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Advances in Engineering Local Drug Delivery Systems for Cancer Immunotherapy

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Abstract

Cancer immunotherapy aims to leverage the immune system to suppress the growth of tumors and to inhibit metastasis. The recent promising clinical outcomes associated with cancer immunotherapy have prompted research and development efforts towards enhancing the efficacy of immune checkpoint blockade, cancer vaccines, cytokine therapy, and adoptive T cell therapy. Advancements in biomaterials, nanomedicine, and micro-/nano-technology have facilitated the development of enhanced local delivery systems for cancer immunotherapy, which can enhance treatment efficacy while minimizing toxicity. Furthermore, locally administered cancer therapies that combine immunotherapy with chemotherapy, radiotherapy, or phototherapy have the potential to achieve synergistic antitumor effects. Herein, the latest studies on local delivery systems for cancer immunotherapy are surveyed, with an emphasis on the therapeutic benefits associated with the design of biomaterials and nanomedicines.

Summary and Outlook

Recent advances in the fields of molecular pharmaceuticals, biomaterials, and micro-/nano-technology have inspired enhanced local delivery systems for cancer immunotherapy, which can enhance efficacy and minimize the risk of adverse effects caused by systemic toxicity. Local administration using intratumoral injection, peritumoral injection, sprayable gel, or transdermal microneedle array can be effective to overcome potential systemic transport limitations and to enhance the retention time of therapeutics at the diseased site. Local administration also partially

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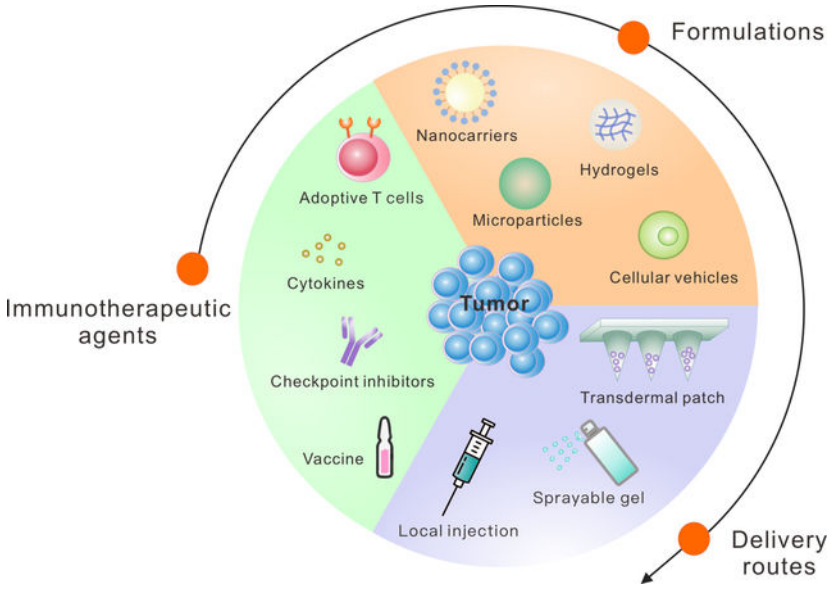
addresses the toxicity issue, as locally administered immunotherapeutics often have lower minimum effective doses than those of systemically administered immunotherapeutics. Sustained release formulations, which utilize materials such as hydrogels and micro-/nano-particles, can further address toxicity issues by establishing control over the release kinetics of the encapsulated therapeutic agents and can sometimes directly serve as adjuvants that help increase activation of the immune system. Importantly, compared to the local administration of small molecule chemotherapeutic drugs, local treatment with immunotherapeutics could also have efficacy toward metastasized sites. Locating and injecting internal tumors could be technically challenging, but these challenges could be addressed by imaging-guided injection and minimally invasive surgical techniques. Examples presented in this review show that locally administered immunotherapeutics can induce systemic antitumor responses specific to the tumor antigens at the injection site, and thus can be effective in inhibiting tumor recurrence and metastasis potential. However, detailed in vivo studies of pharmacokinetics and pharmacodynamics should be evaluated, associated with the distant diseased sites.

A detailed understanding of the intercellular interactions and signaling molecules in the tumor microenvironment can be leveraged to design combination therapies for synergistic antitumor effects. Therefore, continued research aimed at elucidating the immunological mechanisms that play a role in the establishment and development of tumors is crucial for further progress in the field of cancer immunotherapy. Moreover, for accelerating clinical translation of anticancer immunotherapeutic delivery systems, the rational design of formulations and devices in the initial development phase is crucial, especially regarding issues of biocompatibility of materials, feasibility of large-scale manufacturing, and quality control. The selection of formulations and delivery routes should be tightly linked to the clinical needs. For example, hydrogel- based systems are well-suited to deliver multiple types of therapeutics, such as immune checkpoint antibodies and chemo- therapy small molecules. By altering the chemical composition, properties such as the drug release rate, biocompatibility, degradation triggers, and physical properties of the hydrogel can be tuned to the desired specifications. Nano-/micro- formulations can also be incorporated to adjust these properties. Moreover, the location of the tumor and the disease degree should be carefully considered when selecting the delivery systems. For example, in the case of unresectable melanoma, a transdermal patch might be highly suitable. If only a partial surgical resection is possible, an injectable hydrogel may be suitable to replace the volume removed. If a tumor can be fully resected, a sprayable gel may be preferred to treat the post-surgical site with sufficient area covered. By fine-tuning the selection of therapeutics, formulation, delivery methods, and potential combination with other treatment modalities such as radiotherapy, clinical efficacy and safety can be enhanced. As personalized medicine is emerging as an overarching theme in healthcare, the choice of the most suitable therapeutic agent(s), formulation(s), and delivery method(s) should be tailored for each individual patient.

Graphical/Visual Abstract and Caption



Caption: Recent advances in local delivery systems for cancer immunotherapy show promise for enhancing therapeutic efficacy while minimizing toxicity.

Introduction

Cancer treatment strategies include surgical resection (Pavri, Clune, Ariyan, & Narayan, 2016), chemotherapy (Diaby, Tawk, Sanogo, Xiao, & Montero, 2015), radiotherapy (Allen, Her, & Jaffray, 2017), hormone therapy (Forster, Stoffel, & Gasser, 2002), stem cell therapy (T. Wang et al., 2015), and immunotherapy (Velcheti & Schalper, 2016). Representative cancer immunotherapy approaches include immune checkpoint blockade, cytokine therapy, cancer vaccines, and adoptive T cell therapy (Kakimi, Karasaki, Matsushita, & Sugie, 2017; Lohmueller & Finn, 2017; Y. Yang, 2015; Yousefi, Yuan, Keshavarz-Fathi, Murphy, & Rezaei, 2017). The immune system can normally play a key role in eliminating cancer cells. Specifically, the immune system is able to identify, target, and eliminate cancer cells (C. Wang, Ye, Hu, Bellotti, & Gu, 2017). In the cancer-immunity cycle, released tumor-associated antigens are internalized by antigen-presenting cells (APCs) and then presented to naïve T cells, which become cytotoxic T lymphocytes (CTLs) that can recognize and kill cancer cells (Shi & Lammers, 2019). In order to avoid elimination by the immune system, cancer cells can create an immunosuppressive tumor microenvironment that hinders the ability of immune cells to eliminate cancer cells - in effect “pushing the brakes” (Jeanbart & Swartz, 2015). Therefore, the goal of immunotherapy is either to limit the ability of cancer cells to inhibit the immune response and/or to increase activation of immune cells to promote their ability to target cancer cells.

Although the advances in the development of cancer immunotherapy are promising, considerable limitations and risks still need to be addressed. Limitations of immune checkpoint blockade include systemic toxicity and relatively low clinical objective response rates (A. Ribas & Wolchok, 2018). Many studies are attempting to elucidate the biological factors that determine the efficacy or lack thereof of immunotherapeutics. For example, it is

known that the efficacy of immune checkpoint blockade could be affected by factors related to the gut microbiome (Gopalakrishnan et al., 2018; Sivan et al., 2015; Vétizou et al., 2015), signal transducer and activator of transcription 1 (STAT1) signaling, Toll-like receptor 3 (TLR3) signaling, interleukin-10 (IL-10) signaling, and by the number of infiltrating-activated natural killer (NK) cells (Zemek et al., 2019). Limitations of adoptive T cell therapy include the risk of cytokine release syndrome, a severe reaction to therapy that is potentially life-threatening, and limited efficacy towards solid tumors when administered systemically, as a result of issues relating to trafficking and expansion within tumors (Beatty & O'Hara, 2016; Gill, Maus, & Porter, 2016). For these reasons, delivery methods that enhance therapeutic efficacy while mitigating the risk of systemic toxicity and adverse side effects are needed (A. S. Cheung & Mooney, 2015; Hotaling, Tang, Irvine, & Babensee, 2015; Koshy & Mooney, 2016; Marabelle, Kohrt, Caux, & Levy, 2014; Riley, June, Langer, & Mitchell, 2019; Weber & Mule, 2015). Advances in nano-/micro-technologies have the potential to address some of these limitations by enhancing the local delivery of immunotherapeutic agents and by modulating the local tumor microenvironment (Fan & Moon, 2015; Irvine, Hanson, Rakhra, & Tokatlian, 2015; Ji, Zhao, Ding, & Nie, 2013; Shao et al., 2015; Sun et al., 2019). Furthermore, nanomedicine-based immunotherapies have demonstrated the potential to inhibit cancer metastasis in preclinical cancer models (P. Zhang, Zhai, Cai, Zhao, & Li, 2019). Among them, the local delivery systems have potential to enhance the efficacy and safety of immunotherapy by facilitating sustained delivery of immunotherapeutics directly to the disease site, while minimizing the side effects that are often associated with systemic administration (Qian Chen, Wang, Chen, Hu, & Gu, 2018; Gu & Mooney, 2016; Huitong Ruan et al., 2019; Wen, Chen, et al., 2019; Wolf et al., 2019). Therefore, in cases when solid tumors cannot be fully resected due to technical or other reasons, local immunotherapy drug delivery systems could be preferable compared to systemic administration. Furthermore, drug delivery-assisted cancer immunotherapy can be enhanced when combined with other cancer therapeutics, including chemotherapy, radiotherapy, and phototherapy (Qian Chen, Chen, & Liu, 2019; D. G. Leach, Young, & Hartgerink, 2019; H. Wang & Mooney, 2018). In this review, we will discuss advances in enhancing the efficacy of cancer immunotherapeutics through the rational design of local drug delivery systems (Figure 1).

1. Local Delivery of Immune Checkpoint Inhibitors

Immune checkpoints include intercellular inhibitory pathways and immunosuppressive enzymes, such as indoleamine-2,3-dioxygenase (IDO) (Pardoll, 2012; Ricciuti et al., 2019). Because cancer cells can exploit immune checkpoints to avoid immune system-mediated elimination, immune checkpoint inhibition can be an effective anticancer strategy. Food and Drug Administration (FDA) approved immune checkpoint inhibitors work by targeting cytotoxic T lymphocyte-associated protein 4 (CTLA-4 or CTLA4) or the interaction between programmed cell death 1 (PD-1) receptors and the associated programmed cell death ligand 1 (PD-L1) (R. Park, Winnicki, Liu, & Chu, 2019; A. Ribas & Wolchok, 2018). CTLA-4 is a cell surface protein expressed by T cells that inhibits the T cell response against cancer cells by outcompeting for signaling molecules (CD80 and CD86) necessary for effector T cell activation and by facilitating T cell inhibitory signaling (Bengsch, Knoblock, Liu, McAllister, & Beatty, 2017; A. Ribas & Wolchok, 2018). The interaction between

CTLA-4 and CD80/CD86 results in a diminished ability of T cells to kill cancer cells, which permits continued tumor growth. Ipilimumab is an FDA-approved antibody therapy designed to bind to CTLA-4 in order to counteract its inhibitory effects (A. Ribas & Wolchok, 2018). Another inhibitory effect is due to the secretion of Interferon-γ (IFNγ) by T cells in response to cancer cells, which triggers the expression of PD-L1 in cancer cells (Alsaab et al., 2017). When the PD-1 receptors on T cells interact with PD-L1, the antitumor response of the T cells is diminished, allowing continued tumor growth (Alsaab et al., 2017). Several FDA-approved antagonistic antibodies, which inhibit binding of PD-1 to PD-L1 by blocking either PD-1 (Pembrolizumab, Nivolumab) or PD-L1 (Atezolizumab, Avelumab, Durvalumab) are currently in the market (A. Ribas & Wolchok, 2018; Zou, Wolchok, & Chen, 2016). CD47 immune checkpoint blockade is also another promising strategy that has been under recent investigation in clinical trials (Uger & Johnson, 2020). CD47, a cell surface protein often over-expressed on the surface of cancer cells, binds to SIRPα receptors on macrophages, which inhibits their phagocytosis capability and allows cancer cells to avoid phagocytic attack (Jaiswal et al., 2009; Soto-Pantoja et al., 2014; Vonderheide, 2015). The local delivery of immune checkpoint inhibiting antibodies for solid tumors has been reported to enhance therapeutic efficacy and reduce systemic toxicities when compared to systemic administration (Aznar et al., 2017; Huynh et al., 2019; Marabelle, Kohrt, & Levy, 2013; Simmons et al., 2008; C. Wang, Ye, & Gu, 2017). Furthermore, advances in the fields of biomaterials and nanomedicine have facilitated enhanced therapeutic efficacy and delivery of immune checkpoint blockade therapy (Francis & Thomas, 2017; Lamichhane et al., 2019; Marabelle, Tselikas, de Baere, & Houot, 2017; Zhao, Li, Wei, & Luo, 2018). In this section, we will summarize several examples of local delivery systems for immune checkpoint inhibiting antibodies and IDO inhibitors. Additional methods to locally deliver immune checkpoint inhibitors in combination with other cancer therapeutics will be discussed in the combination therapy section.

In one example, Lei et al. reported that functionalized mesoporous silica (FMS) nanoporous supports loaded with anti-CTLA-4 antibodies (aCTLA-4) locally administered at the tumor site could enhance therapeutic efficacy (Lei et al., 2010). The authors had previously reported a method for protein loading and immobilization in FMS (Lei et al., 2006). Unfunctionalized mesoporous silica was modified with -COOH and -NH_2 functional groups to generate FMS and to facilitate electrostatic, hydrophilic, and hydrogen bonding interactions with the loaded protein. The authors demonstrated that controlled release of the antibodies could be tuned by altering the pore size of the FMS and by changing the type and concentration of functional groups present in the FMS. The authors investigated how significant toxicities associated with systemically administered aCTLA-4, such as enteritis and endocrine deficiencies, could be avoided by means of local delivery (J. C. Yang et al., 2007). They found that direct injection of aCTLA-4 loaded FMS into a murine melanoma cancer model effectively inhibited tumor growth. Furthermore, FMS particles exhibited negligible toxicity and could facilitate sustained local release of the antibodies with less systemic exposure compared to directly injected antibodies.

In another work, Fransen et al. conducted a study to investigate the effects of local and systemic delivery on the efficacy of CTLA-4 blockade in murine cancer models (Fransen, van der Sluis, Ossendorp, Arens, & Melief, 2013). The authors reported that an 8-fold lower

dose administered locally was as effective as a systemic high dose, and that mice given the low dose treatment systemically had similar survivorship outcomes to untreated mice. The authors also found that local aCTLA-4 treatment of tumors could decrease the tumor volume of distant tumors. The authors proposed that this phenomenon is due to the effect of aCTLA-4 on tumor-specific $CD8^+$ T cells. The authors also compared the effect of a local, low dose aCTLA-4 in a slow release formulation of Montanide ISA-51, a water in oil emulsion that is also known as incomplete Freund's adjuvant (IFA), to a systemic high dose of aCTLA-4, and found that serum levels of aCTLA-4 were 1,000-fold higher in the systemic high dose condition. By decreasing systemic exposure to aCTLA-4, controlled release formulations could help reduce systemic toxicity. In a related study, Sandin et al. investigated the effects of local vs. systemic delivery of aCTLA-4 for the treatment of a murine model of pancreatic adenocarcinoma cancer (Sandin, Eriksson, et al., 2014). The authors found that an approximately 6-fold higher systemic dose was necessary to achieve a comparable outcome to the local dose.

Rahimian et al. engineered aCTLA-4 loaded poly(lactic-co-hydroxymethyl-glycolic acid) (pLHMGA) polymer-based microparticles and demonstrated their efficacy in treating murine models of cancer (Leemhuis et al., 2006; Rahimian et al., 2015). By varying the polymer concentration, the investigators could control the microparticle size, loading efficiency, duration of release, and burst release properties. Increasing polymer concentration resulted in decreased burst release, and all formulations exhibited a sustained release kinetic profile due to polymer degradation. The authors compared the effects of locally administered aCTLA-4 loaded microparticles with locally administered aCTLA-4 in an IFA formulation and found that serum antibody levels were 5 to 10 times lower using microparticles than with the IFA formulation. Furthermore, the authors observed no remainder of the microparticle formulation at the injection site during postmortem examination, whereas the IFA formulation was still observable.

Microneedle array patches have been engineered to enhance local immune checkpoint inhibition therapy. For the first time, Wang *et al.* demonstrated the delivery of anti-PD-1 antibodies (aPD-1) to treat melanoma using a transdermal microneedle patch (Figure 2) (C. Wang, Ye, Hochu, Sadeghifar, & Gu, 2016). Nanoparticles were prepared using a biocompatible pH sensitive dextran matrix, polyelectrolyte-based surfactant, a glucose oxidase (GOx)-catalase enzymatic system, and aPD-1. The surfaces of the nanoparticles were coated with alginate to achieve negatively charged outer surfaces. The nanoparticles were embedded and concentrated at the tips of a hyaluronic acid (HA)-based microneedles. This system released aPD-1 in a glucose and pH-dependent manner, due to the catalytic effect of the GOx enzyme and the acid degradable polymeric component. By changing the amount of GOx enzyme present, the release kinetics of the aPD-1 could be adjusted to facilitate controlled release. The authors then tested a similar microneedle formulation to load both aCTLA-4 and aPD-1, which outperformed aCTLA-4 alone and aPD-1 alone, validating that simultaneous delivery of aCTLA-4 and aPD-1 immune checkpoint inhibitors could increase treatment efficacy. Ye et al. reported a method to deliver aPD-1 and 1-methyl-DL-tryptophan (1-MT), an IDO inhibitor, *via* a transdermal microneedle patch to treat murine models of melanoma (Y. Ye et al., 2016). IDO is an enzyme that has been shown to limit T effector cell function while promoting the proliferation and differentiation of T

regulatory cells, which together make the tumor microenvironment more immunosuppressive (Munn, 2012). Therefore, by inhibiting this enzyme with 1-MT, the authors sought to further enhance the therapeutic effects of the microneedle-based immune checkpoint inhibitor system previously designed. The authors utilized a 1-MT-conjugated HA microneedle formulation to encapsulate the anti-PD-1 loaded nanoparticles. Accumulation of 1-MT in the tumor site was 3-fold greater in the microneedle formulation with 1-MT than that of free 1-MT, and furthermore, tumor uptake of aPD-1 increased in the 1-MT containing microneedle. The mice treated with the microneedle formulation containing 1-MT had significantly higher survivorship than the mice treated with the microneedle formulation without 1-MT. Recently, Chen *et. al.* developed a transdermal hollow-structured microneedle array patch to facilitate cold atmospheric plasma-mediated aPD-L1 therapy for melanoma treatment (Figure 3) (G. Chen et al., 2020). The microneedle patch facilitated the entry of cold atmospheric plasma into the tumor, causing the release of tumor-associated antigens. The authors found that such release of tumor-associated antigens and PD-L1 blockade inhibited tumor growth in both treated and distant (untreated) tumors, indicating that a systemic anti-tumor immune response had been achieved.

Ultrasound-guided injections can be used to deliver a variety of therapies to internal unresectable tumors in a minimally invasive manner. Van Hooren et al. conducted a study to investigate the effect of local vs. systemic delivery of CTLA-4 and PD-1 checkpoint inhibitors to treat murine models of bladder cancer (van Hooren et al., 2017). The authors first performed a titration experiment wherein mice bearing subcutaneous murine bladder 49 tumors (MB49) were injected with either 3, 10, or 30 μg aCTLA-4 either peritumorally or intravenously. The authors found that only the 30 μg dose was effective, and that both routes of administration exhibited similar therapeutic efficacy. However, intravenous injections produced significantly higher serum levels of aCTLA-4 and IL-6 than local injections at four hours post injection. Lower serum aCTLA-4 levels decrease the risk of adverse events due to the toxicity effects of CTLA-4 therapy. The authors then investigated the effect of an ultrasound-guided intratumoral injection of aCTLA-4 on an orthotopically growing MB49-GFP-Luc bladder tumor and found a more than ten-fold reduction in serum aCTLA-4 levels in comparison to systemic administration.

Ishihara et al. reported a method to locally deliver aCTLA-4 and aPD-L1 *via* conjugation to high affinity extracellular matrix binding peptides to treat murine tumor models of melanoma and breast cancer (Ishihara et al., 2017). Sulfosuccinimidyl-4-(*N*-maleimidomethyl) cyclohexane-1- carboxylate (sulfo-SMCC) was used to conjugate PlGF-$2_{123–144}$ peptides, which have high affinity for cellular extracellular matrices (ECM), to either aPD-L1 or aCTLA-4. The results show that each antibody could be conjugated to about six PlGF-$2_{123–144}$ peptides without altering its target recognition capability. Both PlGF-$2_{123–144}$-anti-CTLA-4 and PlGF-$2_{123–144}$-anti-PD-L1 were found to bind to the ECM proteins, including fibronectin, fibrinogen, vitronectin, osteopontin, and type I, II, III, and IV collagen. The purpose of the modification was to increase the retention of the antibodies at the tumor site, enhance their therapeutic effects, and minimize their toxic side effects by increasing T cell activation and by decreasing systemic exposure. Peritumorally injected PlGF-$2_{123–144}$–antibodies were retained at the tumor site for a longer time than freely administered antibodies. Reduced systemic toxicity in comparison to unmodified antibodies

given *via* peritumoral and intra-peritoneal routes of administration was also achieved with lower serum concentrations of antibodies, tumor necrosis factor–α (TNFα), IFNγ, the liver damage marker, and alanine aminotransferase (ALT) in mice. The combination of PlGF-$2_{123–144}$–anti-CTLA-4 and PlGF-$2_{123–144}$–anti-PD-L1 resulted in T cell activation and increased the number of tumor-infiltrating $CD8^+$ T cells relative to free antibodies.

Since the tumor microenvironment is often acidic, delivery systems that are pH-dependent are desirable for bioresponsive delivery of local immune checkpoint blockade. In one example, Chen et al. designed a sprayable *in situ* formed pH responsive fibrin gel to deliver anti-CD47 antibodies (aCD47) to the post-surgical tumor site (Figure 4) (Qian Chen, Ci, & Gu, 2019; Qian Chen, Chao Wang, et al., 2019). The aCD47 induced tumor associated macrophages to phagocytize cancer cells by blocking the inhibitory ‘don’t eat me’ signaling between cancer cells and macrophages. The dispenser for this therapy contains two compartments; one compartment contains thrombin, and the other compartment contains fibrinogen and aCD47 loaded $CaCO_3$ nanoparticles. When the two liquids mix at the tumor site, the reaction between thrombin and fibrinogen results in the formation of a biodegradable fibrin gel. The nanoparticles degrade in the presence of the relatively acidic tumor microenvironment and release aCD47.

In addition to being relatively acidic, the tumor microenvironment often contains reactive oxygen species (ROS), so ROS-dependent delivery systems are also rational choices for local immune checkpoint blockade. In one example, Yu et al. engineered an injectable ROS-responsive polypeptide-based hydrogel for co-delivery of anti-PD-L1 antibodies (aPD-L1) and dextro-1-methyl tryptophan (D-1MT) to the tumor microenvironment (Figure 5) (Yu et al., 2018). The authors were able to achieve synergistic antitumor effects in tumor-bearing mice using this combination therapy. In another example, Chen et al. designed ROS-sensitive protein complexes for controlled local release of aCD47 and aPD-1 to the tumor site (Qian Chen, Guojun Chen, et al., 2019). Due to the considerable presence of ROS in the tumor microenvironment, the authors hypothesized that the presence of ROS would be a suitable trigger for controlled release of the antibodies. The antibody complexes were prepared by cross-linking aPD-1 and aCD47 using an ROS-sensitive cross-linker: bis-*N*-hydroxy succinimide (NHS) modified 2,2′-[propane-2,2-diylbis(thio)]diacetic acid (NHS-IE-NHS) (Qian Chen, Guojun Chen, et al., 2019). In addition to serving as a trigger for controlled release, the ability of the ROS-sensitive cross-linker-based particles to scavenge the tumor microenvironment for ROS resulted in a significantly reduced population of immunosuppressive cells, including M2-type macrophages and regulatory T cells.

2. Local Delivery of Immunostimulatory Agents

In contrast to immune checkpoint blockade, which inhibits the ability of cancer cells to inactivate T cells, immunostimulatory agents aim to directly activate immune cells; if immune checkpoint blockade limits the ability of cancer cells to “push the brakes,” then immunostimulatory agents can be thought of as “pushing the accelerator.” This can be achieved by several strategies, including targeting co-stimulatory immune cell receptors, delivering cytokines to the tumor microenvironment, or administering immunostimulatory adjuvants.

2.1 Targeting Co-Stimulatory Immune Cell Receptors—Co-stimulatory immune cell receptors, including CD40, OX40 (CD134), and CD137, are potential therapeutic targets for immunotherapy. The binding of agonistic antibodies to these receptors results in downstream signaling as if the natural ligand was bound. This mechanism is the opposite of that used in immune checkpoint blockade, in which antagonistic antibodies that inhibit downstream signaling are used. For example, agonistic antibodies and adenoviral vectors carrying CD40L complementary DNA have been developed to target CD40 (Beatty, Li, & Long, 2017; Lindqvist, Sandin, Fransson, & Loskog, 2009). CD40 is a costimulatory cell surface receptor belonging to the Tumor Necrosis Factor (TNF) superfamily and is often present on APCs, and its ligation to CD40L on T cells causes APC activation and promotes a T cell mediated antitumor response (Vonderheide & Glennie, 2013). Therefore, agonistic CD40 antibodies (aCD40) can promote APC activation and subsequent antitumor immune responses. OX40 is a costimulatory cell surface receptor belonging to the TNF superfamily that can be present on T cells, and its ligation to OX40L on APCs results in enhanced antitumor T cell activity (Aspeslagh et al., 2016). CD137 is a costimulatory cell surface receptor belonging to the TNF superfamily that can be present on T cells and NK cells, and its ligation to 137L on APCs results in enhanced antitumor CTL responses (Pardoll, 2012; Yonezawa, Dutt, Chester, Kim, & Kohrt, 2015).

Sandin et al. conducted a study to compare the local and systemic delivery of aCD40 in MB49 tumor-bearing mice (Sandin, Orlova, et al., 2014). By radiolabeling the aCD40 and measuring serum radiation, the authors determined that local delivery resulted in lower serum antibody levels compared to systemic delivery. The authors reported that local low-dose aCD40 therapy increased survival and decreased toxicity compared to systemic high-dose aCD40 therapy. Furthermore, the locally delivered antibodies could result in tumor regression of both the primary tumor and distant tumors in murine cancer models. To decrease toxicity associated with aCD40 therapy, Fransen et al. developed a delivery system that implemented the slow-release formulation, Montanide ISA-51, to stimulate local tumor antigen-presenting dendritic cells with aCD40 (Fransen, Sluijter, Morreau, Arens, & Melief, 2011). The authors found that a low local dose of aCD40 in Montanide ISA-51 resulted in decreased serum levels of the liver damage markers, ALT and AST, compared to the high systemic dose treatment group.

To enhance the therapeutic efficacy of local delivery of aCD40 therapy and reduce its toxicity, Ishihara et al. utilized super-affinity peptides derived from placenta growth factor-2 (PIGF-$2_{123–144}$) (Ishihara et al., 2018). The authors found that PIGF-$2_{123–144}$ peptides conjugated to aCD40 improved its stability in the tumor microenvironment and decreased systemic toxicity. Another local delivery strategy for CD40 therapy was reported by Rahimian *et al.,* who engineered aCD40 loaded poly (lactic-co-hydroxymethyl-glycolic acid) (pLHMGA) polymer-based microparticles and tested them in murine cancer models (Rahimian et al., 2015). Compared to the IFA formulation, serum aCD40 levels after delivery of aCD40-loaded microparticles were lower, and therefore the risk of adverse side effects due to systemic exposure was mitigated. In another work, Chua *et al.* engineered a nanofluidic drug-eluting seed (NDES) to enhance the therapeutic efficacy of locally delivered aCD40 and agonistic OX40 antibodies (aOX40), and validated the system in

murine models of triple negative breast cancer (Figure 6) (Chua et al., 2018). The authors found that the high interstitial pressure usually associated with intratumoral injection could be avoided by gradually releasing the CD40 and OX40 therapeutic antibodies intratumorally over time using the NDES (Chua et al., 2018). The authors found that the NDES therapy allowed for enhanced controlled release of the antibodies, which resulted in both a local and systemic antitumor immune response. In other works, amphiphilic poly (γ-glutamic acid) nanoparticles and luminescent porous silicon nanoparticles were developed to facilitate local delivery of aCD40 (Broos et al., 2012; Gu et al., 2012).

2.2 Cytokines—IL-2, a pro-inflammatory cytokine, is reported to be the first effective immunotherapy for treating human cancer (Rosenberg, 2014). Other cytokines under investigation for use as immunotherapeutics include Granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-10, IL-12, IL-15, transforming growth factor- β (TGF-β) and TNF-α (Berraondo et al., 2019). Due to the potential lethal toxicity associated with the systemic administration of certain cytokines, it is essential to design drug systems that mitigate the risk of systemic cytokine toxicity while enhancing their antitumor efficacy.

Momin et al. engineered a local delivery system for the immune cytokines IL-2 and IL-12 by fusing them with lumican, a collagen binding protein (Momin et al., 2019). The authors observed that this modification enhanced retention of the cytokines in the tumor microenvironment while decreasing their systemic exposure. Sabel et al. encapsulated IL-12 and TNFα in poly-lactic acid microspheres for intratumoral immunotherapy, and reported that this combination resulted in a systemic and synergistic antitumor response, which was mediated by NK cells and cytotoxic T cells (Sabel et al., 2007). Hydrogels have also been designed to locally deliver cytokines for cancer immunotherapy (C. G. Park et al., 2018). In one example, Zaharoff et al. reported that intratumoral injection of IL-12 in chitosan solution resulted in systemic tumor specific immunity in tumor-bearing mice (Zaharoff, Hance, Rogers, Schlom, & Greiner, 2010). Chitosan was used to facilitate the local and sustained delivery of IL-12 in the tumor microenvironment and resulted in a longer retention time of IL-12 in the tumor region than locally administered free IL-12. The chitosan/IL-12 therapy partially or completely protected the mice from tumor rechallenge at non-injected sites. In another work, Kwong et al. engineered a local delivery system for the IL-2 fusion protein (IL-2Fc) and anti-CD137 by attaching them to the surface of PEGylated liposomes (Kwong, Gai, Elkhader, Wittrup, & Irvine, 2013). Intratumoral injection of anti-CD137-liposomes and IL-2Fc-liposomes in tumor-bearing mice treated most of the primary tumors and also resulted in systemic tumor immunity in a $CD8^+$ T cell dependent manner. Liposome therapy decreased systemic toxicity to negligible levels and slowed the growth of both primary and distant tumors.

2.3 Immunostimulatory Adjuvants—Immunostimulatory adjuvants are a class of immunotherapeutics that are often used in cancer vaccine formulations but can also be used as a monotherapy. Oligodeoxynucleotides containing unmethylated CpG-rich regions (CpG) are favored adjuvants for cancer immunotherapy vaccines due to their ability to activate cells of the innate immune system by binding to TLR9 receptors (Furumoto, Soares, Engleman, & Merad, 2004; Kawarada et al., 2001). For example, intratumoral injection of CpG and

adenovirus type 5 encoding a tumor antigen was shown to have therapeutic benefit in murine cancer models (Geary, Lemke, Lubaroff, & Salem, 2011). In one example, Zhang et al. engineered a CpG delivery system using 3-aminopropyltriethoxysilane (APTES)-modified Fe_3O_4 magnetic (FeNP) nanoparticles (X. Zhang et al., 2018). APTES modification increased CpG loading capacity by creating more CpG binding sites on the FeNPs. The therapeutic effect of this formulation for inhibiting tumor growth in a murine cancer model was superior to that of free CpG. Due to their superparamagnetic properties, FeNP nanoparticles could potentially be concentrated at the tumor site by applying an external magnetic field (Majidi et al., 2016). In another work, Zhu *et al.* engineered DNA-inorganic hybrid nanovaccines (hNVs) for delivery of CpG analogs (Zhu et al., 2016). hNVs were fabricated *via* self-assembly of concatemer CpG analogs and magnesium pyrophosphate (Mg_2PPi), and subsequently injected intratumorally into murine melanoma models. The hNVs were found to dissociate faster in acidic conditions, such as in internalized endosomes, facilitating the release of CpG from the hNVs. In comparison to free CpG, the hNV delivery vehicles carrying CpG significantly inhibited tumor growth, prolonged the retention time of CpG in the tumor microenvironment, and mitigated adverse systemic side effects. Another method to increase the retention time of CpG in the tumor microenvironment was reported by Liu et al., who engineered lipid-conjugated CpG for insertion into the membranes of tumor cells (Liu, Kwong, & Irvine, 2011).

In addition to CpG oligonucleotides, cyclic dinucleotides (CDNs) are also another effective immunostimulatory adjuvant. In one work, Wilson et al. engineered poly(beta-amino ester) nanoparticles to deliver CDNs, which activate the stimulator of interferon genes (STING) in APCs, upon intratumoral injection (Wilson et al., 2018). CDNs can also be delivered locally using hydrogels, as was demonstrated by Leach *et. al.*, who engineered a “STINGel” - a MultiDomain Peptide hydrogel loaded with CDNs (David G. Leach et al., 2018). The authors demonstrated that this MultiDomain Peptide-based hydrogel drug delivery system significantly enhanced the efficacy of CDN immunotherapy *in vivo* by decreasing the release rate of CDNs by 8-fold, in comparison to a collagen-based hydrogel. Such extended release corresponded to a higher local concentration of CDNs and increased survival rate in preclinical experiments.

Recently, tumor-associated macrophages (TAMs), which are capable of supporting tumor cell proliferation and metastasis, have been under intensive investigation as potential therapeutic targets (Komohara, Fujiwara, Ohnishi, & Takeya, 2016; Muraoka et al., 2019; Nie et al., 2017; B. Z. Qian & Pollard, 2010). Depending on their phenotypic polarization, TAMs can either support or suppress tumor growth; M2 polarized macrophages often support tumor growth, metastasis, and angiogenesis, whereas M1 polarized macrophages are tumor destructive (W. Hu et al., 2016; Rodell et al., 2018). An increased number of M2 polarized macrophages within the tumor microenvironment is associated with poor clinical prognosis and limited treatment efficacy (Zheng et al., 2017). Therefore, one strategy for immunotherapy is to increase the number of M1 TAMs while decreasing the number of M2 TAMs within the tumor microenvironment (Mills, Lenz, & Harris, 2016). For example, Jaynes *et. al.* reported that the RP-182 peptide could convert the polarization of TAMs from M2 to M1 upon binding to CD206 receptors, which are expressed on M2 TAMs (Jaynes et al., 2020).

Another strategy for macrophage-mediated immunotherapy involves using the toll-like receptor 7/8 agonist small molecule, R848, to increase macrophage M1 polarization and production of pro-inflammatory immune cytokines (Manome et al., 2018; Rodell et al., 2018). Park et al. demonstrated that extended peritumoral release of R848 from a hydrogel-based scaffold could significantly enhance treatment efficacy compared to local administration of R848 in solution (C. G. Park et al., 2018). Furthermore, the authors demonstrated that perioperative implantation of a hydrogel loaded with R848 could inhibit local and systemic tumor recurrence and metastasis, and could eliminate existing metastases. In a related work, Tsai *et. al.* designed a drug delivery system for Imiquimod (R837), a TLR7 agonist that has similar effects on macrophage polarization as R848, by first generating Imiquimod liposomes using 1,2-Dipalmitoyl-*sn*-glycero-3-phosphatidylcholine, and then loading them into a thermosensitive pluronic F127 hydrogel (Tsai et al., 2019). At cold temperatures, the liposomes and hydrogel are in a soluble form, while at body temperature, the mixture converts into a gel, facilitating the ease of administration.

3. Local Delivery of Cancer Immunotherapy Vaccines

Cancer immunotherapy vaccines aim to induce the host immune system to attack cancer cells by decreasing immune tolerance to tumor antigens, which can be achieved by co-delivery of tumor antigens and immunostimulatory adjuvants (Ali, Emerich, Dranoff, & Mooney, 2009). Many intratumorally administered therapeutic cancer immunotherapy vaccines aim to induce a CTL response against tumor cells by introducing strongly immunogenic epitope(s) of the specific cancer antigen(s) (Berzofsky et al., 2018). Cancer vaccines can contain several types of immunogenic components, including peptides, DNA, whole cell lysates, recombinant viruses, or modified immune cells (Thomas & Prendergast, 2016). Patient-specific neoantigens, which can arise as a result of tumor-specific mutations, can be utilized to formulate patient-specific cancer immunotherapy vaccines (Schumacher & Schreiber, 2015). To date, the FDA has approved Sipuleucel-T (Kantoff et al., 2010), talimogene laherparepvec (Gatti-Mays, Redman, Collins, & Bilusic, 2017; Johnson, Puzanov, & Kelley, 2015), and Bacillus Calmettle-Guerin (Gatti-Mays et al., 2017) for the treatment of prostate cancer, melanoma, and bladder cancer, respectively.

In situ cancer immunotherapy vaccines contain immunostimulatory components that activate host immune cells in the tumor microenvironment and utilize locally present tumor antigens, eliminating the need to pre-identify and deliver tumor antigens (Sheen & Fiering, 2019). Oncolytic viruses can be used to produce tumor-associated antigens for *in situ* vaccination, directly lysing tumor cells, and activating of cells of the innate immune system (P. K. Bommareddy & Kaufman, 2018; P. K. Bommareddy, Shettigar, & Kaufman, 2018; Kaufman & Bommareddy, 2019; Lichty, Breitbach, Stojdl, & Bell, 2014). The underlying immunological mechanisms by which a selected oncolytic virus functions as an immune adjuvant have been investigated (M. C. Brown et al., 2017). Oncolytic viruses have been used in clinic to treat various cancers, such as neuroblastoma, hepatocellular carcinoma, Ewing sarcoma, and retinoblastoma (Cripe et al., 2015; B. H. Park et al., 2008; Pascual-Pasto et al., 2019).

The efficacy of cancer vaccines can be enhanced by increasing the availability of tumor-associated antigens to antigen-presenting cells. An et al. described a method to utilize the cytotoxic effects of intratumorally injected cationic silica nanoparticles to induce necrotic tumor cell death, and by doing so, the release of tumor-associated antigens could activate antigen-presenting cells and induce antitumor immunity (An et al., 2018). To further enhance the activation of antigen-presenting cells, the authors complexed the cationic silica nanoparticles (CSiNPs) with CDN adjuvants, which significantly increased retention time in the tumor. The authors found that c-di-GMP/CSiNPs caused increased $CD8^+$ T cell activation and infiltration into the tumor microenvironment. The sustained local release of a pre-identified tumor antigen could also be an effective way to design a vaccine, as was demonstrated by Umeki et al., who engineered a CpG DNA-containing hydrogel encapsulating the modified model antigen, cationized ovalbumin (Umeki et al., 2015). The electrostatic interactions created between the positively charged antigen and negatively charged hydrogel significantly prolonged the release of the antigen from the CpG DNA hydrogel and produced more potent antitumor effects compared to that of unmodified ovalbumin released from the hydrogel.

Several intratumorally injected cancer immunotherapy vaccine formulations make use of plant virus-based nanoparticles, including cowpea mosaic virus, tobacco mosaic virus, potato virus X, and papaya mosaic virus-based nanoparticles (Lebel et al., 2016; Lee et al., 2017; Lizotte et al., 2016; Murray, Wang, Fiering, & Steinmetz, 2018). In one example, Lizotte *et al.* engineered self-assembling, empty cowpea mosaic virus (eCPMV) nanoparticles and found that intratumoral injection of eCPMV nanoparticles resulted in cancer cell necrosis in the tumor microenvironment and systemic antitumor immunity in tumor-bearing mice (Lizotte et al., 2016). In another work, Murray et al. reported that nanoparticles from tobacco mosaic virus were less potent than CPMV-derived nanoparticles for the treatment of a murine model of dermal melanoma due to differences in immune activation (Murray et al., 2018). Plant virus-based nanoparticles can be standalone therapies or can be used as drug delivery vehicles to carry other therapeutics.

4. Local Delivery of Adoptive T Cell Therapy

Cell-based therapies are emerging as a novel therapeutic strategy to potentially enhance treatment efficacy for many diseases, such as cancer and diabetes (Z. Chen, Hu, & Gu, 2018; Fischbach, Bluestone, & Lim, 2013; Wen, Wang, et al., 2019; X. Xu et al., 2019). In the case of engineered T cells, tumor antigen recognition specificity can be achieved by chimeric antigen receptor (CAR) T cell therapy and T cell receptor (TCR) therapy (Klebanoff, Rosenberg, & Restifo, 2016). In CAR T cell therapy, peripheral blood mononuclear cells are harvested from the patient's blood and are stimulated to become T cells, after which, DNA encoding a CAR engineered to recognize a certain antigen is incorporated into the genome of each cell (Gill et al., 2016). The CAR allows the T cell to recognize and kill cells which have those antigens (Gill et al., 2016; Zhen, Carrillo, & Kitchen, 2017). The modified CAR T cells are cultured to increase the total number of cells and are infused back into the patient, where they initiate an antitumor immune response (Gill et al., 2016; Zhen et al., 2017). Currently, the FDA has approved two CAR T cell therapies to treat blood cancers: Axicabtagene ciloleucel and tisagenlecleucel, which aim to treat refractory diffuse large B-

cell lymphoma and B-cell precursor acute lymphoblastic leukemia, respectively (Leahy, Elgarten, Grupp, Maude, & Teachey, 2018; Roberts, Better, Bot, Roberts, & Ribas, 2018). In addition, several CAR T cell therapies for diseases, including multiple myeloma, human immunodeficiency virus (HIV), and neuroblastoma are under investigation (Ghosh et al., 2018; Gill et al., 2016; Zhen et al., 2017). Systemically administered CAR T cells are often of limited therapeutic efficacy for the treatment of solid tumors due to inefficient delivery to the tumor site(s) (Beatty & O'Hara, 2016). It has been demonstrated in both preclinical cancer models and clinical trials that local delivery of CAR T cells is preferable to systemic delivery for treating solid tumors of several types cancer, including head and neck squamous cell carcinoma and carcinoembryonic antigen peritoneal tumors (Katz et al., 2016; Papa, van Schalkwyk, & Maher, 2015; van Schalkwyk et al., 2013). Several examples of locally delivered CAR T cells are discussed here to highlight the potential advantages of their local delivery as opposed to their systemic delivery for the treatment of solid tumors.

Adusumilli et al. reported that intrapleurally administered CAR T cells were superior to intravenously administered CAR T cells for the treatment of pleural tumors as well as metastatic sites (Adusumilli et al., 2014). The authors engineered mesothelin (a tumor cell surface marker)-targeted CD28 co-stimulated (M28z) CAR T cells and evaluated their efficacy in murine orthotopic models of malignant pleural mesothelioma (MPM). In contrast to the intravenously administered T cells, which produced only a marginal antitumor effect compared to the control CAR T cells, intrapleurally administered CAR T cells produced a stronger antitumor response. Even when administered at a 30-fold lower dose than the intravenous dose, the intrapleural dose produced an enhanced antitumor effect and resulted in systemic tumor immunity at extrathoracic tumor sites. Moreover, a similar amount of intravenously administered CAR T cells accumulated at the tumor site did not generate a comparable antitumor effect.

Katz et al. reported that intraperitoneal infusion of CAR T cells resulted in enhanced protection over systemically infused CAR T cells in murine models of peritoneal carcinomatosis (Katz et al., 2016). The authors found that intraperitoneal injection of CAR T cells resulted in a 37-fold reduction in tumor burden, whereas intravenously injected mice only showed a 3-fold reduction. Priceman *et al.* reported that local intracranial administration of HER2 targeted CAR T cells resulted in significant antitumor effects in orthotopic xenograft murine models of brain tumors resulting from breast cancer metastasis (Priceman et al., 2018). The authors evaluated three delivery routes: intravenous (systemic), intraventricular (regional), and intratumoral (local). Equivalent antitumor efficacy for both regional and local delivery of CAR T cells was observed, although regional delivery sometimes resulted in a delayed response. Systemic delivery of a ten-fold greater dose than that of regional delivery resulted in only marginal tumor regression. Nellan *et al.* reported that regional and intravenous administration of CAR T cells targeting HER2 resulted in tumor regression in murine models of medulloblastoma, although a 5-fold higher dose of intravenously administered CAR T cells was required to produce a comparable effect to intratumorally injected CAR T cells in murine models (Nellan et al., 2018). Murad et al. generated CAR T cells against tumor-associated glycoprotein 72 with a 4–1BB intracellular co-stimulatory signaling domain (TAG72-BBζ) and tested their efficacy in murine models of peritoneal ovarian tumors using a regional, intraperitoneal injection (Murad et al., 2018).

The authors found that mice showed rapid antitumor responses after an intraperitoneal injection of TAG72-BBζ CAR T cells, while an intravenous injection of the same therapy showed only a marginal antitumor response.

The beneficial effects of local delivery of CAR T cells have been investigated in clinical trials; for example, Van Schalkwyk et al. described a clinical trial (NCT01818323) to evaluate the efficacy of intratumoral injection of CAR T cells targeting ErbB in patients with head and neck squamous cell carcinoma (HNSCC) in an effort to minimize toxicity (van Schalkwyk et al., 2013). In another work, Brown et al. reported that intracranial infusion of CAR T cells targeting IL13Rα2 in a patient with glioblastoma (NCT02208362) resulted in regression of all observable intracranial and spinal tumors with no major toxic effects (C. E. Brown et al., 2016).

To aid in the local delivery of engineered T cells, biopolymer implants have been engineered to facilitate sustained local delivery of adoptive T cells directly to solid tumors (Smith et al., 2017; Stephan et al., 2015). Because a large number of T cells must be generated *ex vivo* in a short period of time prior to therapy, techniques that can increase the speed and efficiency of this process are highly desirable. Currently, the commercially available superparamagnetic Dynabeads coated with anti-CD3 antibodies (aCD3) and anti-CD28 antibodies (aCD28) are considered the standard for *ex vivo* T cell isolation, activation and expansion (Neurauter et al., 2007). Biomaterials can facilitate the *ex vivo* expansion and activation of T cells prior to their administration (Lin et al., 2018; Schluck, Hammink, Figdor, Verdoes, & Weiden, 2019). Specifically, hydrogel-based scaffolds have been investigated for use in local cell delivery as well as in the *ex vivo* expansion and activation of T cells (Monette, Ceccaldi, Assaad, Lerouge, & Lapointe, 2016; Weiden et al., 2018). In one example, Tsao et al. engineered a thermosensitive poly(ethylene glycol)-g-chitosan hydrogel to serve as a local T cell delivery depot for the treatment of glioblastoma (Tsao et al., 2014). The authors demonstrated that the T cells released from the hydrogel could kill the glioblastoma model cells, U-87 MG, *in vitro*. In another work, Guasch engineered polyethylene glycol hydrogels cross-linked with integrin-activating fibronectin-derived peptides, decorated with gold nanoparticles functionalized with aCD3, and co-stimulated with aCD28 for enhanced *ex vivo* T cell activation and proliferation (Guasch, Muth, Diemer, Riahinezhad, & Spatz, 2017). Therefore, the authors demonstrated that by providing extracellular matrix interaction cues to T cells, T cell activation and expansion could be enhanced.

In addition to hydrogels, polydimethylsiloxane (PDMS) microbeads coated with aCD3 and aCD28 have also been engineered to enhance T cell *ex vivo* expansion, and were reported to increase T cell population faster than Dynabeads (Lambert et al., 2017). To increase *ex vivo* T cell growth rate, Fadel et al. bound carbon nanotube-polymer composites carrying antigens and biotinylated T cell stimuli to PLGA nanoparticles carrying IL-2 and magnetite, in order to create artificial APC cues to T cells (Fadel et al., 2014). The authors validated the resulting therapeutic T cells in animal tumor models and reported that a 3 order of magnitude higher concentration of IL-2 was necessary to produce an approximately similar number of T cells without the carbon nanotube-based therapy. In a related work, Cheung et al. designed a scaffold to mimic antigen-presenting cells to increase T cell *ex vivo* expansion

rate using fluid lipid bilayers supported by mesoporous silica micro-rods carrying aCD3, aCD28, and IL-2 (Alexander S. Cheung, Zhang, Koshy, & Mooney, 2018). Collectively, these examples demonstrate how recent advances in the fields of biomaterials and nanomedicine can enhance both the local delivery and *ex vivo* preparation of adoptive T cell immunotherapies.

5. Local Delivery of Combination Therapy

Local immunotherapy can be enhanced through combination with chemotherapy, phototherapy, radiotherapy, or another type of immunotherapy (Qian Chen, Muchao Chen, et al., 2019). Several local combination therapies have demonstrated increased efficacy and minimal toxicity in the clinic. For example, Chesney et al. reported that intralesional injection of Talimogene laherparepvec and systemic aCTLA-4 led to significantly higher objective response rates than that of aCTLA-4 alone in patients with advanced, unresectable melanoma (Chesney et al., 2018). Some combinations result in synergistic antitumor effects, while others result in enhanced but no synergistic effects (Q. Hu, Sun, Wang, & Gu, 2016). To lower the additional costs associated with combination therapies (Almutairi et al., 2019), it is desirable to achieve synergistic rather than additive effects since synergistic combinations produce greater results than the sum of both agents. Therefore, it is important to understand the mechanisms by which different therapies function in order to achieve the most potent synergistic antitumor effect (Nam et al., 2019; Smyth, Ngiow, Ribas, & Teng, 2016). For example, it has been reported that cancer vaccine formulation dictates synergy or lack thereof with CTLA-4 and PD-L1 checkpoint blockade therapy (Hailemichael et al., 2018). Furthermore, the timing of different therapeutics administered in combination immunotherapy could be critical for achieving the most potent antitumor effects. For example, Messenheimer et al. reported that OX40 therapy followed by PD-1 therapy resulted in significantly enhanced therapeutic effects compared to PD-1 therapy followed by OX40 therapy (Messenheimer et al., 2017). A thorough understanding of interferon and innate immune signaling pathways as well as cancer immunology is crucial for the rational design of personalized combination cancer immunotherapy treatment strategies (Minn & Wherry, 2016; Moynihan et al., 2016; Huitong Ruan et al., 2019).

5.1 Combination Immunotherapy—Numerous combinations of immunotherapeutics have been reported to result in enhanced antitumor efficacy, including PD-1 blockade and CTLA-4 blockade (Curran, Montalvo, Yagita, & Allison, 2010), PD-1 blockade and IL-21 therapy (Pan et al., 2013), PD-L1 blockade and IL-2 therapy (West et al., 2013), aCD40 and a toll-like receptor-4 agonist (Van De Voort, Felder, Yang, Sondel, & Rakhmilevich, 2013), CTLA-4 or PD-1 blockade and CpG (Mangsbo et al., 2010), PD-1 blockade and aOX40 (Z. Guo et al., 2014), CTLA-4 or PD-1 blockade and a tumor-selective oncolytic vaccinia virus encoding IL-12 and IL-7 (Nakao et al., 2020), and also, a mixture of mRNAs encoding IL-23, IL-36γ, and OX40L (Hewitt et al., 2019). In one study, Sagiv-Barfi et al. investigated the mechanisms by which a combination therapy consisting of an intratumoral injection of aOX40 and CpG results in a synergistic and systemic antitumor host immune response for treating multiple types of cancer (Sagiv-Barfi et al., 2018). It was reported that CpG causes myeloid-derived cells to secrete pro-inflammatory cytokines, including IL-12, IFNγ, and TNFα, which causes $CD4^+$ T cells in the tumor microenvironment to upregulate OX40. The

aOX40 in this formulation then binds OX40, which activates tumor-infiltrating effector T cells (T_{effs}) while inhibiting regulatory T cell (T_{reg}) activity, resulting in a therapeutic antitumor immune response. T_{regs} have been reported to induce tumor-infiltrating CTL dysfunction (Bauer et al., 2014). The authors found that the systemic antitumor immune response was specific to the tumor antigens present at the locally injected site; therefore, this strategy has the potential to reduce the chance of tumor recurrence at metastatic sites. The authors were able to increase antitumor efficacy while minimizing systemic toxicity by using a lower local dose of CpG and aOX40 than if given systemically. Other combination therapies incorporating CpG have shown promising results; Kwong et al. engineered PEGylated liposomes with surface-conjugated CpG and aCD40 for intratumoral injection, and found reduced toxicities associated with systemic exposure to CpG and aCD40 (Kwong, Liu, & Irvine, 2011). In another work, Wang *et al.* designed DNA nanococoons to locally release aPD-1 and CpG in the presence of inflammatory conditions, and reported that this bioresponsive formulation resulted in synergistic therapeutic effects (C. Wang, Sun, Wright, Wang, & Gu, 2016).

Several oncolytic virotherapies have been reported to induce enhanced or synergistic antitumor effects when combined with checkpoint inhibitors in both experimental cancer models and in clinical trials (Bourgeois-Daigneault et al., 2018; C. Y. Chen, Hutzen, Wedekind, & Cripe, 2018; LaRocca & Warner, 2018; Antoni Ribas et al., 2017; Saha, Martuza, & Rabkin, 2018; Zamarin et al., 2014). Intratumoral injection of oncolytic viruses in combination with immune checkpoint blockade can increase objective clinical response rates, especially in patients with immune checkpoint blockade resistant tumors (Shekarian et al., 2019). In one example, Bartee et al. designed a locally injected oncolytic virus, which induced host antitumor responses and caused infected cells to produce a soluble form of PD-1 (Bartee, Dunlap, & Bartee, 2017). The authors found that local treatment with this virus combined with systemic depletion of CD4 resulted in enhanced antitumor effects in melanoma-bearing tumor models. Oncolytic viruses have also been utilized to generate therapeutic immune cytokines at the tumor site; Wang *et al.* designed a tumor-targeted oncolytic virus encoding a modified version of IL-12 to limit systemic exposure to IL-12, and verified its antitumor efficacy in a subcutaneous animal model of pancreatic cancer (P. Wang et al., 2017). Plant virus nanoparticles, which are potentially safer than mammalian viruses due to their lack of infectious properties in mammals (C. Wang, Beiss, & Steinmetz, 2019), have also been under investigation for local combination immunotherapy. For instance, Wang *et al.* demonstrated that *in situ* treatment with cowpea mosaic virus nanoparticles and CD47 blockade achieved the most significant reduction in tumor volume in 4T1 breast tumor model bearing mice (C. Wang & Steinmetz, 2019).

Hydrogels can facilitate the local delivery of multiple therapeutics at the tumor site and can be engineered to degrade and release their contents under certain desired conditions (Bae & Kurisawa, 2016; Elias et al., 2015; Ishii, Kaneko, & Nagasaki, 2016; Sharifzadeh & Hosseinkhani, 2017; Thambi, Li, & Lee, 2017; Van Tomme & Hennink, 2007). For example, Lemdani et al. designed a thermosensitive mucoadhesive hydrogel for the sustained local delivery of two immunomodulators: Heat-killed *Mycobacterium tuberculosis* (HKMT) and GM-CSF (Lemdani et al., 2019). The bioadhesive properties of this gel enhanced its efficacy by prolonging its *in vivo* half-life. In another work, Hori et al. designed an alginate-based

hydrogel for peritumoral delivery of therapeutic dendritic cells and IL-15 superagonist (IL-15SA), and found that peritumoral injections resulted in an approximately 40-fold higher concentration of IL-15SA at the tumor site in comparison to systemically injected IL-15SA (Hori, Stern, Hynes, & Irvine, 2009). In another example, Song et al. engineered an injectable PEG-*b*-poly(L-alanine) hydrogel for sustained local co-delivery of tumor cell lysate, GM-CSF, aCTLA-4, and aPD-1 (H. Song et al., 2019). This therapy resulted in an enhanced tumor specific CTL response, while decreasing systemic exposure to the antibodies. Oh et al. engineered a gelatin-based hydrogel for local delivery of dendritic cells and oncolytic adenoviruses expressing IL-12 and GM-CSF, which enhanced retention of the therapeutic agents at the tumor site (Oh et al., 2017). Nanoscale liposomal polymeric gels with hydrogel cores to deliver IL-2 and a TGF- β inhibitor have also been engineered (J. Park et al., 2012).

5.2 Immunochemotherapy—Immunochemotherapy (or equivalently, chemoimmunotherapy) aims to enhance the efficacy of cancer treatment by combining a chemotherapeutic agent to directly inhibit tumor growth while also potentiating the patient's immune system to attack the tumor using an immunotherapeutic agent (Y. L. Chen, Chang, & Cheng, 2017). In addition to causing cell cycle arrest and subsequent cell apoptosis in both cancerous and non-malignant cells, antineoplastic chemotherapeutic drugs may also indirectly serve as immunotherapeutic agents by activating dendritic cells upon release of "danger" signals, such as HMGB1 from dying tumor cells, or by directly enhancing the antigen-presenting function of dendritic cells (Shurin, Tourkova, Kaneno, & Shurin, 2009; Tanaka, Matsushima, Mizumoto, & Takashima, 2009). Combination oncolytic virotherapy and chemotherapy has made tremendous progress in preclinical cancer models and has advanced to clinical trials (Praveen K. Bommareddy, Aspromonte, Zloza, Rabkin, & Kaufman, 2018; Simpson, Relph, Harrington, Melcher, & Pandha, 2016). In one example, Yang *et al.* reported that a combination therapy consisting of an engineered adenovirus expressing IL-24 and temozolomide induced an antitumor effect in tumor-bearing mice (M. Yang et al., 2018). Several nanoparticle-based local immunochemotherapy delivery strategies have been engineered to enhance treatment efficacy. For instance, Lee et al. found that intratumoral injection of potato virus X (PVX)-based nanoparticles and doxorubicin resulted in an enhanced antitumor response, although PVX nanoparticles carrying doxorubicin produced inferior antitumor effects (Lee et al., 2017). Doxorubicin is a chemotherapeutic drug that interferes with the cellular machinery necessary for DNA replication (Rivankar, 2014). In another example, Lu *et al.* engineered self-assembling indoximod prodrug nanovesicles for local co-administration with the chemotherapeutic drug, oxaliplatin, and validated this immunogenic cell death combination therapy using syngeneic murine models of pancreatic ductal adenocarcinoma (J. Lu et al., 2017). Locally injected formulations, which can potentially enhance the efficacy of immunochemotherapy treatment, include hydrogel-based formulations, porous silica-based formulations, nanogel-based formulations, and engineered nanoparticles.

By facilitating a sustained local release of therapeutic agent(s), hydrogels can enhance the efficacy of immunochemotherapy while minimizing the risk of systemic toxicity (Fakhari & Anand Subramony, 2015; J. Li & Mooney, 2016; Oliva, Conde, Wang, & Artzi, 2017; Singh

& Peppas, 2014). In one example, Li *et al.* reported the local co-delivery of celecoxib and aPD-1 from an alginate hydrogel could synergistically enhance antitumor immunity (Y. Li et al., 2016). The angiogenesis inhibition effect of celecoxib limits the ability of the tumor to obtain the necessary nutrients and eliminate waste, and thereby inhibits tumor growth. The authors found that relatively slow, local, sustained release of both celecoxib and aPD-1 from the alginate hydrogel resulted in enhanced antitumor efficacy in comparison to celecoxib and aPD-1 administered in PBS. In addition to limiting tumor vascularization, anti-angiogenesis therapy has been found to reprogram the tumor microenvironment from an immunosuppressive state to an immune-supportive state (Yi et al., 2019). In another work, Jiang et al. designed a thermosensitive poly(D,L-lactide-co-glycolide)-poly(ethylene glycol)-poly(D,L-lactide-co-glycolide) (PLGA-PEG-PLGA) injectable hydrogel-based drug delivery system containing multifunctional dendritic nanoparticles to locally deliver doxorubicin and L-Arginine to the tumor site (Jiang et al., 2018). L-Arginine serves as the substrate for inducible nitric oxide synthase enzymes present in M1 macrophages in the tumor microenvironment, which then kills tumor cells by producing nitric oxide (NO) (Jiang et al., 2018). A hydrogel formulation that makes use of the abundant ROS present in the tumor microenvironment was reported by Wang *et al.*, who engineered an *in situ* formed ROS-responsive hydrogel-based scaffold to locally deliver gemcitabine and aPD-L1 to the tumor site upon reactive oxygen species-dependent hydrogel degradation (C. Wang et al., 2018). Gemcitabine is a chemotherapeutic agent that inhibits cellular processes necessary for DNA synthesis, and has been reported to reduce the number of myeloid suppressor cells in tumor-bearing animals (Plunkett et al., 1995; Suzuki, Kapoor, Jassar, Kaiser, & Albelda, 2005). In another work, Ruan et al. engineered a pH and reactive oxygen species-dependent hydrogel-based delivery system for local co-delivery of aPD-1 and zebularine, which is a hypomethylating agent that is reported to enhance tumor-associated antigen expression (H. Ruan et al., 2019). A chitosan hydrogel-based delivery system for the local delivery of doxorubicin and vaccinia virus vaccine, expressing a protein that enhances antigenicity to the tumor site, has been reported to result in synergistic antitumor effects (Han et al., 2008). Additionally, hydrogel-based delivery systems for immunochemotherapy have been engineered to locally deliver GM-CSF and doxorubicin, cisplatin, or cyclophosphamide (Seo, Han, Noh, Kim, & Son, 2009), doxorubicin, IL-2 and IFNγ (Lv, He, Quan, Yu, & Chen, 2018), IL-15 and cisplatin (X. Wu et al., 2017), as well as doxorubicin and melittin (Jin et al., 2018).

Several porous silica delivery systems have been reported to facilitate enhanced local immunochemotherapy treatment. For example, Qian et al. investigated the use of intratumorally injected mesoporous silica-zinc oxide micro-rosettes (MS-Zn) containing doxorubicin and polyinosinic-polycytidylic acid sodium salt (PIC) for enhanced immunochemotherapy (G. Qian et al., 2019). PIC is a TLR3 ligand which causes the release of pro-inflammatory cytokines from dendritic cells, which could help counteract the immunosuppressive tumor microenvironment (Han et al., 2016). In addition, the MS and Zn are both immune potentiators that further counteract the immunosuppressive tumor microenvironment (X. Wang et al., 2016). The authors found that this formulation resulted in both local tumor regression as well as systemic antitumor response. Furthermore, the authors engineered the MS-Zn micro-rosettes to exhibit increased release rate of the loaded drugs at

lower pH values. Since the tumor microenvironment is slightly acidic, it is desirable for drug delivery vehicles to exhibit increased release rates in more acidic environments. Mice treated with the MS-Zn-DOX-PIC were found to have a greater $CD4^+$ and $CD8^+$ T cell populations in their splenocytes than mice treated with free DOX-PIC, demonstrating that the MS-Zn delivery vehicle played a crucial role in promoting systemic antitumor immunity.

Nanogels, which are gels with diameters usually less than 100 nm, can be useful drug delivery vehicles for local or systemic immunochemotherapy due to their ability to hold large therapeutic biomolecules and potential to serve as inherent immunostimulatory agents (Purwada et al., 2016; Song et al., 2017; Tahara & Akiyoshi, 2015). In one example, Wu *et al.* engineered nanogel-incorporated chemical and physical hybrid hydrogels for extended peritumoral release of IL-2, IFNγ, and doxorubicin (Xilong Wu, He, Wu, Chen, & Cheng, 2015). By facilitating local and sustained release of IL-2, IFNγ, and doxorubicin, the nanogel-integrated hydrogel carrying system demonstrated a significant synergistic effect in experimental A549 xenograft tumors compared to protein therapy or chemotherapy alone. A syringeable immunomodulatory multidomain nanogel (iGel), formed by positively charged nanoliposomes and negatively charged multi-nanodomain vesicles carrying gemcitabine and R837, was engineered by Song *et al.* for local immunochemotherapy treatment (C. Song et al., 2019). By extending the release time to over one week, the iGel significantly reduced systemic toxicity, inhibited tumor metastasis, and achieved systemic antitumor immunity in murine cancer models. In another work, Phuengkham *et al.* engineered a 3D hyaluronic acid and collagen-based porous scaffold for postoperative peritumoral implantation, which consists of gemcitabine, a whole tumor lysate vaccine, and nanogels loaded with the TLR3 agonist, polyinosinic:polycytidylic acid (Phuengkham, Song, Um, & Lim, 2018). The authors found that postsurgical implantation of this nanogel-containing scaffold inhibited tumor recurrence and tumor metastasis, while inducing a systemic antitumor immune response.

5.3 Photoimmunotherapy—Photothermal therapy (PTT) and photodynamic therapy (PDT) are two types of phototherapy under investigation for use in combination immunotherapy (Sanchez-Barcelo & Mediavilla, 2014). In photothermal therapy, photosensitizer molecules absorb light and convert it into heat to ablate tumor cells and induce the release of tumor antigens (Liang, Xu, Song, & Liu, 2016). Photothermal immunotherapy can enhance the therapeutic efficacy of immune checkpoint blockade, therapeutic cancer immunotherapy vaccines, and CAR T cell therapy (Qian Chen, Quanyin Hu, et al., 2019; T. Wang et al., 2018; X. Ye et al., 2019). However, due to immune-related adverse events observed in patients receiving *in situ* photothermal immunotherapy, it is desirable to engineer local delivery strategies that can mitigate these side effects while also increasing therapeutic efficacy (X. Li et al., 2010).

Chen et al. demonstrated that the release of tumor antigens during local photothermal immunotherapy could induce tumor immunity following intratumoral injection of poly(lactic-co-glycolic) acid (PLGA) nanoparticles carrying a photothermal agent, indocyanine green, and the toll-like receptor-7 agonist, R837, followed by irradiation with a 808 nm laser (Q. Chen et al., 2016). R837 serves as an adjuvant that primes host immune cells to recognize the released tumor antigens and to subsequently induce an antitumor

response. Similarly, Xu et al. engineered upconversion nanoparticles carrying clorin e6, a photothermal agent, and R837 for intratumoral injection (J. Xu et al., 2017). In another work, Cano-Mejia *et al.* designed a photothermal immunotherapy using intratumorally injected pH-sensitive and immunostimulatory Prussian blue nanoparticles with local 808 nm laser irradiation, and found that this therapy could rapidly decrease tumor burden in the murine model of neuroblastoma (Cano-Mejia et al., 2017). These nanoparticles remain stable and reach higher temperatures in the relatively acidic tumor microenvironment (pH 5–6) but degrade at systemic pH (7.4). Therefore, this response increases cancer cell ablation at the tumor site while minimizing systemic activity. Several local photothermal immunotherapy strategies utilize CpG in order to induce immunostimulatory activity of antigen-presenting cells in the tumor microenvironment. For example, Guo *et al.* engineered intratumorally injectable chitosan-coated hollow copper sulfide nanoparticles containing CpG for photothermal immunotherapy (L. Guo et al., 2014). Similarly, CpG-containing hydrogel nanoparticles have been engineered (Dong et al., 2019; Yata et al., 2017). In one work, Tao *et al.* engineered CpG-loaded polyethylene glycol and polyethylenimine dual-polymer-functionalized cationic graphene oxide (GO-PEG-PEI-CpG) for enhanced intratumoral local photothermal immunotherapy (Tao, Ju, Ren, & Qu, 2014). The authors found that GO-PEG-PEI-CpG therapy could induce the local release of cytokines, including TNF-α and IL-6. Li et al. engineered IR-7-loaded liposomes coated with hyaluronic acid-CpG for local photothermal immunotherapy, and reported that this combination therapy could induce tumor cell necrosis and subsequent release of tumor-associated antigens, and could activate antigen-presenting cells (Figure 7) (L. Li et al., 2018).

Single-walled carbon nanotubes are also useful for photothermal therapy due to their ability to convert optical energy into thermal energy upon irradiation. A photothermal immunotherapy approach using intratumorally injected PEGylated single-walled carbon nanotubes exposed to 808 nm laser irradiation in combination with systemic CTLA-4 blockade therapy resulted in significantly reduced lung metastases and inhibited the development of secondary tumors (C. Wang et al., 2014). Another method to enhance the efficacy of single-walled carbon nanotubes for photothermal immunotherapy was reported by Zhou et al., who engineered glycated chitosan modified single-walled carbon nanotubes that could enhance tumor immunogenicity (Zhou et al., 2012). In a related example, Li et al. studied the effects of combined intratumorally injected glycated chitosan modified single-walled carbon nanotubes and intraperitoneally injected CTLA-4 immune checkpoint blockade, and found that this combination resulted in systemic antitumor immunity in murine models of metastatic breast cancer (Y. Li et al., 2019).

Whereas PTT uses heat to locally ablate the tumor, PDT combines photosensitizers, light, and oxygen to generate ROS to induce immunogenic cell death *in situ*. In one example, Lu et al. engineered chlorin-based nanoscale metal−organic framework, TBC-Hf, to locally deliver a small molecule IDO inhibitor and locally generate ROS upon light irradiation (K. Lu et al., 2016). ROS caused the release of tumor associated antigens, which were subsequently presented to T cells, and the authors reported that this combination therapy achieved systemic antitumor immunity in murine cancer models. Another photodynamic-immunotherapy approach was reported by Meng et al., who engineered a photosensitizer-modified catalase poly(ethylene glycol) double acrylate (PEGDA)-based light triggered *in*

situ gelation system for local delivery of R837-loaded PLGA nanoparticles, and *in situ* generation of ROS (Meng et al., 2019). Combination therapies utilizing both photothermal therapy and photodynamic therapy have also been reported (Yan et al., 2019). In one recent example, Wang et al. fabricated antigen-capturing nanoplatforms for simultaneous photothermal and photodynamic immunotherapy *via* the self-assembly of 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[maleimide(polyethylene glycol)-2000] (DSPE-PEG-mal) and indocyanine green onto upconversion nanoparticles, which were further modified with the photosensitizer, rose bengal (UCNP/ICG/RB-mal) (M. Wang et al., 2019). The authors reported that upon intratumoral injection and subsequent NIR laser irradiation, UCNP/ICG/RB-mal therapy could eliminate the treated tumor while significantly decreasing the growth of untreated distant tumors.

5.4 Radiotherapy-enhanced Immunotherapy—Several preclinical studies and clinical trials have investigated the potential therapeutic benefits of combining immunotherapy with radiotherapy (Formenti & Demaria, 2013; Levy, Massard, Soria, & Deutsch, 2016; Mujoo et al., 2018; Reynders, Illidge, Siva, Chang, & De Ruysscher, 2015; Tsui, Mihalcioiu, & Cury, 2018), as well as the underlying biological mechanisms that can explain these benefits (Apetoh et al., 2007; Barker, Paget, Khan, & Harrington, 2015). The mechanisms by which radiotherapy can induce antitumor immune responses were reviewed by Spiotto et al., who reported that radiotherapy increases inflammation in tumors, facilitates dendritic cell maturation following the release of tumor-associated antigens, and increases T cell priming and infiltration into tumors (Spiotto, Fu, & Weichselbaum, 2016). Furthermore, radiation therapy enhances immunotherapy in part by increasing the diversity of T cell receptors in the tumor microenvironment and by causing release of tumor antigens, which play a crucial role in the establishment of tumor immunity (Mujoo et al., 2018; Twyman-Saint Victor et al., 2015). Radiotherapy-induced cell death can cause the release of endogenous immune adjuvants, which alert the immune system to danger (Kono & Rock, 2008). Local radiotherapy combined with immune checkpoint blockade can jump-start a systemic response of tumors previously unresponsive to immune checkpoint blockade alone (Demaria, Coleman, & Formenti, 2016; Kalbasi, June, Haas, & Vapiwala, 2013). In one preclinical study, radiation and PD-L1 therapy resulted in a synergistic antitumor immunity through a CTL–mediated mechanism (Deng et al., 2014). However, due to the toxicities and adverse effects observed in patients receiving combination radiotherapy with immune checkpoint blockade therapy, further work is necessary to mitigate such risks (Hwang, Pike, Royce, Mahal, & Loeffler, 2018). Radiotherapy is able to directly induce cell death at the specific location where it is administered, but usually fails to result in regression of cancer cells at untreated sites, which is known as the abscopal effect (Z. I. Hu, McArthur, & Ho, 2017). Several reports of locally delivered immunotherapeutics in combination with radiation therapy have been reported to achieve abscopal effects. For instance, Yasmin-Karim et al. reported that intratumoral injection of aCD40 in combination with stereotactic body radiation resulted in synergistic regression of untreated tumors in murine models of pancreatic cancer, and achieved an abscopal effect (Yasmin-Karim et al., 2018).

Biomaterials and nanomedicines have been utilized to enhance the efficacy and safety of local immunotherapy administered in combination with radiotherapy. Nanoscale metal-

organic frameworks have recently been utilized in local radiotherapy-enhanced immunotherapy, owing to their biocompatibility and ability to serve as radioenhancers by absorbing X-ray energy. In one example, intratumoral injection of nanoscale metal-organic frameworks loaded with IDO inhibitors in combination with low-dose X-ray radiation treatment resulted in an abscopal antitumor effect in murine models of breast and colorectal cancer (K. D. Lu et al., 2018). Nanoscale metal-organic frameworks have also been engineered to enhance combination immune checkpoint blockade radiotherapy. Ni et al. reported that two porous, Hf-based nanoscale metal-organic frameworks could enhance the efficacy of radiotherapy upon intratumoral injection, and could trigger tumor regression in untreated tumors when combined with PD-L1 checkpoint blockade therapy, thereby achieving an abscopal effect (Ni et al., 2018). In another work, Min et al. fabricated antigen-capturing nanoparticles to enhance the delivery of tumor specific antigens released during radiotherapy to APCs in order to generate an abscopal effect (Min et al., 2017). In another example, Chen *et al.* designed PLGA nanoparticles containing catalase enzymes and the TLR7 agonist, R837, and found that intratumoral injection of these nanoparticles along with systemic administration of aCTLA-4 achieved an abscopal effect in murine models of cancer undergoing radiation therapy (Figure 8) (Q. Chen et al., 2019). The authors found that the ability of the catalase enzyme to decrease hypoxia in the tumor microenvironment enhanced the therapeutic efficacy of radiotherapy.

Plant-derived virus-based nanoparticles have also been reported to enhance radiation therapy. Patel et al. reported that intratumoral injection of cowpea mosaic virus in combination with radiation therapy in animal models of serous ovarian cancer resulted in enhanced therapeutic efficacy in comparison to radiation therapy alone (Patel, Czapar, Fiering, Oleinick, & Steinmetz, 2018). In another work, Hoopes et al. reported that intratumoral injection of plant-based virus-like nanoparticles in two canine oral melanoma patients undergoing hypofractionated radiation effectively treated the local tumor and showed no relapse within 5–9 months post-treatment (Hoopes et al., 2018). Hydrogels have been engineered to facilitate the local delivery of combination immunotherapy-radiotherapy. In one example, Chao et al. engineered a radioactive sodium alginate formulation capable of *in situ* gelation to facilitate the local delivery of therapeutic ^{131}I radioisotopes along with immunostimulatory CpG oligonucleotides (Chao et al., 2018).

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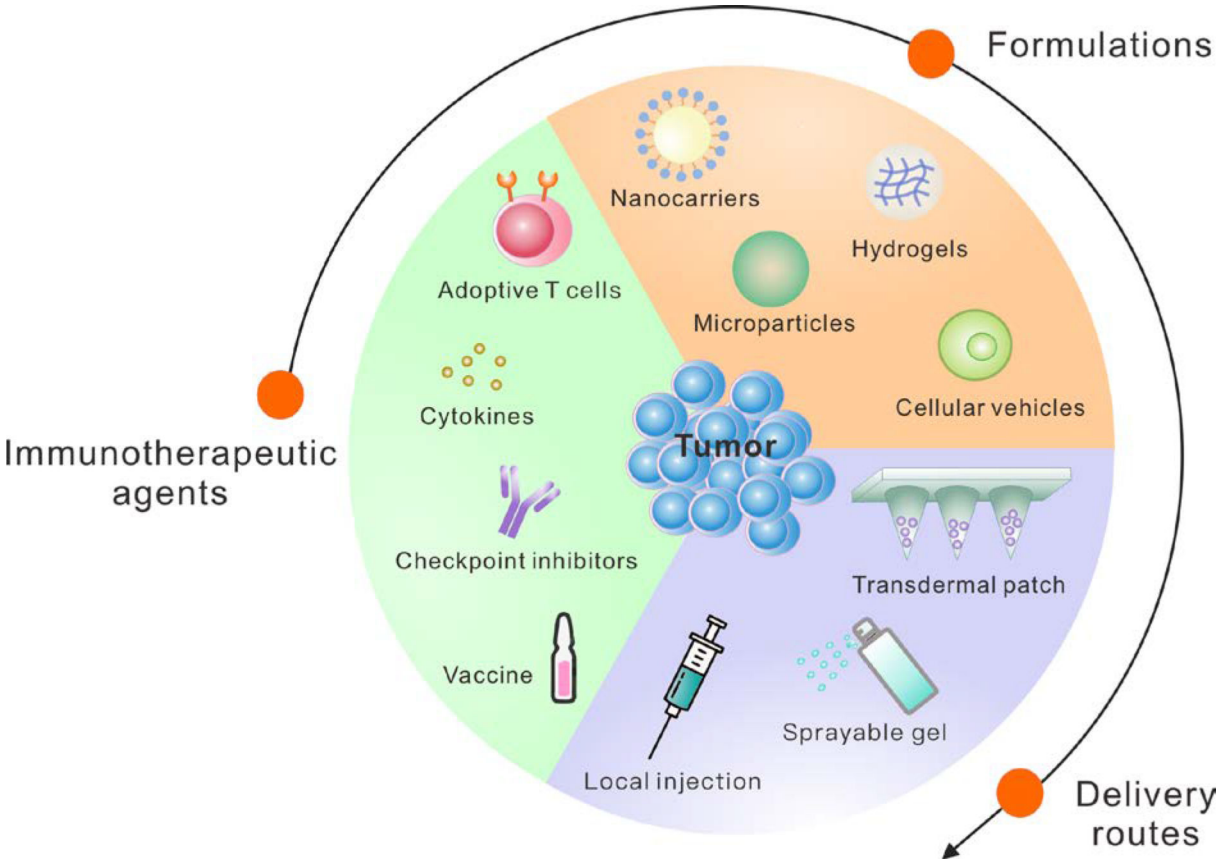

Figure 1: Local Delivery Strategies for Cancer Immunotherapy

Recent advances in local delivery systems for cancer immunotherapy show promise for enhancing therapeutic efficacy while minimizing toxicity.

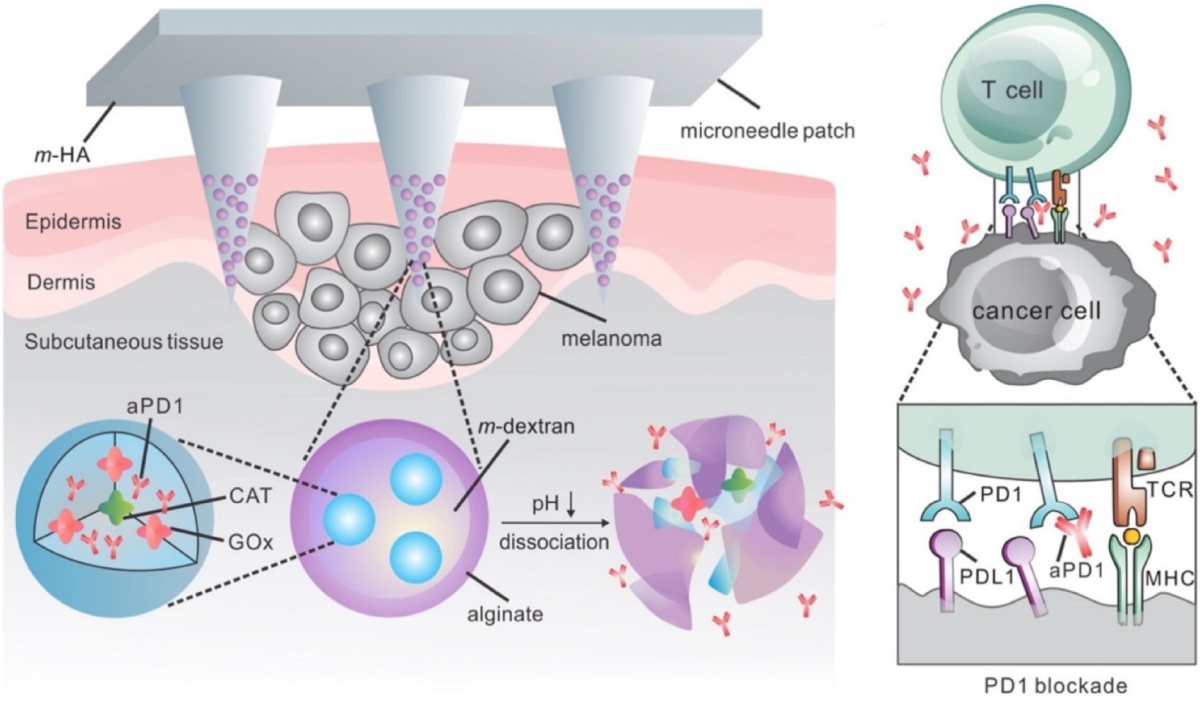

Figure 2: Microneedle Patch Assisted Delivery of PD-1 Blockade

The schematic showing the delivery of PD-1 blockade using nanoparticles released from a microneedle patch for melanoma treatment. Adapted with permission from (C. Wang et al., 2016). Copyright 2016, American Chemical Society.

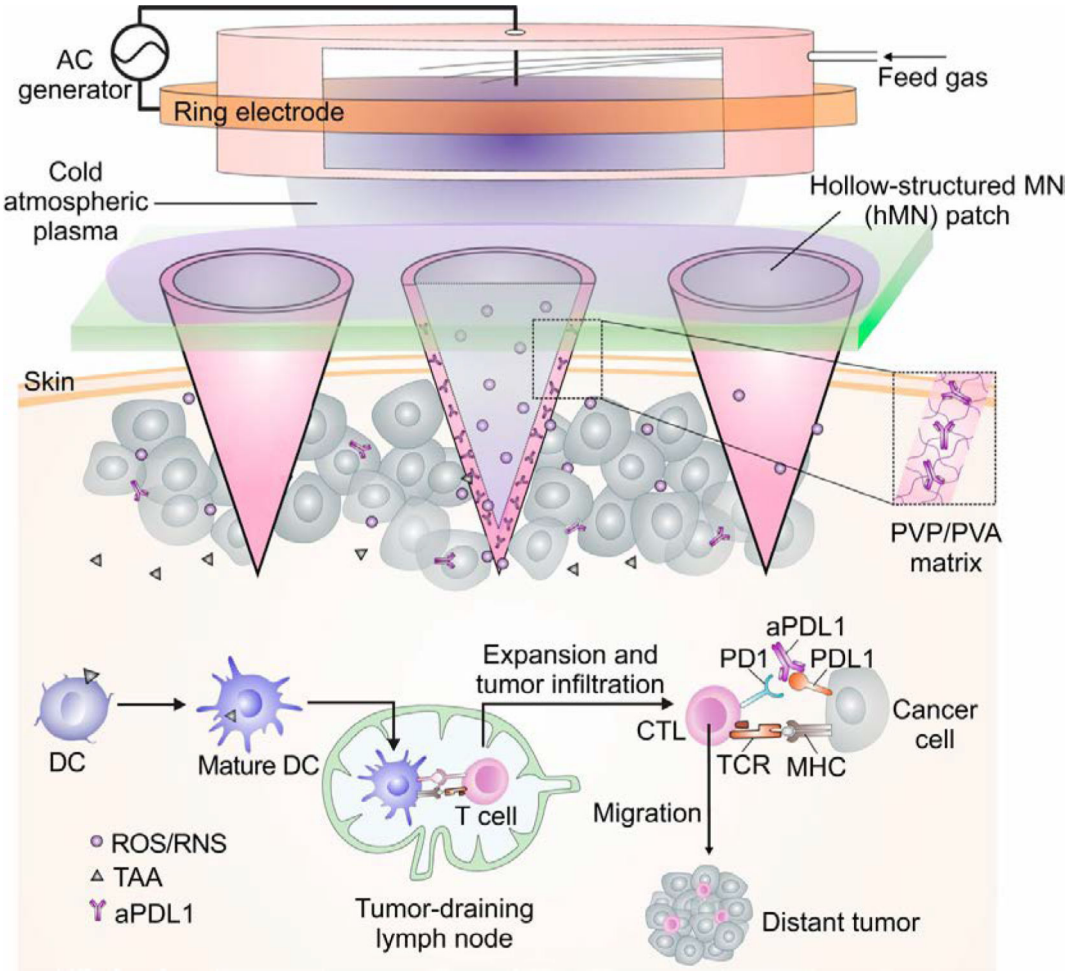

Figure 3: Microneedle Patch Assisted Cold Atmospheric Plasma-mediated Immune Checkpoint Blockade

The schematic for the microneedle patch enabled cold atmospheric plasma-mediated immune checkpoint blockade. Adapted from (G. Chen et al., 2020). Copyright 2020, National Academy of Sciences.

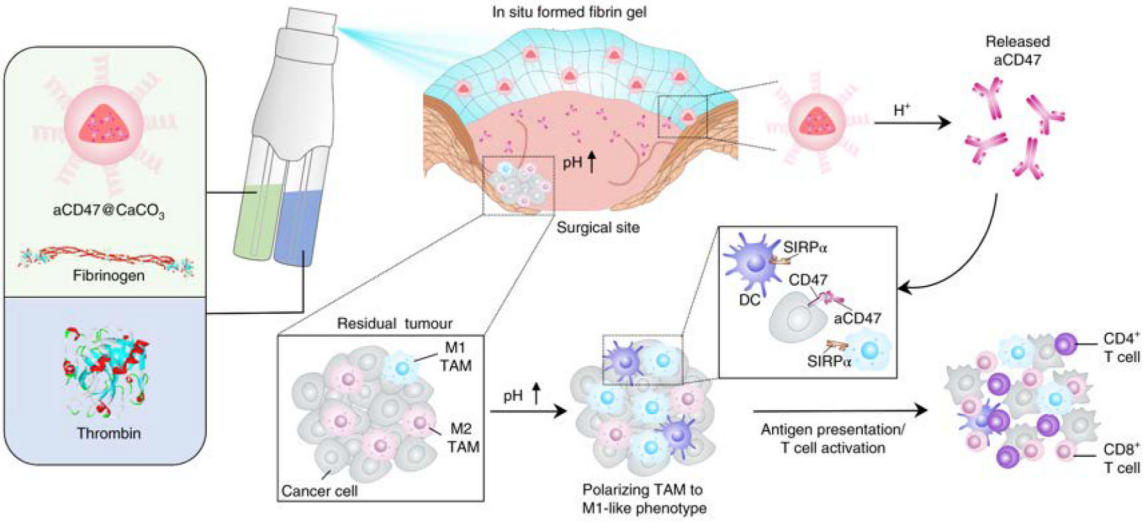

Figure 4: *In Situ* Sprayed Bioresponsive Immunotherapeutic Gel

The schematic of the *in situ* formed fibrin gel containing aCD47-loaded nanoparticles. Adapted with permission from (Qian Chen, Chao Wang, et al., 2019). Copyright 2018, Springer Nature.

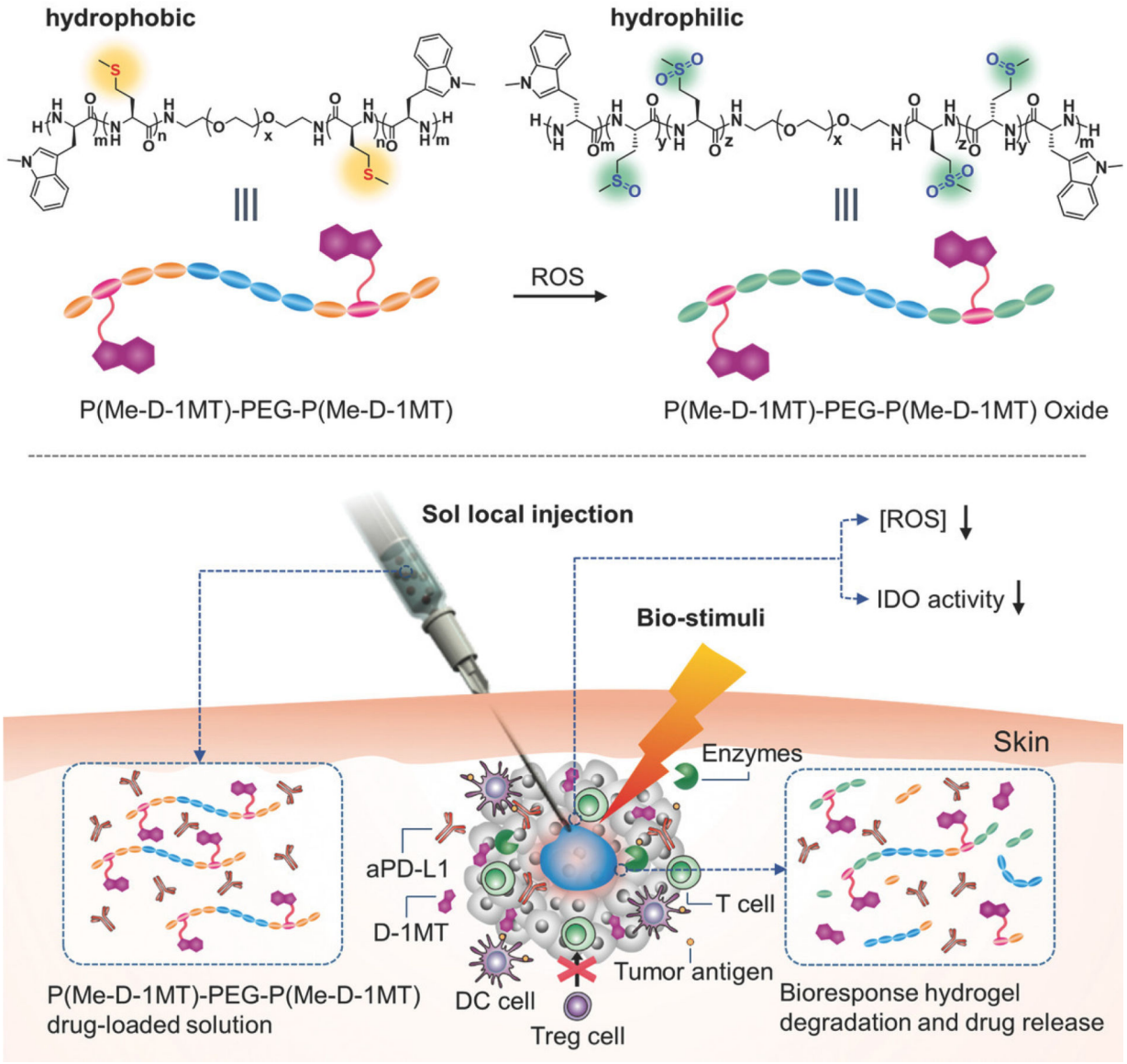

Figure 5: Bioresponsive Hydrogel for PD-L1 Blockade and IDO Inhibitor Delivery

The schematic showing the local delivery of PD-L1 blockade therapy and the IDO inhibitor, D-1MT, using a bioresponsive hydrogel. Adapted with permission from (Yu et al., 2018). Copyright 2018, John Wiley & Sons, Inc.

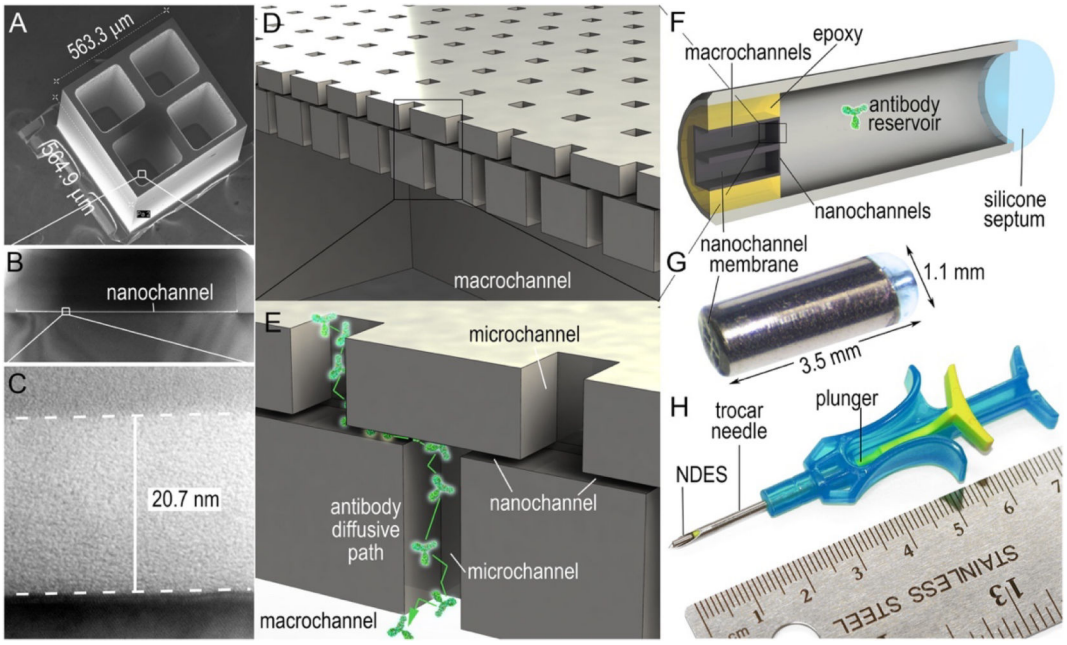

Figure 6: Nanofluidic Drug-Eluting Seed

The panel describing the properties of the nanofluidic drug-eluting seed. Adapted with permission from (Chua et al., 2018). Copyright 2018, Elsevier B.V.

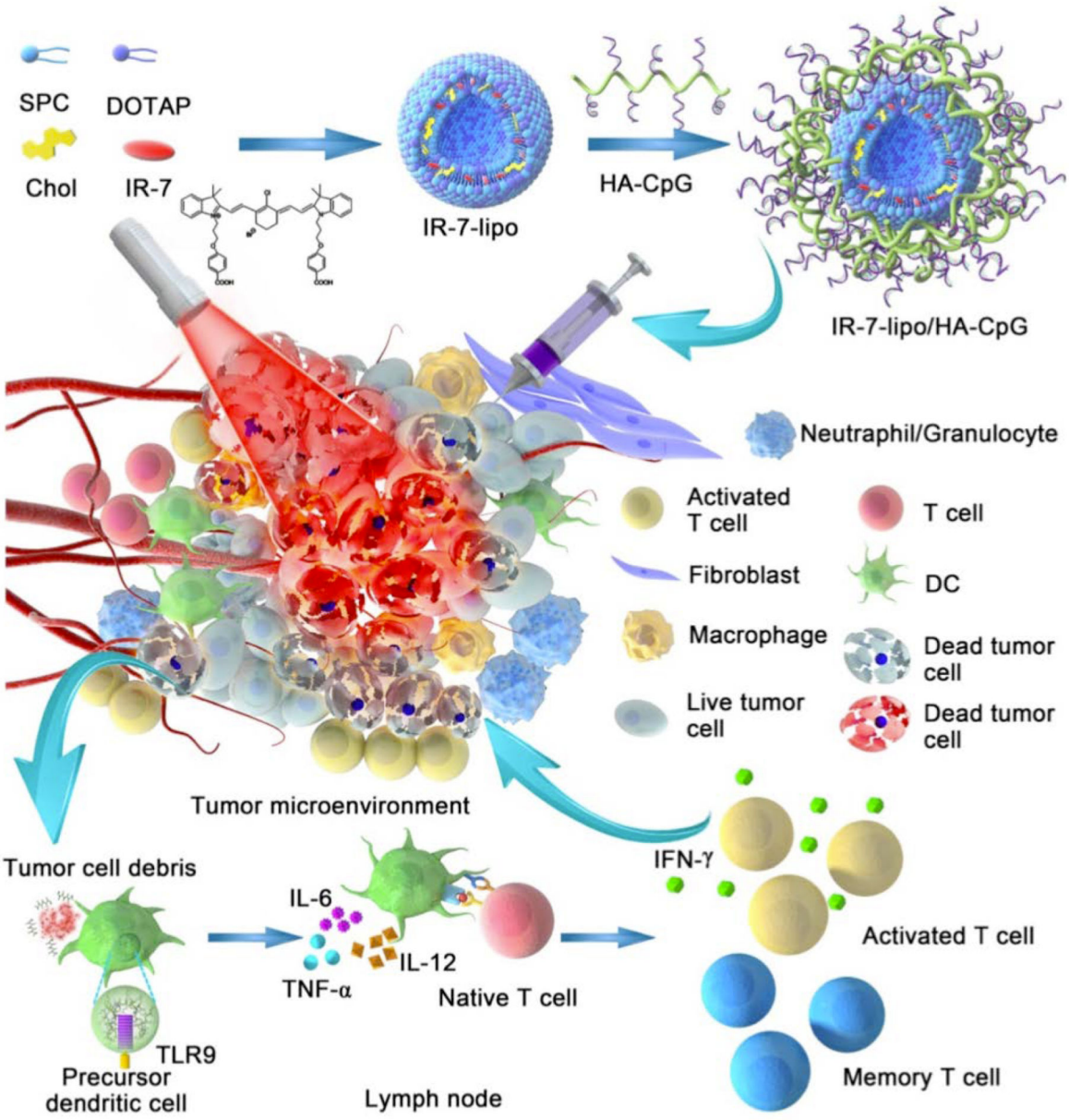

Figure 7: Local Photothermal Immunotherapy

The schematic showing the delivery of IR-7-loaded liposomes coated with hyaluronic acid-CpG for local photothermal immunotherapy. Adapted from (L. Li et al., 2018) under a Creative Commons Attribution (CC BY-NC) license.

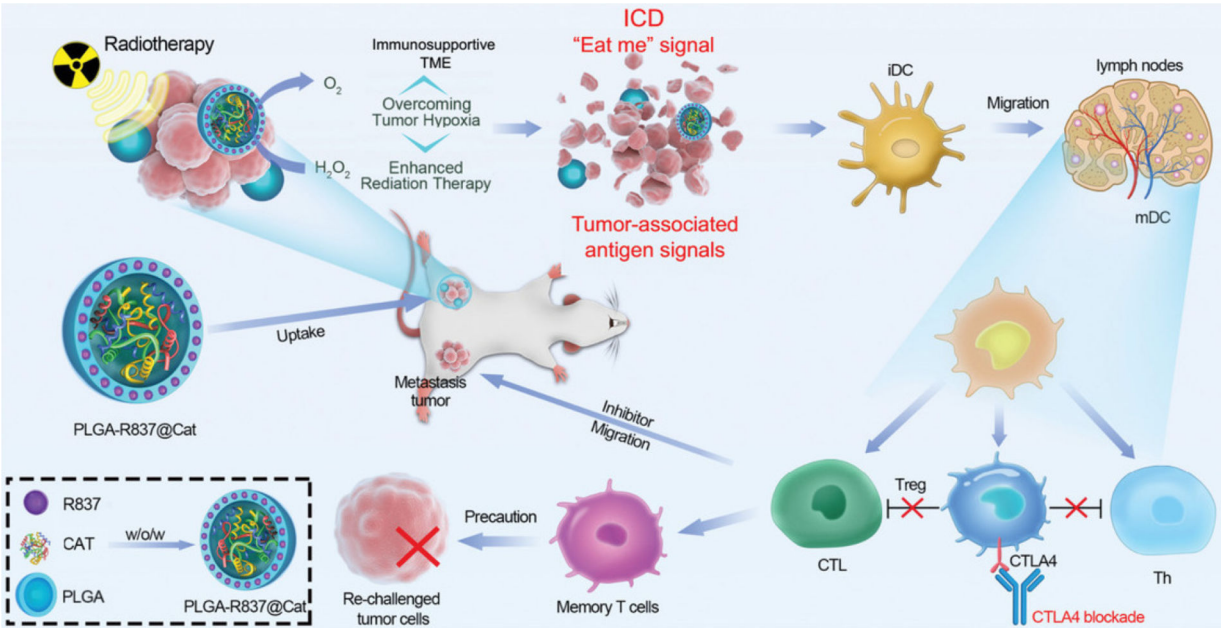

Figure 8: Nanoparticle Assisted Radiotherapy

The schematic showing the mechanism for PLGA-R837@CAT nanoparticle-assisted radiotherapy. Adapted with permission from (Q. Chen et al., 2019). Copyright 2019, John Wiley & Sons, Inc.