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Immunoengineering with Biomaterials for Enhanced Cancer Immunotherapy

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Abstract

Cancer immunotherapy has recently shown dramatic clinical success inducing durable response in patients of a wide variety of malignancies. Further improvement of the clinical outcome with immune related cancer treatment requests more exquisite manipulation of a patient's immune system with increased immunity against diseases while mitigating the toxicities. To meet this challenge, biomaterials applied to immunoengineering are being developed to achieve tissue- and/or cell-specific immunomodulation and thus could potentially enhance both the efficacy and safety of current cancer immunotherapies. Here, we review the recent advancement in the field of immunoengineering using biomaterials and their applications in promoting different modalities of cancer immunotherapies, with focus on cell-, antibody-, immunomodulator-, and gene-based immune related treatments and their combinations with conventional therapies. Challenges and opportunities are discussed in applying biomaterials engineering strategies in the development of future cancer immunotherapies.

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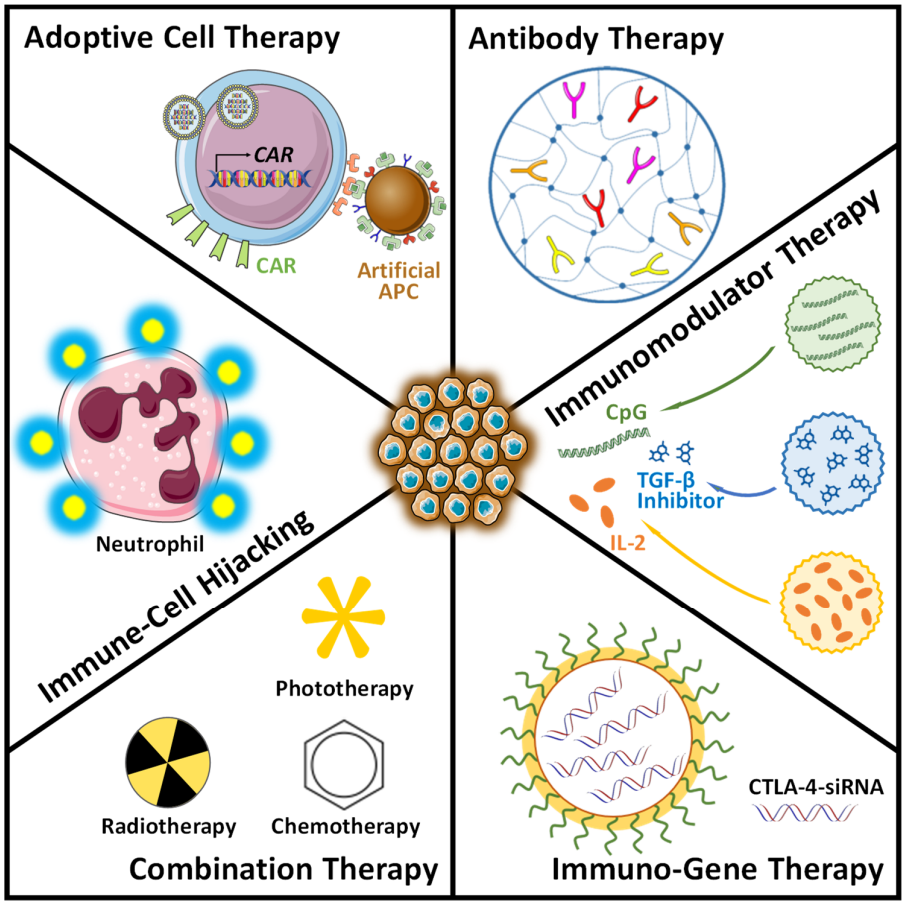

Immunoengineering with biomaterials for enhanced cancer immunotherapy

Introduction

Cancer immunotherapy, a treatment that harnesses the power of a patient's immune system to fight cancer, is transforming the standard-of-care for cancer. Although under investigation for more than a century,[1] only until recently cancer immunotherapy has been demonstrated to be effective in the clinic. In the past decade, breakthroughs have been made in cancer immunotherapy to consistently improve the overall long-term survival of patients with advanced-stage cancers. For example, anti-cytotoxic T-lymphocyte antigen 4 (CTLA-4) antibody, the first Food and Drug Administration (FDA)-approved checkpoint inhibitor, induced durable remission in patients with advanced melanoma.[2] Since then, a number of new checkpoint inhibitors and immunotherapeutic treatments have been carried forward to treat a variety of malignancies including non-small cell lung cancer, kidney cancer, bladder cancer, head and neck cancer, Hodgkin's lymphoma, etc.[3–5]

Immunotherapy represents several different immune-based treatment modalities. Therapeutic vaccine is among the first studied cancer immunotherapies. FDA-approved examples include Bacille Calmette-Guerin and Sipuleucel-T, a dendritic cell (DC) based cancer vaccine therapy. So far, the most broadly efficacious immunotherapy is immune checkpoint inhibitors, antibodies that antagonize CTLA-4 or programmed cell death protein 1 (PD-1) or its ligand (PD-L1). Immune checkpoint inhibitors have shown unprecedented clinical responses in a number of malignancies.[3] Other antibody-based immunotherapies include cancer-targeting monoclonal antibodies for induced innate immunity against cancer,[6] agonist antibodies that stimulate T-cell functions,[7] and bispecific antibodies.[8] Another potent immunotherapy is adoptive T-cell transfer, a clinical treatment with the infusion of a large number of *ex vivo* expanded tumor-infiltrating lymphocytes (TILs), or T-cells engineered with recombinant T-cell receptor (TCR) or chimeric antigen receptors (CARs) that directly target and kill cancer cells. Adoptive cell transfer (ACT), in particular CAR-T cell therapy, has yielded striking clinical results in the treatment of patients with hematological malignancies.[5] Additional approaches of cancer immunotherapies include immunomodulators that stimulate innate immunity (e.g., Toll-like receptor (TLR) agonists), cytokines, small molecule inhibitors that modulate the immunosuppressive tumor microenvironment (e.g., indoleamine 2,3-dioxygenase (IDO) inhibitor), gene therapy based immune-treatment, oncolytic virotherapy, etc.[4]

Although promising, there are several pressing challenges facing cancer immunotherapy that limit its full therapeutic potential.[9,10] One of the major hurdles is the low-response rate of patients treated with immunotherapy. For example, Nivolumab treatment was associated with an overall response rate of 28% in advanced melanoma patients.[11] To improve the response rate, one of the solutions is to develop more potent synergistic combination therapies. However, combination therapies typically come at a cost of dramatically increased toxicity. When concurrently treating patients with Nivolumab and Ipilimumab, response rate raised up to 40%. However, patients receiving the combination therapy also experienced several severe toxicities with the rate of grade 3-4 treatment-related severe adverse events increasing to 53%.[12,13] In general, the broad, nonspecific activation of an immune response is responsible for the widespread adverse events observed in patients treated with mono- or combination immunotherapies. Therefore, a key challenge in the field is to develop more efficacious immunotherapies while avoiding immune toxicities.

In parallel, the advancement in the field of biomaterials engineering and nanomedicine has resulted in numerous novel materials in the form of solid implants, hydrogels, microparticles, or nanoparticles (NPs) which find widespread applications in addressing biomedical issues. In particular, many biomaterials are designed to achieve precisely spatiotemporal control of drug delivery in cancer

therapies. Recently, growing interest has been focused on engineering biomaterials for modulating the immune response in the context of disease treatment, such as cancer, infectious diseases, and autoimmunity.[14–17] Such efforts have given rise to an emerging field, immunoengineering, which characterizes, analyzes and modulates immune responses using various engineering approaches. Applying immunoengineering approaches in cancer treatment has led to the development of a number of promising novel strategies in cancer immunotherapies[18–21] (Figure 1). For example, material and molecular engineering methods have greatly promoted the antitumor efficacy of cancer vaccines.[22,23] Immunoengineering with advanced biomaterials aiming for precisely controlled delivery of immune-therapeutics hold great promise in addressing some key challenges in current immunotherapies such as immune related toxicities through tissue- and/or cell-specific immunomodulation.

In this review, we examine the ongoing efforts in enhancing cancer immunotherapies using biomaterials engineering approaches (Figure 1). We first discuss how biomaterials are designed to enhance various modalities of cancer immunotherapies. Application of biomaterials and nanotechnology in vaccine development has been extensively reviewed recently[24–26] and is not included. For each immune-related treatment approach, we give a brief overview of recent advancement in that field of study. This is followed by a brief discussion on the applications of biomaterials in combination therapies. Finally, we conclude with some thoughts on important future directions in which biomaterial-based immunoengineering could further promote cancer immunotherapy.

BIOMATERIALS ENHANCING CELL-BASED IMMUNOTHERAPY

The fast and complete eradication of cancer cells mostly relies on caner reactive cytotoxic T lymphocytes (CTLs). Direct infusion of a large number of activated tumor antigen-specific T-cells is a potent immunotherapy that quickly reinforces the dysfunctional host cellular immunity. In recent years, adoptive T-cell transfer immunotherapy, such as CAR and TIL, has triggered long-lasting remissions in a subset of patients with hematological malignances leading to the FDA approval of the first CAR-T cell therapy.[27,28] In this section, we will first discuss how biomaterial can enhance adoptive T-cell therapy in the *ex vivo* or *in vivo* phase. Drug delivery hitchhiking on immune cells is also discussed.

Activating Antigen-Specific T-cell by Artificial Antigen-Presenting Cells (aAPCs)

Preparation of natural APCs, such as autologous DCs, is a clinically laborious and expensive process, and the quality of *ex vivo* generated DCs varies, which greatly limits their clinical use. Engineered aAPC has been developed as an alternative to overcome some of these limitations of natural APCs. Compared to natural APCs, aAPC has well-defined compositions and controlled, uniform signal presentation. In addition, aAPCs can be easily manufactured in large scale and developed into an off-the-shelf product.[29] There are two major categories of aAPCs. One is genetically modified cellular aAPC, such as K562 human leukemic cells,[30] NIH/3T3 murine fibroblasts,[31] which has recently been extensively reviewed.[32] Another is synthetic aAPC generated using biomaterial engineering strategies.[33–36] Here, we illustrate the recent progress in engineering synthetic aAPCs using biomaterials and nanotechnologies for cancer immunotherapy.

Key physiochemical parameters, such as size and shape, of aAPCs have been studied for the effect on T-cell activation. Nanomaterials with high aspect ratio may have enhanced interaction with T-cells. For example, Fahmy and colleagues exploited the unique nanoscale topography of carbon nanotubes (CNTs) to present clustered peptide-loaded major histocompatibility complex (pMHC) and a costimulatory ligand for T-cells.[37] Such CNTs were further complexed with interleukin-2 (IL-2)-encapsulating poly(lactide-co-glycolide) (PLGA) NPs to provide the third signal for the stimulation and expansion of T-cells. The aAPC composed of composite materials expanded T-cells more efficiently than peptide-pulsed autologous DCs under conventional conditions and resulted in enhanced therapeutic efficacy in mouse B16-F10-ovalbumin melanoma model. In another example, to mimic the physiological functions of DCs to stimulate T cells, red blood cells were engineered to provide a flexible cell surface with appropriate physiochemical parameters for antigen presentation, which enabled the efficient activation of antigen-specific T-cells.[38]

Magnetic NPs are often incorporated into the aAPC for facile separation and enrichment of antigen specific T-cells. Using paramagnetic nanosized aAPC bearing peptide-based neo-epitopes to stimulate naïve T-cells and then enrich the neo-epitope specific T-cells with a magnetic column, Schneck and colleagues demonstrated an elegant streamlined technology with a single reagent to generate markedly increased number of antigen specific T-cells after *ex vivo* culture.[39] Magnetic NPs response to magnetic fields and provide the possibility for externally spatial and temporal control of T-cell activation process as the magnetic field can exert forces on T-cell surface that is bound with paramagnetic NPs. Perica et al. showed that in the presence of the internal magnetic field, the magnetic aAPCs aggregated on T-cell surface inducing increased size of TCR clusters and thus boosted T-cell activation and expansion *in vitro*.[40] Such aAPC-activated T-cells were then adoptively transferred and mediated enhanced tumor rejection effect in a mouse melanoma model. This novel method based on magnetic NPs is a promising tool to study the T-activation process and activate T-cells with enhanced specificity and efficiency. In a recent study, Yu and colleagues have extended the approach to achieve remotely controlled T-cell activation with single-cell precision using Janus particles that are magnetically responsive on one hemisphere and stimulatory to T-cells on the other side.[41] By controlling the rotation and locomotion of such anisotropic materials under an externally applied magnetic field, the Janus particle selectively activated T-cell at a certain orientation. More recently, Zhang et al. reported a biomimetic magnetosome aAPC that not only exhibited high performance for antigen-specific T-cell activation and proliferation but also visually guided adoptively transferred T-cells into tumor site through magnetic resonance imaging and magnetic control.[42]

Multivalency is known to play an important role in T-cell activation.[43] Hammink et al. reported an elegant study using antibody-functionalized polymer with controlled polymer length and antibody density as a "synthetic DC" to probe the multivalent effect on T-cell activation.[44] Increased multivalency significantly prolonged the activation of the stimulated T-cells and is hence an important design criterion for aAPCs.

Recently, the stiffness of the NPs has been found to play an important role in T-cell activation.[45] Compared to the rigid polystyrene beads, mechanically soft polydimethylsiloxane beads activated T-cells more efficiently *in vitro*. This finding provides a new method to improve the efficiency of the adoptive T-cell platform.

Besides the application in *ex vivo* T-cell activation and expansion, aAPC can also be employed for direct *in vivo* T-cell activation and enhancing cancer immunotherapy.[46] For example, Kosmides et al. showed that when co-administered *in vivo* aAPC based on biodegradable PLGA polymer NPs synergized with anti-PD-1 and enhanced the therapeutic effect of adoptively transferred naïve tumor-reactive $CD8^+$ T-cells.[46] However, the intravenously (i.v.) injected aAPCs are subjected to the rapid clearance by monocytes and macrophage in circulation and therefore their effect might be diminished substantially. In a recent report, compared with aAPC without CD47 functionalization, CD47-coated aAPC showed inhibited phagocytosis but non-compromised capability in activating and expanding antigen-specific T-cells leading to augmented anti-tumor efficacy when administered together with the adoptive transfer of tumor reactive T-cells in a mouse B16-SIY tumor model.[47]

Enhancing the Efficacy of Adoptive T-cell Transfer

Although promising in triggering durable remissions in some blood cancers, few clinical successes have been achieved in the treatment of solid tumors with adoptive T-cell therapy. Due to the highly immunosuppressive microenvironment in solid tumor, adoptively transferred T-cells are prone to lose effector function and switch to exhausted phenotype.[48] In clinic, supporting transferred T-cells with adjuvant drugs, e.g., stimulant cytokines and co-stimulatory agonist, is necessary to prolong the persistence and functionality of T-cells. However, systematic administration of such adjuvant drugs often induces severe toxicities.[49] Targeted delivery of T-cell supporting drugs to specifically expand and support tumor-reactive T-cells becomes an attractive strategy to enhance the efficacy while minimizing systemic toxicities due to non-specific immune stimulation.

Recently, nano- or implantable materials are designed to assist adoptively transferred T-cells to overcome the immune suppression in tumors. One strategy is “backpacking” tumor-reactive T-cells with NPs *ex vivo* prior to the adoptive transfer. These NPs encapsulating T-cell promoting drugs release the adjuvant drugs after the T-cells are transferred together with the NP backpacks and induce an autocrine stimulation of the T-cell proliferation and functionality *in vivo*. Irvine and colleagues demonstrated the backpacking strategy by chemically conjugating a liposome NPs to the surface thiol groups of activated anti-tumor T-cells and showed markedly increased *in vivo* expansion of the transferred T-cells leading to enhanced anti-tumor efficacy in a mouse B16 melanoma model.[50–52] Such liposome NPs can load with common γ-chain cytokines such as IL-2, IL-15 and IL-21, or small molecule inhibitors that inhibit negative regulators of T-cell activation and function.[51]

Instead of backpacking T-cells *ex vivo*, NPs can also be designed to target tumor-reactive T-cells *in vivo* and support their expansion and function. Zheng et al. recently reported T-cell targeting liposomes encapsulating transforming growth factor targeted a CD90 isoform expressed exclusively by the donor T-cells when administered i.v. and led to greater tumor regression over equivalent doses of the free systemic drug. This study demonstrated a broadly applicable strategy to target exogenous or endogenous T-cells with modulatory drugs for enhanced therapy.[53]

Bulk biomaterials, for example, implantable hydrogels, are also designed to support T-cell expansion and function in tumors. Stephan et al. recently reported an alginate-based polymer implant capable of delivering, expanding and dispersing tumor-reactive T-cells.[54] These polymer implant harboring the transplanted T-cells contained T-cell stimulant-encapsulating microparticles (IL-15 superagonist, anti-CD3, anti-CD28 and anti-CD137 antibodies) for substantially promoted T-cell expansion *in vivo* and resulted in enhanced efficacy against tumor relapse and metastasis. This biodegradable polymer

scaffold-supported T-cell implant as a localized immunotherapy is particularly useful to treat locally advanced, unresectable or incompletely resected tumors. Similarly, Monette et al. reported an injectable chitosan thermogel for increased T-cell proliferation and gradual release, which provided site-specific CTLs to enhance the efficacy and safety of adoptive T-cell therapy.[55]

CAR T-cells are typically generated by genetically programming patient-derived T-cells *ex vivo* and expanded to a large number for reinfusion back to the patient. However, this process is complex, labor intensive, and expensive, and remains one of the major obstacles for implementing ACT as an off-the-shelf cancer treatment. Efforts have been made to try to solve this problem using nanotechnology. In an elegant example, polymeric nanocarriers for DNA were designed to target lymphocytes *in vivo* and program them into tumor-reactive T-cells directly without T-cell isolation or the *ex vivo* modification procedures (Figure 2). Stephan and colleagues have recently demonstrated this exciting new strategy was applicable in a mouse leukemia model.[56] They developed a T-cell targeting poly(β-amino ester) polymer-DNA complex NPs to deliver leukemia-specific CAR genes and hyperactive iPB7 transposase gene into host T-cells *in situ* and generate CD19-specific CARs with large quantity and comparable efficacy to the conventional CAR T-cells transduced *ex vivo*.

Hijacking Immune Cells for Drug Delivery

Immune cells, as well as some other cells in blood, are exploited as carriers for targeted and controlled drug delivery in immunotherapy due to their unique trafficking behaviors. In response to tumor microenvironment, circulating leukocytes have the capability to infiltrate solid tumor via chemotaxis.[57] For instance, tumor antigen specific T-cells home to tumor or tumor draining lymph nodes and mediate specific responses against the tumor.[5] Tumor growth could induce neutrophil polarization and recruit neutrophils as well as other myeloid-derived suppressive cells.[58] Recruited $CCR2^+$ monocytes could differentiate into tumor-associated macrophages.[59]

Healthy lymphocytes are known to traffic to lymphoid organs where lymphomas home. Taking advantage of this intrinsic trafficking ability, Huang at al. activated and expanded autologous polyclonal T-cells *ex vivo* while maintaining their lymphoid tissue homing receptors and exploited these activated T-cells as live carriers to enhance tumor-specific delivery of chemotherapy[60] (Figure 3(a)). By conjugating NPs loaded with SN-38, a potent topoisomerase I poison, to the surface of the T-cells, they showed that T-cells mediated 90-fold greater amount of SN-38 delivered to lymph nodes than the free drug administered systemically at even 10-fold higher dose. Surprisingly, the T-cells with surface bound SN-38 encapsulating NP were resistant to SN-38 but mediated efficient killing of lymphoma cells *in vitro*. The T-cell based delivery approach substantially improves the anti-tumor efficacy of free SN-38 or the SN-38 encapsulating NP alone. In addition, the T-cell surface bound NPs can also be loaded with imaging contrast reagents for diagnosis. Meir et al. labeled melanoma-specific T-cells with gold NPs and used X-ray computed tomography (CT) to track these T-cells *in vivo* through whole-body CT imaging.[61] T-cell can be employed as not only a live carrier but also an active trigger to control the drug release from the cell surface bound NPs. Jones et al. recently found that perforins secreted by cytotoxic T-cells upon recognition of peptide-MHC-I complex lysed the cell surface bound liposome drug carriers resulting in antigen-recognition-triggered release of drug cargos.[50]

Neutrophils, the “first responders” for the inflammation and the most abundant granulocytes, are important for defending the body against evading pathogens through phagocytosis and secretion of cytokines and reactive oxygen species (ROSs).[62] Targeting neutrophils with therapeutic NPs can treat

inflammation and infection.[63,64] Wang and colleagues recently expanded this neutrophil-targeting strategy for cancer immunotherapy by developing an ethanol-denatured albumin NPs which were specifically internalized by activated neutrophils when administered i.v. in mice. The NPs loaded with pyropheophorbide-A, a photodynamic therapeutic agent, hijacked the neutrophils and accumulated in tumor mediated by the neutrophils resulting in improved antitumor efficacy through synergistic effect with an anti-tumor antibody.[63] More recently, Xue et al. reported another neutrophil hijacking strategy in the mouse. After *ex vivo* uptake of liposomes that contain paclitaxel (PTX), i.v. injected neutrophils could penetrate the blood-brain barrier and suppress the recurrence of surgically resected glioma. The local inflammatory microenvironment after tumor resection recruited the neutrophils into the inflamed brain and triggered the release of liposomal PTX. This delivery strategy efficiently slowed the recurrent growth of tumor.[65] Similar, NPs are also designed to hijack monocytes[66] or macrophages[67] for tumor targeting.

Platelet, an important component of blood functioning to stop bleeding by clumping and clotting blood vessel injuries, are known to accumulate in wound sites and interact with circulating tumor cells triggering inflammation and tissue repair.[68] Wang et al. recently presented an elegant strategy of conjugating a monoclonal antibody against PD-L1 to the surface of platelets to reduce post-surgical tumor recurrence and metastasis in a mouse model with partially removed primary melanoma (B16-F10) or triple-negative breast carcinoma (4T1)[69] (Figure 3(b)). The release of platelet-bound anti-PD-L1 was triggered by platelet-derived microparticles upon platelet activation specifically in the tumor post-surgery leading to prolonged survival (Figure 3(c)-(e)).

BIOMATERIALS ENHANCING ANTIBODY-BASED IMMUNOTHERAPY

Antibody-based therapy is one of the most actively pursued cancer immunotherapies. Antibodies targeting tumor antigens are among the earliest developed antibody based cancer therapies. Many of those antibodies are designed to induce effector function through immune-mediated cancer cell killing mechanism, for example, rituximab, an anti-CD20 antibody.[70] Other antibodies directly modulate the immune response of T-cells or APCs, such as checkpoint blockade antibodies and immunostimulatory antibodies. All these approaches have shown success in clinic and led to the FDA approval. Here, we discuss the recent progress in biomaterial-assisted antibody-based immunotherapies that exploit various mechanisms for cancer cell killing.

Tumor Targeting Antibody

Antibody based therapies can be designed to target tumor specific antigens inducing antibody-dependent cell-mediated cytotoxicity and/or complement dependent cytotoxicity for cancer cell killing. However, challenges remain for this type of antibody therapy in cancer treatment. Many of the targets for the antibodies are not truly cancer specific but also distributed in healthy tissues leading to toxicities against normal tissues.[71,72] Moreover, concentration of the antibody dose in tumor can be greatly hampered due to limited tissue penetration into the disease site distal to blood vessels.[73] To meet these challenges, biomaterials are being developed to achieve specific and controlled delivery of antibody activities to tumor. For example, antibodies have been conjugated to gold NPs,[74] polyethylenimine (PEI),[75] or multilayered hydrogel capsule[76] for enhanced stability *in vivo* and tumor targeting. Recently, Erster et al. reported a novel and elegant strategy to achieve site-specific targeting

of antibody activity using a protease-activated masked probody.[77] In a related study, Desnoyers et al. applied this probody strategy to target EGFR for cancer therapy with an antibody that remains masked against antigen binding until activated locally by proteases overexpressed in the tumor.[78] Using recombinant technology, they modified cetuximab by introducing an identified binding peptide extension at the N terminus of the light chain with a cleavable substrate linker inserted. The substrate was designed to respond to proteases known to be up-regulated in tumor. The probody formulation of cetuximab remained relatively inert in healthy nonhuman primates, but specifically activated and efficacious in mouse xenograft models.

In situ secretion of a therapeutic antibody *in vivo* using implantable artificial "bioreactor" is an interesting strategy to pursue. Aliperta et al. recently developed an implantable immunotherapeutic organoid harboring human mesenchymal stromal cells genetically modified to secrete anti-CD33-anti-CD3 bispecific antibodies for triggering T-cell mediated anti-tumor response.[79] The artificial organoid comprised of biocompatible star-shaped poly(ethylene glycol) (PEG)-heparin cryogel scaffold and MSCs as a therapeutic machinery for the treatment of acute myeloid leukemia. The macroporous biohybrid cryogel platform enabled constant release of a sustained level of the bispecific antibodies *in vivo* overcoming some common limitations associated with the administration of soluble bispecific antibodies or direct injection of bispecific antibodies-secreting cells such as frequent re-dosing, systemic toxicity, cell loss and high cost.

Therapeutic antibodies typically show a low level of tissue penetration in the solid tumor due to the large molecular weight, which greatly limits the efficacy of antibody-based immunotherapy.[80] To enhance the tumor penetrating ability, a wide variety of protein scaffolds have been designed as alternatives.[81] Compared to the large-sized antibody (150 KDa on average), these small scaffolds have molecular weights ranging from 2 to 20 KDa, which confer them largely enhanced tissue penetrating abilities. Among those scaffolds, bicyclic peptide, a linear peptide of 9-15 amino acids cyclized by 1,3,5-Tris(bromomethyl)benzene to form two peptide loops, is the smallest (2 KDa).[82] Pollaro et al. recently developed a bicycle peptide inhibiting the serine protease urokinase–type plasminogen activator, a protease playing an important role in tumor growth. By conjugating to an albumin binding tag, the bicycle peptide showed a long plasma half-life and diffused deeply into tumor tissues achieving nanomolar concentrations.[83]

Checkpoint Blockade Antibody

Immune checkpoint blockade therapy, represented by anti-PD-1/ PD-L1 and anti-CTLA-4, has shown dramatic clinical results in the treatment of a variety of cancers.[84] However, both PD-1 and PD-L1 have a role in maintaining the normal immune homeostasis, which is evidenced by the fact that the genetic deletion of PD-1/PD-L1 leads to severe autoimmunity. Similarly, CTLA-4 is not only expressed in tumor infiltrating T-cells, but also expressed on peripheral regulatory T-cells that keep peripheral tolerance. Thus, anti-CTLA-4 treatment in patients also induced significant autoimmune toxicities.[9,85] Anti-PD-1 is generally better tolerated but could induce sever toxicities when used in tandem with other therapies, such as BRAF inhibitor.[86]

Using responsive material, one may focus the checkpoint blockade antibody activity specifically in tumor tissue and thus reduce the toxicity. For example, Wang et al. recently developed an inflammation-triggered anti-PD-1 checkpoint blockade cancer immunotherapy with a responsive CpG oligodeoxynucleotides (CpG ODNs) Nano-cocoon.[87] The Nano-cocoon was first synthesized with

repeatedly spaced CpG sequences and cutting sites for restriction enzyme HhaI, and loaded with anti-PD-1 antibodies. In order to achieve the responsive release of anti-PD-1, HhaI enzymes caged in triglycerol monostearate (TGMS) NPs were attached to the nano-cocoons; HhaI became liberated through the cleavage of the ester bond between TGMS and the enzyme mediated by esterases and matrix metalloproteinases presented at the inflammatory sites after tumor resection. Liberated HhaI degraded the nano-cocoon leading to tumor-specific release of anti-PD-1 antibodies to minimize non-specific toxicity.

Local delivery is another approach to minimizing the systemic toxicity of checkpoint blockade antibodies. For example, responsive local delivery of antibody can be achieved using microneedle patch. Gu and colleagues recently reported an innovative degradable microneedle patch for the sustained delivery of anti-PD-1 in the physiological environment[88] (Figure 4). The microneedle is composed of biocompatible hyaluronic acid integrated with pH-sensitive dextran NPs loaded with anti-PD-1 and glucose oxidase, an enzyme converting blood glucose to gluconic acid. Once exposed in transdermal, the microneedles generated acidic environment and promoted the self-dissociation of anti-PD-1-encapsulating NPs and release of anti-PD-1. It has been demonstrated that a single administration of the microneedle patch induced enhanced immune responses in a B16-F10 mouse melanoma model. Similar microneedle system could also be utilized to deliver combination therapies, for example, anti-CTLA-4 or immunosuppressive enzyme IDO inhibitor[89] together with anti-PD-1. Other examples include using implantable bulk biomaterials or microparticles for the local delivery of checkpoint blockade antibodies. For instance, Lei et al. synthesized an implantable functionalized mesoporous silica material which can be loaded highly efficiently with anti-CTLA-4 and facilitate gradually released locally *in vivo* under physiological conditions.[90] Improved anti-tumor efficacy has been demonstrated in a mouse melanoma model. Similarly, Li et al. recently utilized an alginate hydrogel system to locally deliver celecoxib and anti-PD-1 to treat mouse B16-F10 melanoma and 4T1 metastatic breast cancer through peritumoral injection.[91] They showed that the alginate hydrogel delivery system significantly improved the antitumor activities of celecoxib, anti-PD-1, or combined. In another strategy, Rahimian et al. developed a poly(lactic-co-hydroxymethyl-glycolic acid) microparticles to load with anti-CTLA-4 and anti-CD40 to achieve sustained control release of the antibodies upon subcutaneous injection in mice bearing MC-38 tumors.[92]

Immunostimulatory Antibody

Agonistic antibodies can provide co-stimulatory signals necessary to prime an anti-tumor immune response for immunotherapy. So far, significant tumor remission has been noticed in clinical trials for agonistic antibodies, such as anti-CD40.[93] However, these agents are prone to eliciting serious side effects following systemic infusion as they instigate the peripheral lymphocytes to break the tolerance.[94] Thus, clinical application of agonistic antibodies is greatly limited by the dose-limiting inflammatory toxicity. Targeted or local delivery strategies using biomaterials may restrict the activity of the immuno-agonists in the tumor tissues in order to minimize the toxicities.

Agonists against CD40, a co-stimulatory receptor expressed on the surface of APCs, strongly activate APCs and thus prime potent anti-tumor cytotoxic T-cell responses. Simply i.v. injection of anti-CD40 results in server toxicities including cytokine release syndrome.[95] In order to achieve the full therapeutic potential of anti-CD40, Kwong et al. prepared a PEGylated liposome bearing surface-conjugated anti-CD40 and CpG to anchor immuno-agonist compounds to the liposome in order to

retain the bioactivity of therapeutics in the local tumor tissue and tumor-draining lymph nodes.[96] Following intratumoral injection, anti-CD40/CpG-liposomes successfully restricted anti-CD40 and CpG in tumor, preventing their leakage into systemic circulation while allowing draining to the tumor-proximal lymph node, and markedly increased the safety and efficacy of these two immunostimulatory agents. Targeting costimulatory receptor CD137 (4-1BB) expressed on the surface of activated T-cells, natural killer (NK) cells, and DCs with agonist antibody is another potent immunotherapy. However, systemically administration of agonistic antibody targeting CD137 elicits hepatic inflammatory damage and disordered lymphocyte migration.[97] In a related study, the same group developed a combined immunotherapy by anchoring anti-CD137 agonistic antibodies and engineered IL-2Fc to the surfaces of PEGylated liposomes.[98] Through intratumoral injection, the liposome surface bound anti-CD137 antibodies and IL-2Fc could reach the tumor parenchyma and tumor draining lymph nodes but were protected from leaking into systemic circulation. In B16-F10 mouse melanoma model, intratumoral injection of anti-CD137 + IL-2Fc anchored liposome was able to cure established primary tumors while preventing the lethal inflammatory toxicities, which were observed in treated mice with soluble anti-CD137 + IL-2Fc.

OX40 (CD134), a tumor necrosis factor (TNF) receptor expressed mainly on activated T-cells, is another costimulatory receptor that transmits a potent costimulatory signal once engaged.[99] Agonistic anti-OX40 antibody enhances tumor immune response leading to therapeutic effects in mouse tumor models. However, when tested in phase I clinical trials it did not show objective clinical activity in patients with metastatic or locally advanced tumors.[7] Chen et al. reported a novel strategy of NP-mediated delivery of anti-OX40 to efficiently induce CTL responses *in vitro*.[100] The biodegradable PLGA-NPs were covalently conjugated with anti-OX40 on NP surface and capable to induce CTL proliferation and antigen-specific cytotoxicity against cancer cells *in vitro* in a more potent manner than free anti-OX40. The NP had an average diameter of 86.0 ± 14.1 nm and may potentially provide an effective delivery system for agonist antibodies *in vivo*.

BIOMATERIALS ENHANCING IMMUNOMODULATOR-BASED THERAPY

In this section, we discuss how biomaterials can be designed to enhance therapeutic efficacy and safety of immunomodulators other than antibodies. Reagents including agonists for pattern recognition receptors (PRRs),[101] cytokines,[102] small molecule inhibitors,[103] etc., can vigorously modulate the immune response and thus are all potentially cancer immunotherapies. For example, IL-2, a cytokine which potently stimulates T-cell proliferation, is the first FDA-approved cytokine-based immunotherapy.[104] High dose IL-2 has shown a significant clinical response in melanoma or renal cancer.[105,106]

Ligand of Pattern Recognition Receptor

As a key feature of innate immune cells, PRRs enable the detection of infections through binding with certain general types of molecules that are expressed across pathogens but absent or restricted in vertebrates. Agonists of PRRs, such as TLRs, can activate the immune system and potentiate antibody and cytotoxic T-cell responses to antigens, and have demonstrated the therapeutic potential in cancer in preclinical and clinical studies.[107–109] The best-understood family of PRRs is the TLRs. One of the major therapeutic applications of TLR ligands is to adjuvant vaccines. This specific application has been

thoroughly reviewed elsewhere.[110,111] Here, we discuss the direct applications of these agents in cancer treatment by eliciting innate immunity. TLR ligands can induce antitumor efficacy as a monotherapy or in combination with other therapies, such as antibodies, chemotherapies, etc. However, systemic application of immunostimulatory TLR ligands may induce non-specific stimulation of the immune system and hence causes inflammatory toxicities, which are the major hurdle for the clinical application of these agents. Biomaterials-based delivery strategies may prevent systemic dissemination of TLR ligands and minimize toxicities.

Agonist for TLR9 has shown therapeutic potential in cancer treatment. Activation of TLR9 receptor increases the production of pro-inflammatory cytokines and the presentation of costimulatory receptors on T-cells, and directs APCs toward priming potent, Th1-dominated T-cell responses.[112] Liu et al. recently developed a cell-membrane-inserting unmethylated cytosine-guanosine motifs (CpG-ODNs), a synthetic oligonucleotide-based TLR9 ligand, to facilitate stable tumor cell surface anchoring of CpG-ODNs upon intratumoral injection and therefore enhance the local stimulation of APCs responding to apoptotic/necrotic tumor cells leading to improved antitumor efficacy.[113] To avoid systemic immune activation, in another example, Bourquin et al. delivered CpG to draining lymph nodes with cationized gelatin-based NPs that potentiated antigen-specific cytotoxic T-cells and antibody response when administered subcutaneously together with antigens.[114] The NP with an average size of 272 nm and slightly positive charge almost exclusively focused the CpG in draining lymph nodes and prevented the systemic dissemination of CpG, which otherwise caused the systemic release of proinflammatory cytokines and destruction of lymphoid follicles. In another report, Radovic-Moreno et al. synthesized the spherical immunomodulatory nucleic acids using gold NPs as templates.[115] Comparing to the free oligonucleotides, such spherical immunomodulatory nucleic acids induced significantly enhanced activation of innate immunity and potentiated more potent humoral and cellular immune response. Likely the 3D structure and orientation of the oligonucleotide on the shell of NP played a role in the potent and durable immune activation. Other delivery approaches have also been explored. For instance, long DNA sequence integrated with tandem CpG synthesized through rolling circle reaction self-assembled into a nanoflower structure for the delivery and protection of CpG.[116]

Covalent conjugation of TLR agonists to macromolecules is another applicable approach for reduced side effect. TLR7/8 agonists activate APCs and induce high levels of type I interferon (IFN) and IL-12 that direct potent Th1 and cytotoxic T-cell activity. Geest and colleagues conjugated imidazoquinoline (IMDQ), a small molecule TLR7/8 agonist, through amide bond formation to a 50-nm degradable polymeric nanogels prepared by self-assembly of an amphiphilic block copolymer. The IMDQ-ligated nanogels were restricted in the draining lymph nodes for focused innate activation and thus limit the inflammatory toxicity by preventing systemic dissemination and non-specific activation induced by soluble IMDQ as evidence in IFN-β reporter mice[117] (Figure 5). Similarly, Wu et al. synthesized a novel TLR7 agonist, 4-[6-amino-8-hydroxy-2-(2-methoxyethoxy)purin-9-ylmethyl] benzaldehyde (UC-1V150), bearing a free aldehyde for the covalent coupling to proteins such as mouse serum albumin (MSA). The UC-1V150/MSA conjugate induced the prolonged local release of cytokines when administered in the lung whereas the free TLR7 ligand caused acute systemic cytokine release with resultant toxicity.[118]

In order to effectively activate PRRs in the tumor, material engineering approaches have been developed to target TLR agonists to the tumor associated macrophages (TAMs) and/or tumor

infiltrating dendritic cells (TIDCs). Huang et al. recently reported the construction of a nanocomplex containing a high number of mannose moieties and a pH-responsive modified alginate, for tumor targeted delivery of let-7b, a synthetic microRNA mimic functioning as a TLR7 agonist, and reprogramming of the functions of TAM and TIDC and reversing the suppressive tumor microenvironment.[119] Effective anti-cancer therapeutic efficacy was achieved in a murine breast cancer model. In another example, CpG, together with IL-10 and IL-10RA antisense oligonucleotides were complexed with galactosylated cationic dextran modified with a pH-sensitive motif, i.e. PEG-histidine-modified alginate, for the tumor specific release in response to the acidic microenvironment and targeted delivery toward TAMs.[120]

Other agonists of PRRs have also been explored for cancer treatment in addition to TLR ligands. For example, agonists of stimulator of interferon genes (STING), a cytosolic immune adaptor protein, have shown therapeutic potential in cancer treatment.[121] Activation of STING pathway leads to DC maturation, production of type I interferons, which are critical for the induction of anticancer T-cell response. Systemic delivery of STING agonist, such as 2'3'-cyclic guanosine monophosphate-adenosine monophosphate, is challenging due to possible off-target inflammation or autoimmunity. To realize its full therapeutic potential, Koshy et al. developed a novel delivery strategy for STING agonist using cationic liposome for enhanced cellular uptake and STING pathway activation.[122] The liposomal delivery of STING agonist also increased the retention of STING agonist in tumors and colocalization with tumor-associated APCs, inducing regression of tumors and durable protective immunity against the challenge of the same tumor cells. In a another strategy, delivery of STING agonist with implantable biopolymers was reported to synergize with CAR T-cells by stimulating immune responses to eliminate tumor cells that escape from recognizing by adoptively transferred CAR T-cells.[123]

Cytokine

Cytokines including interleukins, IFNs, and TNFs, can vigorously stimulate immune responses.[102] Unmodified cytokine molecules are small in size (~15 kDa) and suffer from the short circulatory half-life. Systemic administration of cytokines is often accompanied with inflammatory toxicities which create another major hurdle for the clinical application of the therapeutic agents of this kind.[124]

PEGylation, a protein modification strategy that conjugates PEG to a protein molecule, is a well-studied approach for enhanced circulation half-life and reduced side effect of cytokines.[125,126] PEGylated granulocyte colony-stimulating factor, interferon α-2a and interferon α-2b have already been FDA-approved.[127] Beyond PEGylation, there are a number of alternative polymers for the conjugation with cytokines for increased safety and efficiency,[128] such as Poxylation (poly(2-oxazoline) polymers).[129]

Biomaterial-based delivery strategies for cytokines have been developed for effective tumor targeting. For example, Park et al. developed a system called nanolipogel, a nanoscale liposomal polymeric gel, for the co-delivery of transforming growth factor beta (TGF-β) inhibitor and IL-2 using a core-shell structure that facilitated the entrapment of the drug-loaded β-cyclodextrins and IL-2 in a biodegradable polymer matrix with a PEGylated liposomal coating.[130] The nanolipogel through systemic administration significantly inhibited tumor growth and increased survival of tumor-bearing mice likely by increasing the activity of intratumoral-activated $CD8^+$ T-cell and NK cells. In another example, Wang et al. designed tumor-microenvironment responsive NPs loaded with IL-12.[131] The

polymer backbone of such NPs was pH-sensitive and enabled the responsive release of IL-12 in acidic tumor-microenvironment. The released IL-12 subsequently reprogramed the phenotype of TAM from tumor-supportive M2 to tumor-suppressive M1, which improved anti-tumor immunity and retarded the tumor growth.

As the receptors for cytokines are expressed on the plasma membrane, it is important to minimize the internalization of the cytokine-loaded carriers for effective presentation of cytokines to the surface receptors. In a recent report, Sun et al. developed a tumor microenvironment-responsive and transformable nanocarrier for efficient cell membrane targeted delivery of tumor necrosis factor-related apoptosis-inducing ligand.[132] Using a phospholipase A2 degradable liposome as a shell, and complementary DNA nanostructures decorated with cytokines as the cores, they showed that the nanocarriers could transform from a spherical structure into nanofibers upon PLA2 activation in the tumor microenvironment and hence retain the TRAIL on the plasma membrane and reduce endocytosis.

Small molecule inhibitor

Small molecule therapies can show strong immunomodulatory functions. For example, TGF-β inhibitor or TGF-β receptor inhibitor has shown therapeutic potential in treating cancer. As discussed above, co-delivery of TGF-β inhibitor and IL-2 by nanolipogel showed a synergistic effect for enhanced efficacy.[130] Shp1 and Shp2 are key phosphatases that downregulate TCR activation in the synapse. Targeting these two phosphatases will potentially enhance T-cell anti-tumor activity. Stephan et al. recently described a NP functionalized with maleimide for the delivery of NSC-87877, a dual inhibitor for Shp1 and Shp2. The NPs were covalently conjugated to the free thiol groups expressed on plasma membrane of tumor-specific T-cells for ACT for effective delivered into the T-cell synapse upon antigen recognition. In a mouse model of advanced prostate cancer, this delivery strategy promoted T-cell expansion at the tumor site and enhanced survival of treated animals.[51]

Pharmaceutical inhibitors acting on cells other than T-cells have also been investigated to enhance cancer immunotherapy. Recently, Soleimani et al. developed micellar nanocarriers for the delivery of signal transducer and activator of transcription 3 (STAT3) dimerization inhibitors to melanoma tumors. STAT3 dimerization inhibitors S3I-1757 encapsulated in methoxy poly(ethylene oxide)-b-poly(ε-caprolactone) PEO_{114}-b-PCL_{22} and methoxy poly(ethylene oxide)-b-poly(α-benzyl carboxylate-ε-caprolactone) PEO_{114}-b-$PBCL_{20}$ micelles with high encapsulation efficiency and controlled slow release profile under physiological conditions were able to significantly increase the IL-12 production of immunosuppressed DCs in tumor inducing a potent cell-mediated immune response.[133]

Cancer immunotherapy that intervenes certain metabolic pathways has drawn increasing attentions.[134] Several metabolic checkpoints have been discovered recently.[135] Among others, IDO blockade has exhibited strong synergic effect with anti-CTLA-4 and anti-PD-1 antibodies to reenergize T-cells, showing promise in clinical trials.[136] Li and colleagues recently reported an immunostimulatory nanocarrier composed of a prodrug conjugate of PEG with NLG919, an IDO inhibitor. The nanocarrier alone was effective to enhance T-cell response; when combined with a chemotherapy drug, PTX, the nanocarrier inhibited the tumor growth in multiple mouse tumor models.[137]

BIOMATERIALS ENHANCING IMMUNO-GENE THERAPY

Gene therapy based immune related cancer treatment is another promising modality among all the immunotherapies. *Ex vivo* genetic modification has already been used extensively in engineering TCR and CAR T-cells for adoptive T-cell therapies; cells with tumor homing capability, such as Tie2-expressing monocytes, are genetically engineered and exploited for tumor-targeted expression of cytokines, for example, interferon-α, or other therapeutic proteins for immunotherapy.[138,139] Typically, *ex vivo* transfection is mediated by viral vectors in most cases. However, these viral approaches are in general associated with safety concerns and moderate transfection efficiency.[140] As a promising and safer alternative, synthetic biomaterial based vector engineered with tissue or cell targeting capability is being developed for improved safety and transfection efficiency, in particular, for the applications of *in vivo* gene therapy. For example, *in vivo* programming of endogenous T-cells into leukemia-specific CAR T-cells have recently been demonstrated using DNA-carrying NPs as aforementioned.[56] Risk of non-specific transfection is one of the major hurdles for the clinical translation of in vivo gene therapy. However, well-designed biomaterials may provide the opportunities to achieve cell[56]- or tissue[141–143]-specific expression of therapeutic genes including immunomodulators. In this section, we will discuss how biomaterials are being developed to enhance immune-related gene therapy for cancer treatment by highlighting some recent examples for the delivery of plasmid DNA and small interfering RNA (siRNA).

Gene Transfection and Expression

Specific expression of immunomodulators in the tumor lesion provides several advantages over systemic administration of the agents directly in enhancing safety and efficacy. Among diverse cytokine-based immunotherapies, interleukin-12 (IL-12) is an ideal candidate for activating both innate and adaptive immune response. However, serious immune toxicity has halted the clinical application of this potent immune modulatory agent.[144] Targeted expression of IL-12 via gene therapy may maintain a low but effective level of IL-12 in the disease and hence reduce toxicity.[145] Recently, Liu et al. developed a novel gene delivery system by the self-assembly of several components together including methoxy poly (ethylene glycol)-poly(lactide) (mPEG-PLA), 1,2-dioleoyl-3-trimethylammonium-propan (DOTAP), and plasmid IL-12 (pIL12) for local gene therapy.[141] In both subcutaneous and peritoneal colorectal cancer models, local administration (intratumoral and intraperitoneal injection, respectively) of the DOTAP/mPEG-PLA-pIL12 complex significantly increased the secretion of IL-12 as well as TNF-α and IFN-γ in tumors or ascites resulting in induction of tumor cell apoptosis, inhibition of tumor angiogenesis, and stimulation of CTL function. In the meantime, no significant toxicity was observed in the vital organs such as liver, kidney, and lung because the expression of IL-12 was restricted locally.

IL-15, another antitumor cytokine that activates varieties of immune cells, especially NK cells and T-cells, has shown therapeutic promise. However, systemic administration of IL-15 causes significant side effects, including fever, liver injury, weight loss, etc.[146] Recently Liang et al. addressed the tumor-targeted delivery of IL-15 plasmid by developing a engineered lipoplex complexed with recombinant IL-15 plasmids (PLP/pIL15) to target folate receptor α (FRα), a surface marker overexpressed in human colorectal cancer cells.[142] Intraperitoneal administration of FRα-targeted PLP/pIL15 in a CT26 colon cancer model in mice delivered a significantly increased level of IL-15 in ascites without detectable

toxicity. The delivery of IL-15 into tumor by gene based therapy promoted the activation of T-cells and NK cells leading to tumor growth inhibition.

Biomaterials mediated targeted expression of protein antagonists in tumors could overcome some of the issues, such as poor tissue penetration and systemic toxicity, for systemically delivered monoclonal antibodies. In an elegant design, plasmid DNA encoding for CXCL12 and PD-L1 targeting traps, trimeric fusion proteins designed to bind target molecules with high affinity, was delivered using liposome-protamine-DNA NPs to target orthotopic pancreatic cancer in mice and reprogram the immunosuppressive tumor microenvironment.[143] These liposome-protamine-DNA NPs showed significant accumulation in tumors besides liver through intravenous injection likely due to the enhanced permeation and retention effect of tumor tissues.[147] Tumor specific expression of the two trap binders enhanced the penetration and effector function of T-cells in tumor leading to significant antitumor efficacy and prolonged survival. These examples show the promise of using NPs to achieve either active[142] or passive[143] tumor targeting of immune-gene therapies diminishing non-specific transfection and toxicity *in vivo*.

Oncolytic adenovirus ($Onco^{Ad}$) is another potent cancer gene therapy.[148] $Onco^{Ad}$ functions as a tumor-lysing agent as originally designed, and/or a viral transfection agent delivering immune-stimulating agents triggering anti-tumor immunity in cancer therapy.[149,150] However, due to the immune response against Ad[151] and hepatic sequestration,[148] the therapeutic potential of systemically administered $Onco^{Ad}$ is largely hindered. To address these problems, Chen et al. modified the $Onco^{Ad}$ with hybrid materials including an inorganic mineral, a lipid, and a polymer to form PEG/lipids/calcium phosphate-$Onco^{Ad}$ (PLC-$Onco^{Ad}$) NPs for the delivery of IL-24 gene into the tumor[152] (Figure 6). IL-24 is a cytokine discovered as a tumor suppressing protein. Compared to the non-modified $Onco^{Ad}$, PLC-$Onco^{Ad}$ showed reduced liver sequestration and systemic toxicity even at a high-dose, and evaded the innate immune response and the neutralization of pre-existing antibodies. Intravenous administration of a high dose of PLC-$Onco^{Ad}$ enhanced the anti-tumor efficacy in a mouse subcutaneous tumor model of Huh-7 hepatocellular carcinoma without inducing severe toxicity demonstrating a promising immune-gene therapy based on the biomaterials-$Onco^{Ad}$ hybrid vector (Figure 6).

RNA interference

siRNA can intervene target gene expression and silence specific gene sequence by inducing messenger RNA (mRNA) degradation and thereby inhibiting target protein production. siRNA based therapy has been applied in multiple diseases including cancer. However, lacking an effective delivery strategy for siRNA to the target tissues or cells greatly hampers its clinical application. To meet this challenge, various biomaterials are being developed for the delivery of siRNAs.[153] For example, Warashina et al. successfully developed a novel delivery system for siRNA using a non-viral cationic lipid YSK12-C4, which formed a multifunctional envelope type nano-device (YSK12-MEND) with siRNA and could target DCs for cancer immunotherapy.[154] Compare to a commercial carrier, Lipofectamine RNAiMAX, the gene silencing efficiency of YSK12-MEND increased to 90% vs. 60% for RNAiMAX in mouse DCs *in vitro*. Meanwhile, the median effective dose decreased 16.7 fold. YSK12-MEND loaded with a siRNA silencing the cytokine signaling 1, a factor that downregulates the cytokine production and antitumor activity of DCs, endowed the transfected DC an enhancement in cytokine production leading to the significant retard of tumor growth in both preventive and therapeutic mouse lymphoma models. In a recent study, Xu et al. showed that liposome-protamine-hyaluronic acid NP delivered TGF-β siRNA to

tumor effectively and knocked-down 50% of TGF-β expression in late stage subcutaneous B16F10 melanoma model in mice. Such down-regulation of TGF-β level in tumor microenvironment greatly boosted the efficacy of a nanoparticulate anti-cancer vaccine.[155]

NP could be designed to target siRNA to tumor tissues when administered through different routes. In a recent report, Van Woensel et al. showed that intranasal delivery of siRNA targeting galectin-1 (siGal-1) with chitosan NPs could induce silencing of Gal-1 in the tumor microenvironment in a mouse glioblastoma model.[156] Chitosan NPs were formed spontaneously by complexing positively charged chitosan polymers and negatively charged sodium tripolyphosphate and siRNA. Intranasal delivery of siGal-1 NPs remarkable enhanced the antitumor immunity in the tumor microenvironment by increasing the number of $CD4^+$ and $CD8^+$ T-cells while reducing the number of regulatory T-cells and myeloid-derived suppressor cells, as well as inducing biased polarization of macrophages to pro-inflammatory M1 phenotypes. Combination of siGal-1 NPs with PD-1 checkpoint blockade immunotherapy triggered significant synergistic effect leading to the enhanced survival of tumor-bearing mice.

As discussed above, immune checkpoint inhibitors, such as anti-PD-1/PD-L1 and anti-CTLA-4 antibodies, have shown exciting clinical results. However, antibody-based checkpoint blockade may be damped by limited tissue penetration due to the relatively large size of protein molecules and the deprivation by Fc-receptor-expressing phagocytes.[157] siRNA-based checkpoint blockade that inhibits the expression of inhibitory receptors within the cytosol represents a promising alternative. Wang and colleagues developed a cationic lipid-coated PEG-PLA NP to deliver CTLA-4 siRNA (siCTLA-4) into T-cells for enhanced proliferation and activation both *in vitro* and *in vivo*. In a mouse B16 melanoma model, systemically administrated siCTLA-4-NPs were internalized by tumor-infiltrating $CD4^+$ and $CD8^+$ T-cells, and increased $CD8^+$ T-cells/regulatory T-cells ratio in tumor leading to enhanced antitumor activity.[158] In another example, Cubillos-Ruiz et al. used linear PEI-based NPs to achieve effective delivery of both non-targeting siRNA and PD-L1 siRNA to $CD11c^+$ $PD\text{-}L1^+$ regulatory DCs in ovarian cancer.[159] Combining the intrinsic agonistic capability of PEI and non-targeting siRNA for TLR5 and TLR3/TLR7, respectively, with the silencing activity of gene-specific siRNA (PD-L1) in such PEI-NP efficiently reversed the tolerogenic phenotype of ovarian tumor-associated DCs, resulting in T-cell-mediated tumor regression and prolonged host survival.

BIOMATERIALS ENHANCING COMBINATION THERAPY

Combination of immunotherapies with conventional therapies, such as chemotherapy, radiotherapy, photodynamic therapy (PDT), or targeted therapies has shown promising synergistic effect. Some of such combination therapies are currently being evaluated in the clinic.[160] The rationale design of those combinations has been systemically reviewed elsewhere.[161,162] Here, we discuss some of the important directions in the development of biomaterials-assisted synergistic combination therapies by highlighting several recent examples.

Chemotherapy and radiotherapy

Chemotherapy that functions by blocking tumor cell division, killing tumor cells through disrupting DNA replication, cellular metabolism, and so on, has been in clinical use to treat cancer in the last decades. Certain chemotherapies have recently been reported to activate immune stimulatory

mechanisms in preclinical and clinical studies.[162,163] Biomaterials are designed and synthesized to promote the targeted co-delivery of chemo- and immuno-therapeutics to tumor tissues. For example, Wu et al. recently developed a nanocomplex system using a cationic polymer, N,N,N-trimethyl chitosan, to efficiently encapsulate both the cytotoxic chemotherapeutic agent, doxorubicin, and T-cell mitogenic cytokine IL-2.[164] The surface modification with *cis*-aconitic anhydride enabled the controlled tumor-specific release of both cargos from the nanocomplexes in response to acidic pH. Such co-delivery system significantly increased the number of tumor-infiltrating CTLs and delayed the tumor growth in a mouse hepatic tumor model. In another report, Lin and colleagues developed a combination strategy using chemotherapy and photodynamic therapy to potentiate checkpoint blockade cancer immunotherapy via core-shell nanoscale coordination polymers.[165] In this research, nanoscale coordination polymer (NCP) core-shell NPs were designed to load oxaliplatin in the core and a photosensitizer, pyropheophorbide-lipid conjugate (pyrolipid) in the shell (NCP@pyrolipid). When combined with check point blockade anti-PD-L1, NCP@pyrolipid synergistically elicited potent immune responses for cancer cell killing.

Radiotherapy has a long history in treating cancer and preventing recurrence after surgery in oncology through the mechanism of producing ROSs for DNA damages in cancer cells. Upon radiation damage-associated molecular patterns such as adenosine triphosphate and high mobility group box 1 are released into the tumor microenvironment, which promotes the internalization of tumor antigens by APCs, leading to the tumor destruction by APC-primed CTLs.[166,167] NPs were utilized to facilitate the tumor-antigen presentation and induce the abscopal effect by capturing the tumor-derived antigens released during radiotherapy, which enhanced the synergistic effect between radiotherapy and checkpoint blockade immunotherapy.[168] In addition, the irradiation may moderate TAM reprogramming by influencing the M1/M2 polarization at the right doses.[169] For example, Klug et al. found a low dose of gamma irradiation can program macrophage differentiation into an inducible nitric oxide synthase positive ($iNOS^+$) M1 phenotype in a mouse insulinomas model. Upregulated iNOS is required for macrophages to activate endothelial cells and to express Th1 chemokine CCL5. As a result, irradiation-reprogramed macrophages normalize the aberrant vasculatures and cause the tumor rejection by recruiting tumor-specific T-cells.[170] Currently, a number of clinical trials are ongoing for evaluating the combination of radiotherapy and immunotherapy.[171,172] Given the advantages of biomaterial-assisted cancer radiotherapy in specificity and sensitivity,[173,174] tumor-targeted co-delivery of radiosensitizers and immunotherapeutic agents enabled by biomaterials engineering may be promising for the future development of synergistic combination therapies.

Photodynamic and photothermal therapies

As a noninvasive or minimal invasive therapeutic approach for cancer treatment, PDT or photothermal therapy (PTT) can destroy tumor cells to release tumor antigens that may induce antitumor immune responses. When immunotherapy combines with PDT or PTT, a synergistic effect can be achieved for strong host immune response and long term anti-tumor immunity.[175] For example, Lin group designed a chlorin-based nanoscale metal-organic frameworks (nMOFs) loaded with IDO inhibitor (IDOi).[176] This delivery system combining nMOF-enabled PDT and IDOi-based immunotherapy was demonstrated to show a synergistic effect in achieving positive immune response with increased T-cell infiltration in the tumor microenvironment and effective local and distant tumor rejection in mouse colorectal cancer models including CT26 and MC38. In addition, checkpoint blockade immunotherapies were shown to reinforce the anti-tumor responses induced by PTT or PDT[177–180]. For example, Liu and

colleagues developed a nanoparticulate therapy to combine PTT with anti-CTLA-4 checkpoint blockade immunotherapy[178] (Figure 7). The NP was composed of a PTT agent, indocyanine green, a TLR7 ligand, imiquimod (R837), and PLGA; all three are clinically approved components. The NP could eliminate primary tumor with near-infrared laser-triggered photothermal ablation. Tumor-associated antigen released due to PTT-mediated tumor cell death was increasingly uptaken by DCs for antigen presentation in the presence of co-delivered R837 leading to enhanced systemic anti-tumor immune responses. Together with anti-CTLA-4 checkpoint blockade therapy, this strategy significantly inhibited tumor metastasis and recurrence.

Conclusion

As illustrated by many examples here, rationally designed biomaterials showed great promise in enhancing cancer immunotherapies by improving the efficacy while reducing the toxicity. In addition to promoting vaccine delivery, rapid advances have been made in developing engineered biomaterials for promoting a broad spectrum of various modalities of cancer immunotherapies. Our increasing capability of exquisitely controlling the structure and function of biomaterials and delivery systems will likely enable more precisely controlled immunomodulation for safer and more efficacious immunotherapies. While the toolbox of novel cancer immunotherapies is being expanded and synergistic combination therapies are being identified, major challenges remain in applying immunoengineering strategies for the future development of next-generation cancer immunotherapies. For example, one of such challenges is to develop more effective delivery systems to augment the therapeutic function of antibodies or their alternatives while eliminating non-specific toxicities. Antibody based therapy will likely continue to be the mainstream in immune-related therapeutic development. However, given some intrinsic limitations of antibodies, such as high cost of development, large molecular weight and limited tissue penetration, as well as widely distributed targets within the entire immune system, smart strategies based on materials immunoengineering for targeted delivery of antibody activity to the disease related tissues or cells are of great interest for future immunotherapy development. Alternative therapeutic agents to the traditional antibody, such as nanobody, small molecule ligand, siRNA, etc., offer great promise to overcome some of the limitations of antibody therapies. Novel delivery systems with sophisticated design are required for those agents to overcome issues such as undesired pharmacokinetics, low bioavailability, etc.

Another challenge is to develop advanced nucleic acid delivery systems for specific immunomodulation *ex vivo* and *in vivo*. Effective delivery of biodegradable and negatively charged molecules such as plasmid or oligonucleotides to immune cells or diseased cells *in vitro* or *in vivo* remains a major hurdle for gene-based therapies. Recently, significant advancement in genome editing, i.e. CRISPR/Cas9 system, has led to a number of biomedical applications based on this novel technology.[181] Non-viral genetic editing with CRISPR/Cas9 system for more efficient generation of CAR T-cells is currently being pursued.[182] Effective delivery systems are also highly desired to achieve *in vivo* gene-therapy based immunomodulation with various therapeutic nucleic acids, such as oligonucleotides, antisense oligonucleotide, microRNA, etc. In addition, combination therapy is most likely the direction to go for increasing response rate of patients with durable disease control. Another looming challenge is how to engineer biomaterials to achieve effective co-delivery of two or multiple components for the optimized synergistic effect.

The final challenge is to translate biomaterial-assisted immunotherapy to clinical application. It is important to integrate lessons learned in fields of applying biomaterials for cancer drug delivery and tissue engineering to the development of biomaterials based novel immunotherapies. Challenges facing the researchers include manufacturing scale-up of the materials in clinical standard when translating research finding to clinical evaluation. Design and use of biocompatible biomaterials with scalable synthesis are the keys to success. One good example is the ongoing Wyss Institute-funded Phase I trial of implantable vaccine scaffolds.[183] We could foresee more and more biomaterial-enabled effective and safe cancer immunotherapies will potentially be evaluated in clinic.

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Figures and Captions

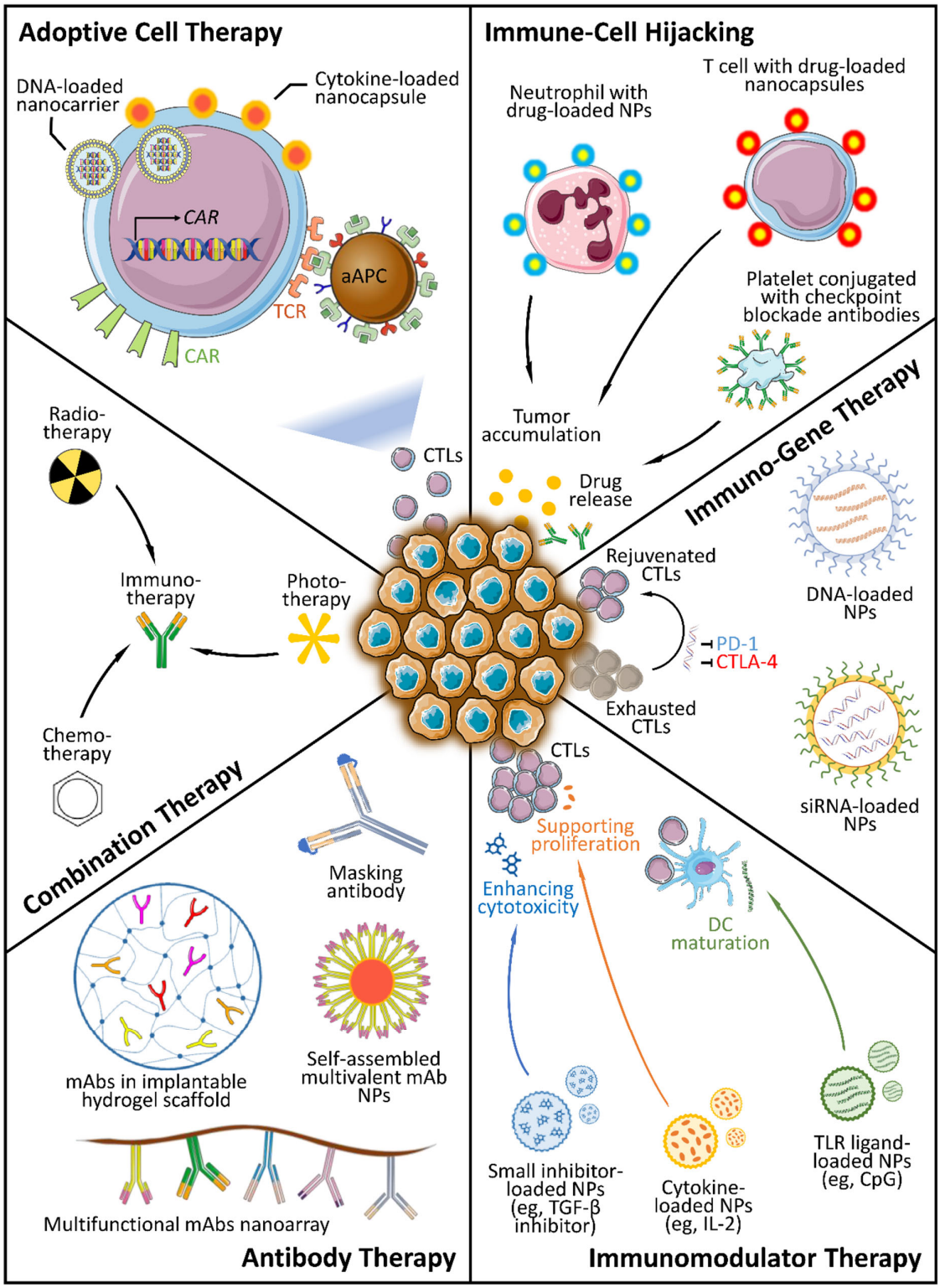

FIGURE 1 | Schematic view of examples of immunoengineering strategies for enhancing different modalities of cancer immunotherapies. CTL: cytotoxic T lymphocyte. CAR: chimeric antigen receptor. TCR: T-cell receptor. aAPC: artificial antigen-presenting cell. NPs: nanoparticles. PD-1: programmed

cell death protein 1. CTLA-4: cytotoxic T-lymphocyte-associated protein 4. siRNA: small interfering RNA. DC: dendritic cell. TLR: Toll-like receptor. mAb: monoclonal antibody.

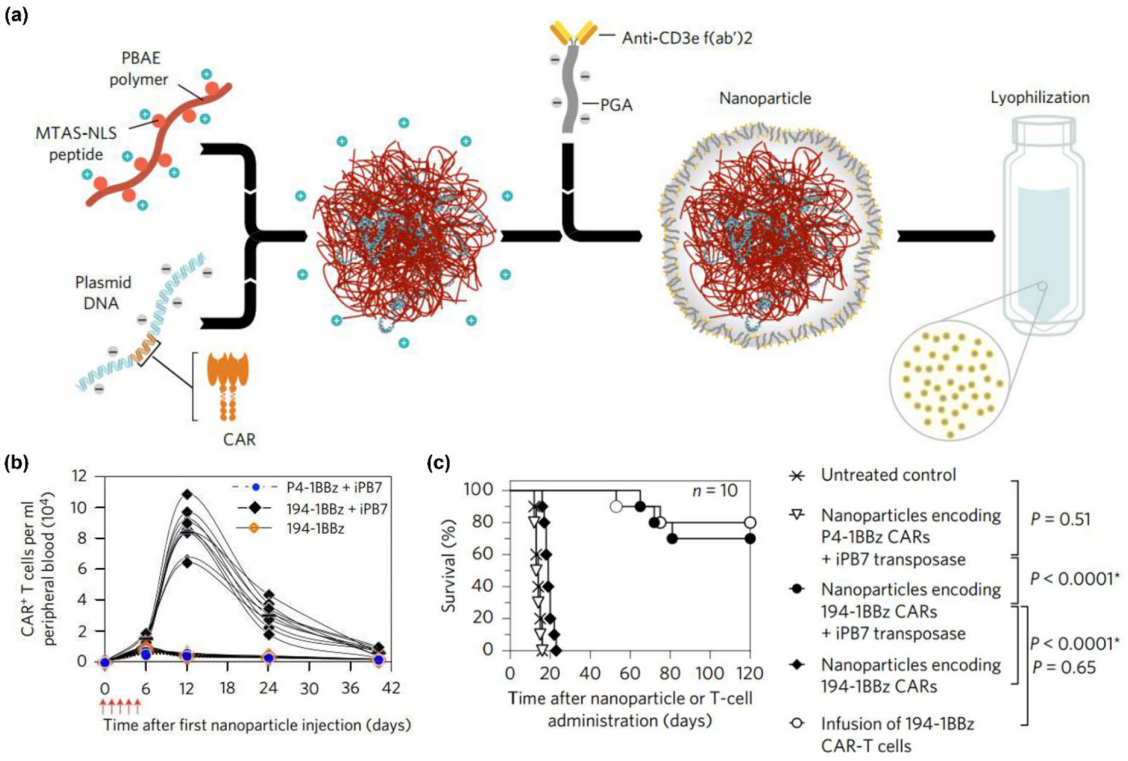

FIGURE 2 | *In vivo* programming of circulating T-cells into antigen-specific T-cells by synthetic DNA NPs. (a) Design and manufacture of lymphocyte-programming DNA NPs. The plasmid DNA encoded the leukemia-specific 194-1BBz chimeric antigen receptor (CAR) and the hyperactive iPB7 transposase was mixed with poly(β-amino ester) (PBAE) polymer functionalized with microtubule-associated-nuclear localization (MTAS-NLS) peptides to form the DNA NPs. The surfaces of PBAE NPs was then coupled with T-cell-targeting anti-CD3e f(ab')$_2$ fragments, which selectively enabled CD3-mediated endocytosis by T-cells. (b) CAR$^+$ peripheral T-cells frequency following the injection of NPs delivering DNA that encoded leukemia-specific 194-1BBz with iPB7, tumor-irrelevant P4-1BBz CAR genes, or 194-1BBz transgene alone. (c) NPs-programmed CAR-T cells induced tumor regression and increased overall survival similarly as adoptively transferred T-cells transduced *ex vivo*. (Reprinted with permission from Ref 56. Copyright 2017 Nature Publishing Group)

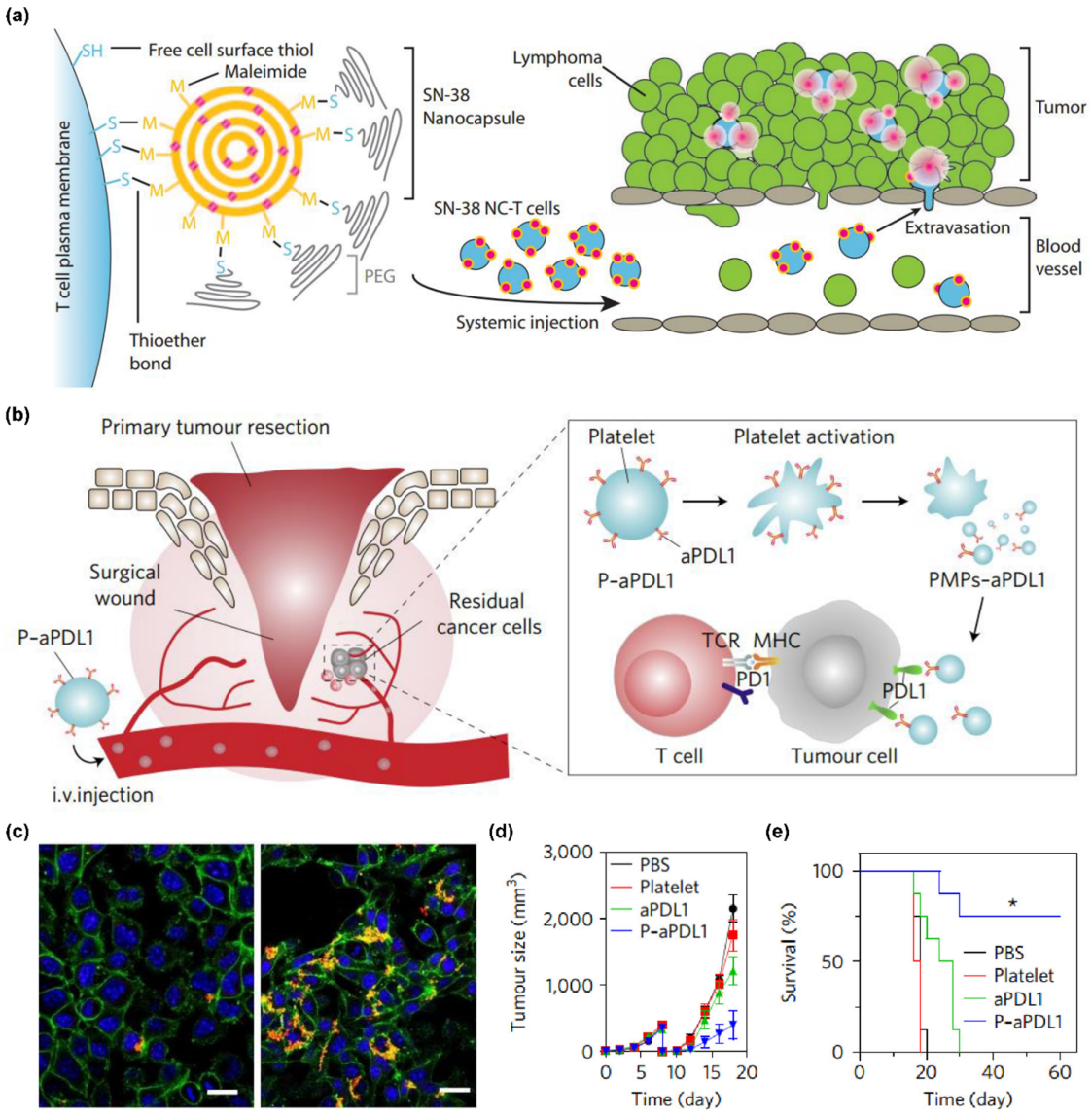

FIGURE 3 | Hijacking immune cells for drug delivery. (a) Schematic view of T-cell functionalization and cell-mediated delivery of topoisomerase I poison SN-38 nanocapsules (NCs) into tumors. (Reprinted with permission from Ref 60. Copyright 2015 American Association for the Advancement of Science) (b) Schematic illustration of the delivery of anti-PD-L1 antibody (aPDL1) to the primary-tumor resection site by platelets. TCR: T-cell receptor; MHC: major histocompatibility complex; PMPs: platelet-derived microparticles; P–aPDL1: aPDL1-conjugated platelets. (c) Confocal immunofluorescence images of B16 cancer cells co-incubated with non-activated (left) and activated (right) P–aPDL1 in a transwell system (pore size: 1 µm). P–aPDL1 and B16 cancer cells were cultured in upper and lower compartments, respectively. Red, blue and green fluorescence indicates aPDL1, nucleus and plasma membrane, respectively. Scale bar, 20 µm. (d, e) Recurrent tumor growth (d) and survival curves (e) of mice bearing a mouse melanoma model with incomplete-tumor-resection. B16-F10 tumors were surgically resected in part followed by i.v. injection of phosphate-buffered saline (PBS), platelets, aPDL1 or P–aPDL1 (dose of aPDL1, 1 mg kg^{-1}). (Reprinted with permission from Ref 69. Copyright 2017 Nature Publishing Group)

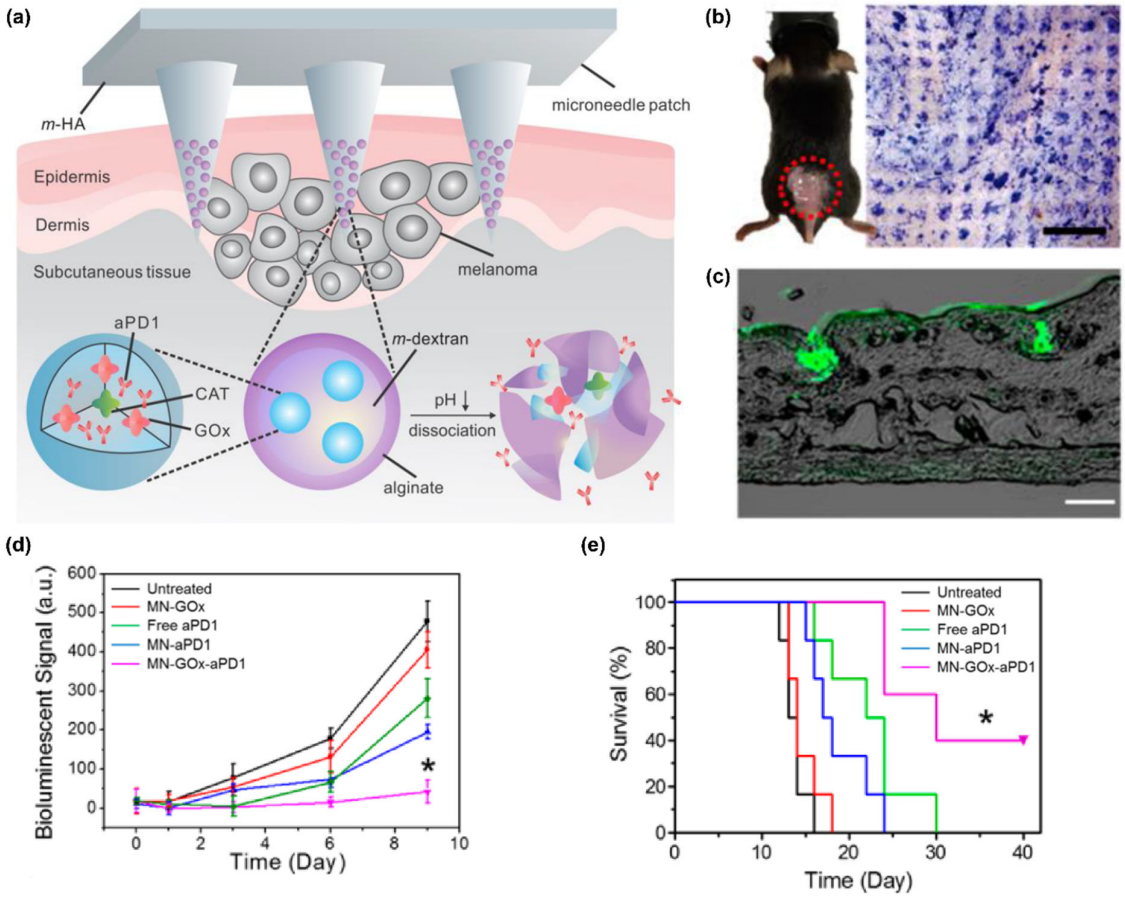

FIGURE 4 | Microneedle patch for enhanced efficacy of checkpoint blockade antibody therapy. (a) Schematic view of the anti-PD-1 antibody (aPD1) delivered by a microneedle (MN) patch loaded with physiologically self-dissociated NPs. With glucose oxidase/catalas (GOx/CAT) enzymatic system immobilized inside the NPs by double-emulsion method, the enzyme-mediated conversion of blood glucose to gluconic acid promoted the sustained dissociation of NPs, subsequently leading to the release of aPD1. (b) Mouse dorsum and relevant skin (the area within the red dashed line) was transcutaneously treated with a MN patch (left), with the image of the trypan blue staining showing the penetration of MN patch into the mouse skin (right) (scale bar, 1 mm). (c) Merged fluorescence and bright field image of the mouse skin penetrated by MNs loaded with fluorescein isothiocyanate (FITC)-labeled aPD1(shown in green) (scale bar, 200 μm). (d) Quantified bioluminescence signal of the subcutaneously implanted B16-F10 tumors in mice treated with MN patch (with GOx), free aPD1, or aPD1-loaded MN patch with or without GOx through a single local administration at the tumor site. (e) Kaplan−Meier survival curves for the treated and untreated mice. (Reprinted with permission from Ref 88. Copyright 2016 American Chemical Society)

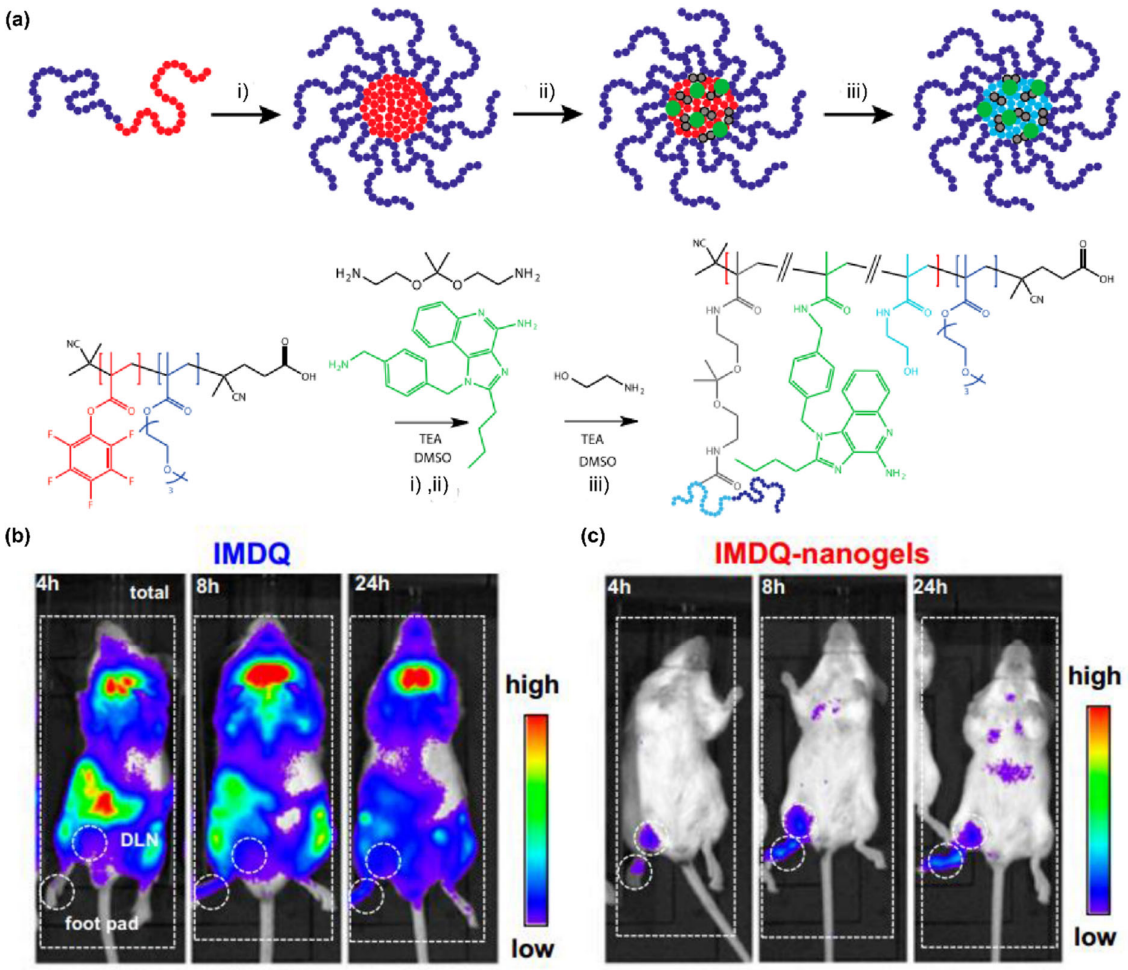

FIGURE 5 | Lymph node-focused delivery of small-molecule Toll-like receptor (TLR) agonist for cancer immunotherapy. (a) Schematic overview and corresponding chemical structures of degradable immune-stimulatory nanogels. i) Block copolymers self-assemble in dimethyl sulfoxide (DMSO) into NPs; ii) Covalent ligation of 1-(4-(aminomethyl)benzyl)-2-butyl-1H-imidazo[4,5-c]quinolin-4-amine (IMDQ), a TLR7/8 agonist (green) and cross-linking. iii) Conversion of residual pentafluorophenyl ester with 2-ethanolamine yielding fully hydrated nanogels after transferring to the aqueous phase. (b, c) *In vivo* bioluminescence in interferon-β reporter mice. Images recorded at 4, 8, and 24 h following injection of soluble IMDQ (b) and nanogel-ligated IMDQ (c) in the footpad (each at 10-μg IMDQ equivalents). DLN: draining lymph node. (Reprinted with permission from Ref 117. Copyright 2016 National Academy of Sciences, USA)

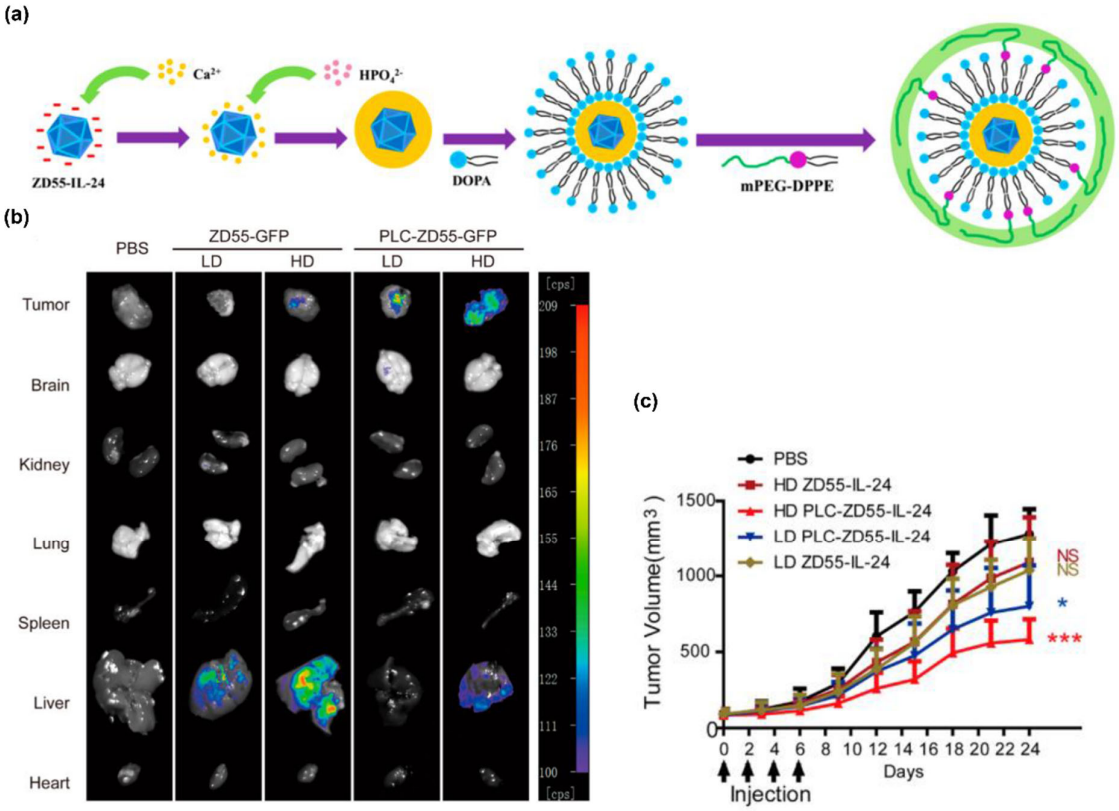

FIGURE 6 | Chemically modified oncolytic adenovirus () for immuno-gene therapy. (a) Synthetic scheme of polyethylene glycol (PEG)/lipids/calcium phosphate (CaP)-Onco^{Ad} (PLC-Onco^{Ad}) delivery system for ZD55-IL-24, an Onco^{Ad} that carries the IL-24 gene. CaP and ZD55-IL-24 were coprecipitated to produce an electron dense biomineral layer. Dioleoylphosphatydic acid (DOPA), an amphiphilic phospholipid, strongly interacted with cations at the interface to stabilize CaP/ZD55-IL-24. The mPEG2000-1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (mPEG-DPPE) formed a hydrophilic protective layer around the DOPA/CaP/ZD55-IL-24 complexes and facilitated long circulation time after intravenous administration. (b) Fluorescence images of excised tumors and organs 4 days after the intravenous injection of ZD55-GFP or PLC-ZD55-GFP for the delivery of GFP as a model gene in nude mice bearing Huh-7 xenograft. GFP: green fluorescence protein. (c) Tumor growth curves of subcutaneous Huh-7 tumors in nude mice injected with PLC-Onco^{Ad} encoding IL-24 (PLC- ZD55-IL-24). LD: low dose = 7.5×10^9 viral particles (VPs); HD: high dose = 1.5×10^{10} VPs. (Reprinted with permission from Ref 152. Copyright 2016 American Chemical Society)

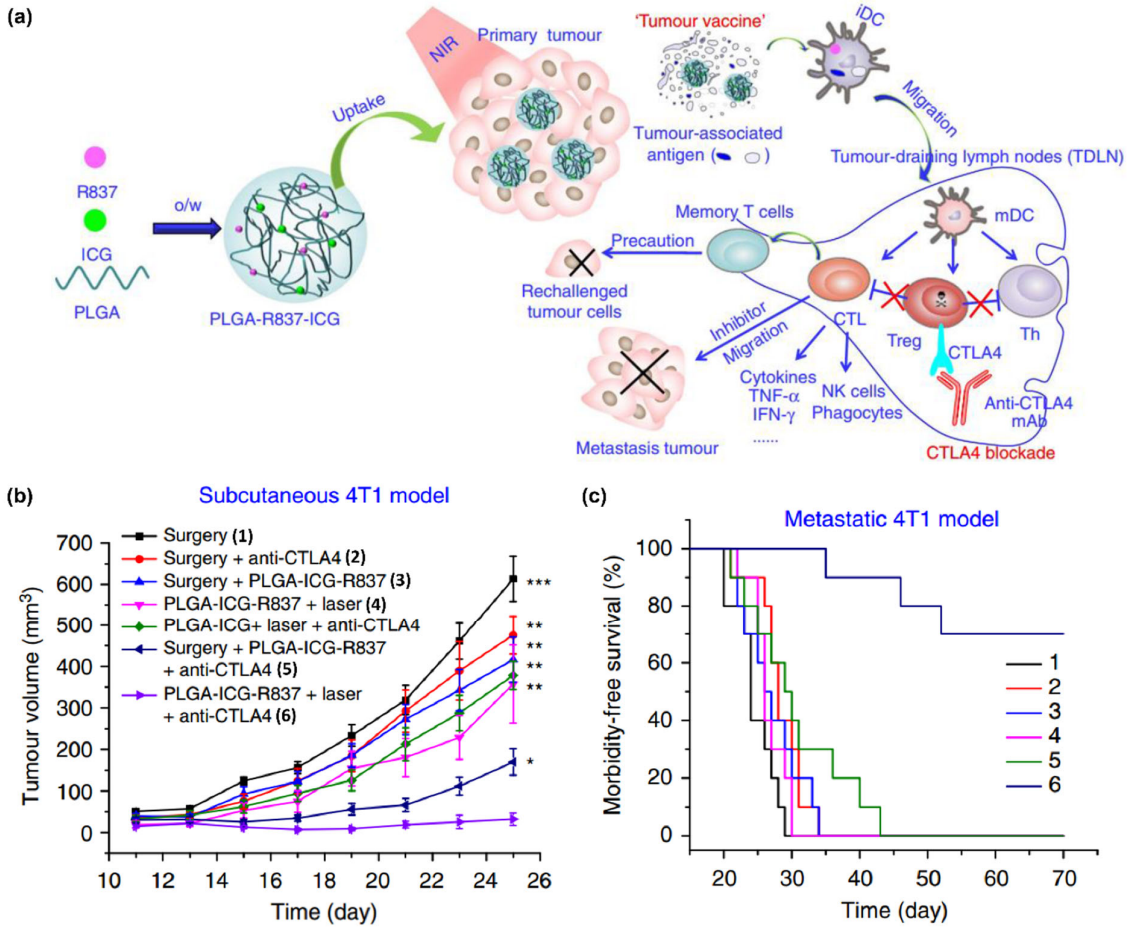

FIGURE 7 | Immunotherapy in combination with photothermal therapy (PTT). (a) The mechanism of anti-tumor immune responses induced by a NP-based PTT in combination with anti-cytotoxic T-lymphocyte antigen-4 (CTLA-4) checkpoint-blockade. Indocyanine green (ICG), a photothermal agent, and imiquimod (R837), a Toll-like-receptor-7 agonist, were co-encapsulated by poly(lactic-co-glycolic) acid (PLGA) to form the NP for PTT. DC: dendritic cell; Th: helper T lymphocyte; CTL: cytotoxic T lymphocyte; NK: natural killer cell; Treg: regulatory T-cell; mAb: monoclonal antibody. (b) Secondary tumor growth curves of different groups of mice with subcutaneous 4T1 tumors after various treatments to eliminate their primary tumors. (c) Morbidity-free survival of different groups of mice with metastatic 4T1 tumors after various treatments indicated to eliminate their primary tumors (the numbers labeling the curves indicate the corresponding treatments in (b)). (Reprinted with permission from Ref 178. Copyright 2016 Nature Publishing Group)