoptively transferred back to patients.

expensive *ex vivo* T cell manipulation [52]. More research may be needed to demonstrate the efficacy and safety of this new *in situ* approach in humans in the future.

4. *In situ* vaccines

As some cancer therapies may fail in most patients with solid tumors, *in situ* vaccination can provide another prospect of driving a systemic anti-tumor immunity. *In situ* vaccination exploits local intratumoral treatment to simultaneously destruct tumor tissue and provides the immune system with an antigen source for the induction of antitumor immunity [53, 54]. Unlike traditional vaccines where selected tumor-associated antigens are used, *in situ* vaccination exploits complete tumor-related antigenic repertoire, including tumor-specific neoantigens derived from non-synonymous mutations [55]. Further, *in situ* vaccines can set the stage for potent antitumor immunity by inducing inflammation and facilitating the recruitment and activation of immune cells to the tumor. Thus, *in situ* vaccine approach provides opportunities for broad, more effective and less toxic treatment strategies to promote systemic antitumor immunity. This approach also bypasses the difficulties of isolating and preparing individualized vaccine *ex vivo*, providing a personalized treatment for cancer patients.

A variety of intratumoral treatments (e.g., radiation, cryotherapy) have been delivered directly to the tumors to induce tumor cell death, release tumor antigens while providing pro-inflammatory signals, which result in systemic activation of anti-tumor T cell responses, followed by inflammatory infiltration of T lymphocytes into the tumor [55–59]. While these early studies demonstrate the potential of *in situ* tumor destruction in promoting both T cell and humoral responses, the efficacy and wide-spread adoption of *in situ* vaccination have been limited. The major challenge lies in the relatively weak antitumor immunity following primary tumor destruction. For example, radiofrequency ablation or cryotherapy allows *in situ* tumor destruction and releases large amount of tumor antigens, but only induce a weak and transient immune response

which fails to prevent tumor relapse [57]. Preclinical and clinical studies combining tumor ablation with local administration of CpG-containing oligonucleotides (single-stranded oligonucleotides containing unmethylated cytosine-guanine motifs that bind TLR-9and serve as potent molecular adjuvants) can boost the induction of systemic antitumor effects [57]. Recent results of clinical trials and pre-clinical models demonstrated that intralesional treatment with cytokines, small drugs of immune checkpoint and radiation led to systemic anti-tumor immunity with limited toxicity [60, 61]. In Phase I/II clinical trial in non-Hodgkin's lymphoma, treatment of intratumoral injection of CpG and low-dose radiation safely induced objective responses at distal non-treated sites in nearly 30% of patients [62]. However, rapid dissemination of unformulated CpG from injection site often leads to systemic toxicity [63]. Conversely, immobilizing CpG ODNs or other immunostimulants [64, 65] in synthetic scaffolds at the tumor site blocks the systemic toxicity.

Overall, *in situ* vaccination represents an alternative and attractive approach to tackle the issues related with neoantigens due to gene mutations in tumor cells. By harnessing the power of nanotechnology as well as molecular adjuvants, it is possible to induce effective immune responses while at the same time overcoming the local immunosuppression at the tumor sites.

5. Nanoparticle-based vaccines

Nanoparticles have emerged as the platform of choice to improve the efficacy and safety of subunit vaccines. Nanoparticles have long served as versatile carriers and been extensively used for the delivery of therapeutic agents, including drugs, antigens, adjuvants, cytokines and other immune modulators. Nanomaterials are known interact with immune cells and carry vaccines to LNs through the interstitial flow, which exists in the lymphatic circulation with velocities of 0.1–1 μm/s [66–68]. This is because nanoparticles are able to mimic the sizes, shape, charge and surface features of virus particles, facilitating the entrance to the lymphatic capillary. Hubbell and Swartz showed that 25 nm diameters polypropylene sulfide (PPS) nanoparticles were transported and captured by APCs in the LN more efficiently than the same nanoparticles with 100 nm diameters [68]. Inorganic nanoparticles such as gold nanoparticles (AuNPs) have also successfully been shown in an animal model for localization of the sentinel LNs following intradermal injection [69–72], and have extensively used as improved vaccine carriers. Additionally, nanoparticles are ideal co-delivery platform in that multiple components can be conjugated or encapsulated in a single particle, fulfilling the requirement of co-delivery of antigens and activation signals in vaccines. We have developed a silica nanoparticle-based delivery platform (SiNPs) which targets tumor antigen and TLR-9 agonist to APCs in the LNs following subcutaneous injection [73]. Vaccine loaded SiNPs led to dramatically enhanced induction of antigen-specific B and T cell responses as compared to soluble vaccines, which in turn drove a protective antitumoral immunity in a murine tumor model [73]. Additionally, SiNPs vaccines greatly reduced the production of systemic proinflammatory cytokines and completely abrogated splenomegaly, key systemic toxicities of TLR-9 agonist that limit its advances in clinical applications. Our results

demonstrate structure-optimized silica nanocarriers can be used as an effective and safe platform for targeted delivery of subunit vaccines [73].

Liposome represents a versatile and convenient approach for vaccine delivery [74]. However, its application is limited by the in physical stability *in vivo*. To improve liposome stability, interbilayer-crosslinked multilamellar vesicles (ICMVs) was recently developed. These physically crosslinked vesicles were relatively stable but rapidly release their vaccine cargos when internalized by DCs. Results from this nanoparticle-based vaccine in mice showed striking enhancement on cellular and humoral responses, characterized by 30% antigen-specific $CD8^+$ T cell expansion and nearly 1000 times increase in antigen-specific antibody titer compared with unformulated vaccine [75]. Nanoparticles can also be used to deliver a full set of tumor associated antigens to DCs to induce anti-tumor immunity. A novel study assessed the therapeutic efficacy of PLGA (poly(lactic-co-glycolic acid)) nanoparticles (100 nm) coated with tumor cell membranes [76]. Membrane-coated PLGA nanoparticles were decorated with a TLR 4 agonist monophosphoryl lipid A (MPLA) which readily activated DCs to license the proliferation and differentiation of $CD8^+$ T cells in a melanoma model. This artificial biomimetic nanoparticle formulation proposed a unique targeting approach that could be utilized for cancer immunotherapy. But it remains to be determined whether tumor membrane-coated nanoparticles can simultaneously elicit broad T cell immune responses against various tumor associated antigens.

Another approach of using nanoparticles for cancer vaccines is artificial antigen presenting cells (aAPCs) [77]. aAPCs, functioning as direct activating units for T cells expansion, are emerging as a prominent and desirable strategy to reverse immunosuppression microenvironment in tumors and activate highly avid tumor-specific T cells. Nanoparticles-based aAPCs are a new approach to efficiently present tumor antigen while at the same time avoid the tolerogenic mechanisms associated with traditional antigen presenting cells. Nanoparticle aAPCs typically have a nanoparticle core coated with peptide/MHC and T cell stimulatory signals. Nanomaterials have been used include polymer (e.g., PLGA), inorganic particles (e.g., iron-oxide), and biomaterials (e.g., liposomes). Immune checkpoint inhibitors have also been conjugated on particle surfaces. The administration of artificial APCs coated with HLA-peptide tetrameric complexes and anti-CD28 mAb together boosted the specific activation of antigen-specific $CD4^+$ T cells [78]. *In vivo*, adoptive transfer of aAPCs obviously restrained tumor growth of a melanoma model in mice, along with IL-2 treatment [79]. While the therapeutic efficacy of these aAPCs needs more evaluation and trials, they certainly boost the development and advancement of cancer vaccine design.

Generally, nanoparticle-based vaccines hold great promise and tremendous potential in the treatment of cancers, and therapeutic efficacy generated by nanoparticle-based approach greatly promotes the development of next-generation cancer vaccines. Although some nanoparticles are commercially available and effective in cancer immunotherapy, it is still critical to physically and chemically orchestrate the design of nanoparticle-based vaccines on a structural basis. By optimizing the rationale of vaccine design and the routes of administration, we may conquer the underlying challenges associated with nanoparticles, which may include potential cytotoxicity to tissues and unexpected accumulation in local sites.

6. Molecular vaccines

The use of nanoparticles for vaccines application has also raised safety concerns. Nanoparticles are typically encapsulated or conjugated with vaccines and their surface are modified with immune cell targeting ligands. However, it remains difficult to design nanocarriers which meet all the criteria for vaccine targeting. Most current nanoparticles do not reach a clinic application primarily due to requirements for complex designs including surface engineering to reduce host immune response, hydrophobic modification to enhance drug encapsulation, and incorporation of ligands to maintain immune cells targeting [80, 81]. Possible stability and toxicological issues including immunogenicity also greatly restrict the nanocarrier's clinical application in the short-term [80, 81]. We recently devised an 'albumin-hitchhiking' molecular approach which uniquely delivers vaccines to APCs in the LNs by binding to and transporting with endogenous albumin [63, 82]. In this approach, molecular vaccines are conjugated to a structure-optimized lipophilic albumin-binding tail linked by a solubility-promoting polar polymer and follow subcutaneous injection, bind tightly to albumin protein. Albumin binding increases the hydrodynamic size of molecular adjuvants, prevents them from rapidly flushing into the bloodstream and re-targets them to lymphatics and draining LNs, where they are filtered by APCs and accumulate. Meanwhile, because most vaccine components are trapped in the LNs, 'albumin-hitchhiking' vaccine also greatly enhances the safety profile by reducing systemic dissemination. We show that a long diacyl lipid (≥16 carbons) and a long polyethylene glycol (≥36 EG units) favors the albumin binding and LN accumulation *in vivo* [63, 82]. Subsequent immunization with the structure-optimized 'albumin-hitchhiking' vaccines exhibited massive antigen-specific T cells priming and improved anti-tumor efficacy. Administration of low dose of albumin-binding TLR-9 agonist and peptide antigens resulted in dramatically increased antigen-specific $CD8^+$ T-cell expansion relative to unmodified vaccine, as demonstrated by dramatic increases in the frequency of antigen-specific T cells measured in the peripheral. Importantly, efficient LN targeting achieved by albumin-binding vaccines also greatly reduces acute systemic side effects of TLR-9 agonist which had made it less attractive as a prophylactic vaccine adjuvant.

Although amphiphilic vaccines are prominent and excellent candidates in treating tumor-bearing mice, more study and work are required to translate this approach to clinical trials in human cancer models to validate the therapeutic and safety benefits. Additionally, the potential toxicity to LNs may be considered and addressed, and finding lipid-modified adjuvants that can function in human immune system is also urgently needed.

Another molecular vaccine which has emerged as an alternative cancer immunotherapy regimen is the DNA vaccine. DNA vaccination holds great potential in clinical translation because of their simplicity, safety and low cost [83]. In DNA vaccines, genetically engineered DNA encoding immunogenic antigens and immunostimulatory factors are injected into the host, and subsequently traffic into the cells for *in vivo* expression of therapeutic agents by using the hosts' protein expression machineries. In this way, DNA vaccines represent an innovative

strategy to induce specific anti-tumor immune response and circumvent immune escape. The injected DNA partially functions as an immunological adjuvant to stimulate the innate immune system due to its bacterial origin [84]. On the other hand, the antigens, expressed by plasmid-transfected host cells, can be processed and subsequently presented by MHC molecules which are critical to license the activation of antigen-specific $CD8^+$ T cells. One of the studies revealed that DNA vaccine encoding alphavirus replicon activated the innate immunity and induced cellular responses against self-tumor associated antigen tyrosinase related protein 1, showing impressive efficacy in reversing immunosuppression in tumor [85]. Another innovative work that elaborated the design of DNA vaccine also showed remarkable effectiveness in overcoming immune escape in tumor models [86]. In this study, the plasmid DNA was engineered to encode a secreted chimeric protein consisting of a single-chain trimer (SCT) of MHC I heavy chain, β2-microglobulin, and peptide antigen linked to IgG. The chimeric protein derived from this plasmid DNA was able to form a dimer which bound avidly to antigen-specific $CD8^+$ T cells and elicited T cell stimulation and expansion directly, bypassing antigen presenting cells. This design simplified the process of antigen presentation and potentially avoided suboptimal activation associated with traditional antigen presentation. Additionally, the IgG domain in this construct enabled chimeric proteins to target the Fc receptor on APCs which initiated the subsequent cascade of immune activation in LNs. Based on this creative design, intradermal administration of this DNA vaccine induced potent Trp2-specific $CD8^+$ T cell dominant immune responses and showed enhanced therapeutic efficacy in B16 melanoma tumor model in mice [86]. DNA vaccines have also been tested in clinical trials to evaluate their efficacy in human cancer, melanoma [87], breast cancer [88], prostate cancer [89], and cervical cancer [90].

DNA vaccination provides an innovative and attractive platform for cancer immunotherapy with additional advantages like low cost, well-defined safety. Technically, DNA vaccines can be readily customized and engineered, which makes large-scale production possible. In addition, DNA vaccines are widely recognized as safe therapeutics in both animal and human clinical trials [89, 91, 92]. Despite a variety of advantages of DNA vaccines, the intrinsic poor immunogenicity have made DNA vaccine less successful in generating desirable therapeutic efficacy in most cancers. Therefore, future development of DNA vaccines may need to focus on their rationale design to greatly improve the immune potency of DNA vaccines in cancers.

7. Combined immunotherapy

Although monotherapy of most cancer vaccines can achieve therapeutic efficacy in cancer treatment to varying extent, therapeutic benefits may be further improved if these cancer vaccines can be administrated in a combinational way to complement each other against cancer. Theoretically, when effector T cells are activated, co-inhibitory molecules CTLA-4 and PD-1 can also be expressed and up-regulated on T cell due to the suppressive microenvironment of tumors, which may compromise the efficacy of vaccine-based cancer therapy. To minimize the impact of the expression of co-inhibitory molecules, a combinational therapy of cancer vaccines and immune checkpoints inhibitors may achieve a cure to cancer treatment. The idea has been realized and supported by several preclinical studies [93, 94]. The first study revealed that breast cancer derived immunogenic multi-peptide vaccine plus anti-PD-1

antibody functioned as a combinational therapy approach and thus prolonged the vaccine-induced progression-free survival period in breast tumor-bearing mice, along with augmented expansion of Tc1 and Tc2 CD8 T cells [93]. Another study demonstrated that anti-PD-1 antibody and GVAX synergistically enhanced the anti-tumor immune responses with great therapeutic efficacy in established melanoma tumor-bearing mice. In contrast with monotherapy of vaccine or PD-1 inhibitors, only a simultaneous administration of both therapies achieved repeated expansion of antigen-specific $CD8^+$ T cells [94]. Similar strategy using GVAX and anti-CTLA-4 antibody has also been utilized for treating metastatic pancreatic cancer, giving rise to objective response in 20% of tumor-bearing patients who have been resistant to chemotherapy [95]. Cancer vaccine-based immunotherapy may weaken the resistance of some cancers to immune checkpoint inhibitors, whereas immune checkpoint inhibitors may make up the drawbacks for cancer vaccines by decreasing the possibility of immune escape in tumors and thus enhancing the efficacy of vaccination. Other combination, such as vaccines plus adoptive T cell transfer might synergistically amplify the antitumor immunity, as demonstrated in recent studies. In summary, the combinational therapy is emerging as a more powerful and comprehensive strategy to address the immune escape associated with tumors and fuel the tumor-antigen specific T cell immune responses. But more studies are needed to test the clinical efficacy of this combinational therapy and assess the potential issues related to it, such as systemic toxicity and anti-drug antibody response.

8. Conclusion and future perspective

Immunotherapies have demonstrated their potential to generate robust antitumor responses and are continuing to grow as a new treatment modality for cancers when administrated alone or as an addition for other "physical" or "chemical" therapies. Strategies based on immunotherapy mainly focus on the induction of potent immune response, especially effector T cell response, against tumor antigens and variants due to genetic mutations, and the decrease or blockade of intrinsic immunosuppression in tumors. The immune system is a sophisticated and complicated entity, which may require elaborate design and engineering of therapeutic agents to reverse tumor-induced immune imbalance. As previously discussed, each single immunotherapy may not be perfect for cancer treatment. The future work may continue improving the rational design of cancer vaccines to maximize their efficacy while minimizing side effects. To date, several immunotherapies have been approved by FDA and dozens more are under clinical evaluation. Indeed, we are at the dawn of a whole new era for cancer treatments. With the rapid technological advancement in the field, cancer vaccines, in combination with traditional cancer treatment, may ultimately lead to a miracle cure for the vast majority of cancer patients.

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Conflict of interest

The authors declare no competing financial interest.

Author details

Chunsong Yu and Haipeng Liu*

*Address all correspondence to: haipeng.liu@wayne.edu

Department of Chemical Engineering and Materials Science, Wayne State University, Detroit, MI, USA

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