Advancing Cancer Immunotherapies with Nanotechnology

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Cancer immunotherapies can elicit long term, durable responses in only a fraction of patients. As such, there is a need to increase the number of patients who can benefit from cancer immunotherapies. By virtue of their versatility and nanoscale, nanoparticles have unique properties that can be exploited to enhance the efficacy of cancer immunotherapies. This review first outlines key concepts in nanotechnology and immunotherapy. Then, it highlights nanotechnology-mediated improvements to the efficacy of immune checkpoint inhibitors, cancer vaccines, and adoptive cellular therapies. Next, the insights derived from nanoparticle-mediated imaging of immune cells in both preclinical and clinical studies are reviewed. Afterwards, the roles of nanotechnology in combination therapies to augment antitumoral immunity are summarized. Finally, the challenges facing this emerging field combining nanotechnology with immunotherapies are discussed. Given the exciting, novel approaches that can arise from nanotechnology, there is great potential for nanotechnology to advance immunotherapies.

1. Introduction

The conventional pillars of cancer treatment—surgical resection, radiotherapy, and chemotherapy—have struggled to eradicate metastases and residual disease. Cancer immunotherapies under investigation include cytokines, immune checkpoint blockades, cancer vaccines, and adoptive cellular therapies. A description of these therapies, and their challenges are summarized in Table 1. While many efforts have been invested into these therapies, only a handful are clinically approved to-date. The most high profile of the approved immunotherapies are immune checkpoint inhibitors, anti-cytotoxic T-lymphocyte-associated protein 4 (anti-CTLA-4) and anti-programmed death-1 (anti-PD-1) monoclonal antibodies, which can induce spectacular tumor regression and long-lasting remission in a fraction of cancer patients. These drugs illustrate the potential of the immune system to eliminate metastases, while generating immunememory to prevent tumor recurrence. However, a minority of patients achieve long-term survival. Additionally, there are safety concerns, as patients often experience autoimmune reactions. Nanotechnology holds promise to improve the efficacy and safety of existing immunotherapies, and may increase the fraction of patients who can achieve durable, long-term responses. This review will focus primarily on nanotechnology’s applications to enhance efficacy of cancer immunotherapies, as improving the safety of immunotherapies using nanotechnology has been reviewed extensively elsewhere.

Nanoparticles are materials sized within 1–100 nm that have unique properties compared to their molecular or bulk counterparts. Nanoparticles are broadly classified into organic and inorganic subtypes (Table 2). Organic nanoparticles include polymers, liposomes, and dendrimers, whereas inorganic nanoparticles include gold, iron oxide, silica, and quantum dots. As this is a non-exhaustive list, readers are referred to reviews on nanoparticles elsewhere. Due to this diversity in composition, nanoparticles represent a versatile platform that can be tailored for specific applications including imaging and drug delivery. As exemplified by applications in tumor imaging...
and therapeutics, nanoparticles have numerous advantageous properties. First, the shape, size, and surface charge of nanoparticles can be easily altered to optimize for drug delivery kinetics and biodistribution,\(^1\) Second, nanoparticles can enhance drug accumulation at the target tumor site.\(^3\) Due to the abnormal, leaky tumor vasculature, nanoparticles can passively accumulate at the tumor over time. Incorporation of polyethylene glycol (PEG) into nanoparticles can prolong circulation half-life of a drug, compared to the free drug, and further enhance tumor accumulation. Through functionalization of a nanoparticle with a targeting ligand, nanoparticles can specifically target tumor cells to promote tumor cell internalization.\(^6\) Third, nanoparticles can mediate controlled release of their cargo in response to environmental stimuli.\(^7\) These “smart” nanoparticles can release their payload in the presence of enzymes, changes in pH, or redox, as well as to external stimuli, such as temperature or light. Fourth, nanoparticles can serve as both therapeutic and diagnostic (“theranostic”) agents.\(^8\) There are many multifunctional nanoparticles that can deliver chemotherapies, or mediate phototherapies, while simultaneously acting as a fluorescent, computed tomography (CT), positron emission tomography (PET), or magnetic resonance imaging (MRI) contrast agent. Preclinically, theranostic nanoparticles may help to determine the mechanisms of action and biodistribution of immunotherapies. Clinically, theranostic nanoparticles may help to monitor response, and inform dosage and scheduling decisions. Furthermore, these multifunctional nanoparticles may serve as a single platform that can facilitate combination therapies. Due to these advantageous properties, nanoparticles have been intensively investigated for cancer imaging and therapy.

Stemming from these efforts are clinically approved nanoparticle contrast agents and therapies. For instance, sulfur colloid radiolabeled with technetium-99m (\(^{99m}\)Tc) are approved for single photon emission computed tomography of patients with breast tumors.\(^9\) Other Food and Drug Administration (FDA)-approved nanoparticles include ferumoxyl, iron oxide nanoparticles which are used clinically to treat iron deficiency anemia in individuals with chronic kidney disease. Ferumoxyl is also under investigation as an MRI contrast agent for mapping metastatic lymph nodes and liver tumors. Meanwhile, the utility of nanotechnology for cancer therapy is exemplified by Doxil, a clinically approved liposomal PEGylated formulation of doxorubicin. Compared to free drug, liposomal doxorubicin has prolonged blood circulation, which increases tumor accumulation, resulting in improved efficacy and overall response rates.\(^10\) Additionally, liposomal doxorubicin has reduced cardiotoxicity, compared to the free drug. Other clinically approved nanoparticles and their uses have been reviewed elsewhere.\(^11\)

These lessons learned from the more mature field of cancer nanomedicine can be exploited by immunotherapies to address issues with drug delivery and toxicities. This review will provide a tutorial of key concepts in cancer immunology. We will highlight seminal developments at the intersection of cancer immunotherapy and nanotechnology and describe the advantages that nanotechnology can offer cancer immunotherapies in furthering their preclinical and clinical development. Then, we will discuss applications of nanotechnology in combination therapies to enhance antitumoral immunity. Finally, an outlook on the emerging field of nanoimmuno-oncology will be provided.

### 2. Overview of the Immune System

Activation of an immune response is a tightly regulated process that begins with the innate immune system. The innate immune system detects pattern recognition molecules that comprise either damage-associated molecular patterns (DAMPs) that are released when cells are damaged, or pathogen-associated molecular patterns (PAMPs), which are common motifs found on various pathogens.\(^11\) Detection of DAMPs or PAMPs is mediated through pattern recognition receptors (PRRs), which can

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be found anchored on the plasma membrane, in intracellular vesicles, and in the cytoplasm of immune cells, or as soluble molecules in the extracellular space. Of the various pattern recognition receptors, Toll-like receptors (TLRs) and Nod-like receptors (NLRs), in particular, are important receptors that are activated by adjuvants, substances that strengthen the immune response to vaccines. Depending on the immune cell on which a PRR is engaged, different responses can be elicited. If PRRs on natural killer (NK) cells are activated, NK cells will initiate lysis of the target. Should PRRs on a neutrophil, macrophage, or dendritic cell be activated, phagocytosis can occur. Phagocytosis is the engulfment of matter—pathogens, cellular debris, and macromolecules. Neutrophils are the first responders in inflammation and tend to engulf and eliminate pathogens, whereas dendritic cells (DCs) and macrophages will phagocyte dead cells, cell debris, and macromolecules. Refer to Table 3 for a summary of immune cell populations.

With their ability to phagocyte debris and macromolecules, DCs and macrophages can serve as antigen-presenting cells (APCs): DCs and macrophages internalize, process, and present foreign material, called antigens, to T-cells. DCs are the crucial link between the innate and adaptive immune systems, as they prime naive T-cells to effector T-cells, which can indirectly and directly kill pathogens and tumor cells. Upon internalization of an antigen, DCs mature and upregulate antigen-processing machinery to degrade the antigen into peptides that are then presented on the DC plasma membrane surface with a major histocompatibility complex (MHC) molecule. Endocytosed antigens are processed within endocytic vesicles and are presented with MHC class II molecules to CD4+ T-cells. An alternate pathway of processing occurs in the cytoplasm, after which antigens are presented with MHC class I molecules to CD8+ T-cells. Upon maturation, DCs migrate to the lymph nodes, where they interact with and prime T-cells. Regardless of whether T-cells have the CD4 or CD8 co-receptor, activation of a naïve T-cell comprises three signaling steps (Figure 1). Signal one consists of specific recognition by the T-cell for the antigen via the T-cell receptor for the MHC/antigen complex presented on the dendritic cell. Next, co-stimulation (signal two) must occur; receptors on DCs such as CD80/CD86 bind to co-stimulatory receptors on T-cells, such as CD28. Finally, signal 3 occurs when cytokines secreted from DCs stimulate T-cells. Cytokines are signaling molecules secreted by immune cells and comprise interleukins, interferons, and growth factors. As a consequence of signal 3, T-cells proliferate and differentiate. CD4+ T-cells differentiate into regulatory T-cells (Tregs) and subsets of T helper (Th) cells, including Th1, Th2, and Th17 (Figure 1). Tregs dampen immune responses to prevent autoimmunity, whereas Th cells provide support to various immune responses through secretion of cytokines, such as interferon-gamma (IFN-γ), interleukin-2 (IL-2).
Table 2. Overview of different nanoparticle types and their features.

| Nanoparticle               | Organic/inorganic | Features                                                                 |
|----------------------------|-------------------|--------------------------------------------------------------------------|
| Carbon Nanotube            | Inorganic         | - Can have either single or multi-walled layers of graphite              |
|                            |                   | - Demonstrates good electronic and thermal conductivity                  |
|                            |                   | - Drug can be encapsulated into nanotube, adsorbed on surface            |
| Gold                       | Inorganic         | - Comprised gold atoms                                                   |
|                            |                   | - Shape, size, surface can be easily modified                            |
|                            |                   | - Have size- and shape-dependent optical and chemical properties         |
|                            |                   | - Can absorb light, fluoresce, and enable surface-enhanced Raman scattering |
|                            |                   | - Biomedical applications in phototherapies, imaging, drug delivery      |
| Iron Oxide                 | Inorganic         | - Comprised of either magnetite (Fe₃O₄) or maghemite (Fe₂O₃) cores, with a hydrophilic coat of dextran or other biocompatible material |
|                            |                   | - Have superparamagnetism, enabling imaging with MR                      |
|                            |                   | - Two superparamagnetic iron oxide nanoparticles (SPIONs) have been FDA-approved (ferumoxide, ferucarbotran) |
| Quantum Dot                | Inorganic         | - Semiconductor particles typically composed of a cadmium selenide core and zinc selenide cap |
|                            |                   | - Have long fluorescence lifetimes and stable against photobleaching     |
|                            |                   | - Size-dependent electronic and optical properties                        |
|                            |                   | - Biomedical applications in imaging                                      |
| Silica                     | Inorganic         | - Can have pores (mesoporous) or lack pores (nonporous); pores facilitate greater drug loading |
|                            |                   | - Easy to functionalize with ligands                                      |
|                            |                   | - Transparent, biocompatible                                              |
| Upconversion               | Inorganic         | - Upon near-infrared irradiation (NIR), nanoparticles emit photons of higher energy between the UV and visible range |
|                            |                   | - NIR facilitates greater depth penetration of tissues so nanoparticles can be used for deeper imaging |
|                            |                   | - Composed of inorganic crystals doped with lanthanides                   |
| Dendrimer                  | Organic           | - Repetitively branched particles made of amino acids, sugars, or nucleotides |
|                            |                   | - Water-soluble                                                          |
|                            |                   | - Drugs can be loaded into core or interact with branches                |
|                            |                   | - Also investigated as a gene delivery carrier                           |
| Liposome                   | Organic           | - Self-assembling spherical vesicles with single or many phospholipid bilayers |
|                            |                   | - Aqueous core of liposomes can be loaded with drugs, while membrane can be functionalized with ligands for specific targeting of cell populations |
|                            |                   | - Clinically approved liposomal formulations of doxorubicin, daunorubicin, vincristine, irinotecan for treatment of various cancers |
| Micelle                    | Organic           | - Water-free core enables drug delivery of hydrophobic molecules          |
|                            |                   | - Can be comprised amphiphilic polymers or lipids                        |
|                            |                   | - Biocompatible, safer than inorganic nanoparticles                      |
|                            |                   | - Clinically approved micellar formulation of paclitaxel (Genexol-PM) for treatment of breast, lung, and ovarian cancers in Korea |

(Continued)
Table 2. Continued.

| Nanoparticle | Organic/inorganic | Features |
|--------------|-------------------|----------|
| Nanoemulsion | Organic           | - Dispersion of oil droplets in an aqueous phase, stabilized by emulsifying agents  
- Can solubilize large amounts of hydrophobic drugs, making it an excellent candidate carrier for oral drug delivery  
- Facile generation of large quantities by shear stress or extrusion |
| Polymer      | Organic           | - Comprised biocompatible, biodegradable block-copolymers with varying hydrophobicity  
- Heavily investigated for delivery of hydrophilic and hydrophobic small molecule drugs, and proteins  
- Can be functionalized with targeting ligands to improve drug uptake |

Citations: [4, 75].

and interleukin-5 (IL-5) (Table 4). Additionally, Th cells provide signals to B-cells (“T-cell help”), which can then differentiate into plasma cells that secrete antibodies to mediate antibody-dependent cellular cytotoxicity (ADCC) and eliminate pathogens. Meanwhile, antigen-specific CD8+ T-cells, also known as cytotoxic T-cells, seek out and directly kill cells presenting the antigen via secretion of molecules such as perforin/granzyme or Fas ligand binding. Both CD4+ and CD8+ T-cells can also differentiate into memory cells, which initiate a stronger and more rapid immune response upon secondary exposure to an antigen.\(^{[11]}\)

3. Immune Checkpoint Blockade

3.1. Introduction to Immune Checkpoint Blockade

Immune checkpoint blockade relies on inhibition of negative regulatory pathways to enhance antitumoral immunity. To-date, monoclonal antibodies against the CTLA-4 and PD-1 receptors have been approved by the FDA for the treatment of various tumors including melanoma, lung, and renal cancers. During T-cell activation, the co-stimulatory molecule CD28 on T-cells must bind with DC ligands CD80 or CD86 (signal 2 of TCR activation).\(^{[12]}\) However, CTLA-4 competes with CD28 for binding to CD80/CD86 to prevent T-cell activation. Blockade of CTLA-4 with its monoclonal antibody allows signal 2 to proceed, resulting in the activation of T-cells. Meanwhile, T-cell activity is attenuated by the binding of PD-1 on T-cells to its ligands programmed death ligand 1 (PD-L1) and programmed death ligand 2 (PD-L2), which are expressed on DCs and tumor cells. Therefore, blockade with a monoclonal antibody against PD-1 or PD-L1/2 restores T-cell activity. However, only a subset of cancer patients has durable, long term responses to these immune checkpoint inhibitors. Moreover, various immune-related adverse events (irAEs) have also been associated with these immune modulators. A meta-analysis of 22 clinical trials of αCTLA-4 monoclonal antibodies administered to oncology patients revealed a high 72% overall incidence of irAEs with 24% of patients experiencing severe-grade irAEs.\(^{[13]}\) The most common toxicities affected the skin (44%) and the gastrointestinal tract (35%). In contrast to CTLA-4, the toxicity profiles of αPD-1/PD-L1 monoclonal antibodies are less severe. Both αPD-1/PD-L1 monoclonal antibodies commonly induced rashes, diarrhea, colitis, hypophysitis, hepatitis, and pneumonitis.\(^{[14]}\) Other immune checkpoint molecules are under investigation, including positive regulators whereby receptor activation promotes antitumoral immunity. Positive regulators of interest include tumor necrosis factor receptor superfamily membrane 9 (4-1BB) and tumor necrosis factor receptor superfamily member 4 (OX-40), whereas negative regulators include T-cell immunoglobulin and mucin domain (TIM-3) and lymphocyte activation gene-3 (LAG-3).\(^{[15]}\)

3.2. Nanotechnology Can Improve Efficacy of Immune Checkpoint Blockade Therapy

Employing nanotechnology in the delivery of immune checkpoint inhibitors can improve their tumor accumulation, enable co-delivery of two different immune checkpoint drugs, allow for monitoring of delivery in real-time, and even facilitate novel delivery approaches.

As demonstrated by Nikpoor et al., encapsulation of αCTLA-4 monoclonal antibodies into PEGylated liposomes can increase the tumor accumulation and therapeutic efficacy of immune checkpoint inhibitors, compared to free antibody.\(^{[16]}\) In mice bearing subcutaneous CT26 colorectal tumors, tumor accumulation of PEGylated liposomes loaded with anti-CTLA-4 antibodies was sevenfold greater than free antibodies 18 h post injection. This greater tumor accumulation translated into greater therapeutic efficacy, as evidenced by significantly prolonged survival of tumor-bearing mice treated with the PEGylated liposomes loaded with antibody (median survival time of 35 days), compared to free antibody (median survival time of 30 days). Therefore, the delivery of immune checkpoint inhibitors via nanoparticles can increase tumor accumulation via the enhanced permeability and retention effect, and facilitate greater therapeutic efficacy. However, the translational potential of the enhanced permeability and retention effect is controversial, as nanoparticle accumulation in tumors in humans varies depending on tumor type, heterogeneity, and perfusion.\(^{[5, 17]}\)
Table 3. Overview of immune cell populations.

| Immune cell       | Function                                                                 | Markers                                                                 |
|-------------------|---------------------------------------------------------------------------|-------------------------------------------------------------------------|
| Natural Killer (NK) Cells | - Lyses virally infected cells but lacks specificity of T-cells<br>- Produces cytokines including interferons<br>- Have Fc receptors so can recognize and lyse targets coated with soluble antibodies via antibody-dependent cell-mediated toxicity | CD56+                                                                   |
| Neutrophils       | - Phagocytoses pathogens<br>- Degranulates to destroy pathogens that cannot be phagocytosed<br>- "First responder" to inflammation | CD15+CD16+                                                              |
| Macrophages       | - Phagocytoses antigens<br>- Presents antigens to memory and effector T-cells to activate them<br>- Produces cytokines and chemokines<br>- Found in all tissues and organs | CD11b+CD68+                                                            |
| -M1-like          | Canonically, pro-inflammatory subtype                                    | MHC class II⁺ CD68+                                                    |
| -M2-like          | Canonically, anti-inflammatory subtype                                    | CD163⁺IL-12⁺IL-10⁻⁺                                                  |
| Dendritic Cells (DCs) | - Phagocytoses antigens for antigen presentation to naive T-cells; important link between innate and adaptive immunity<br>- Produces cytokines<br>- Subsets include classical, plasmacytoid, inflammatory, and follicular dendritic cells | CD80/86+                                                                |
| CD4+ T-cells      | Class of T-cells with CD4 co-receptor; have many subsets                 | CD3⁺CD4⁺                                                              |
| -Treg             | - TGF-β and IL-10 needed for activation of transcription factor, STAT3, to trigger differentiation into Tregs<br>- Induces peripheral tolerance<br>- Tregs exert anti-inflammatory effects via secretion of IL-10, TGF-β and can promote immune tolerance to tumors<br>- Four main subsets of Tregs: natural Tregs (nTreg), induced regulatory T-cells (iTreg), Th1, Th3 cells | CD25⁺Foxp3⁺(for nTreg, iTreg)<br>CD25⁺⁺ Foxp3⁻(for Th1, Th3) |
| -Th1              | - IFNγ, IL-12, IL-27 trigger activation of transcription factor, STAT4, to initiate differentiation into Th1 cell<br>- Upon antigen exposure, transcription factor, T-bet, is activated for effector function (i.e., secretion of IFNγ, IL-2, lymphotokin)<br>- Provides help to CD8⁺ T-cells in responses against viruses, intracellular bacteria<br>- Promotes antitumoral immunity via secretion of cytokines that recruit and stimulate innate leukocytes | IL-12R⁺                                                                |
| -Th2              | - IL-4 needed for activation of transcription factor, STAT6, to initiate differentiation | IFNγR⁺                                                                |
| -Th17             | - TGF-β, IL-6, and IL-21 triggers activation of transcription factors, STAT3 and IRF4, to initiate differentiation<br>- Upon antigen exposure, transcription factor RORyt is activated; results in secretion of IL-17, IL-21, IL-22, IL-26 to combat bacteria and fungi not cleared by Th1 and Th2 cells<br>- Promotes antitumoral immunity via secretion of IL-17, IFNγ, GM-CSF to increase neutrophil recruitment, support CTL function | IL-23R⁺                                                                |
Table 3. Continued

| Immune cell | Function | Markers |
|-------------|----------|---------|
| -Memory T-cells | - Upon re-exposure to antigen, memory T-cells initiate a faster, stronger immune response | CD45RO+CD62LCDR2+/CCR7+ (Tcm) CD45RO+CD62L+ CCR7+ (Tem) |
| - Two major classes: central memory T-cells (Tcm), effector memory T-cells (Tem) | | |
| - CD4+ Tcm reside in secondary lymphoid organs; secrete IL-2 upon activation | | |
| - CD4+ Tem circulate; upon recruitment secrete IL-4, IL-5, IFNγ | | |
| Class of T-cells with CD8 co-receptor | | |
| CD8+ T-cells | - IL-2 from Th cells activates a naive T-cell, induces proliferation to generate pre-CTL precursor cells | IFNγ+TNF+ |
| - In the presence of IL-6, IL-12, IFNγ, pre-CTL precursor cells differentiate to CTLs | | |
| - CTLs lyse target cells via secretion of granules containing perforin and granzyme, activation of Fas apoptosis pathway, secretion of cytotoxic cytokines (i.e., IFNγ, TNF, lymphotoxin) | | |
| - Two major classes: central memory T-cells (Tcm), effector memory T-cells (Tem) | CD45RO+CD62Lhi CCR7hi (Tcm) CD45RO+CD62Llo CCR7lo (Tem) |
| - CD8+ Tem have high granzyme B expression, which upon activation, results in cytolysis | | |
| - Can serve as antigen presenting cells | | |
| - Recognizes T-independent antigens, where T-cell interaction is unnecessary for B-cells to generate antibodies | | |
| - Recognizes T-dependent antigens, in which B-cells need T-cell help from Th cells to activate and differentiate into either plasma or memory B-cells | | |
| - Antibodies generated in response to T-dependent antigens undergo somatic hypermutation, isotype switching to generate diverse IgG, IgE, or IgA antibodies that are specific to an antigen | | |
| - Secretes antibodies which can clear antigen via neutralization, classical complement activation, opsonization, or antibody-dependent cell-mediated cytoxicity | CD138+ |
| - Can be short-lived and produce low affinity IgM antibodies since they do not undergo isotype switching, or somatic hypermutation | | |
| - Can be long-lived to produce high affinity antibodies upon re-exposure to antigen | | |

B-cells

- Recognizes T-independent antigens, where T-cell interaction is unnecessary for B-cells to generate antibodies

CD19+CD20+

Plasma cells

- Secretes antibodies which can clear antigen via neutralization, classical complement activation, opsonization, or antibody-dependent cell-mediated cytoxicity

Citations: [11].

To further stimulate T-cells, nanoparticles can provide a platform to spatially co-localize monoclonal antibodies against inhibitory immune checkpoint receptors with ones for a co-stimulatory receptor. Mi et al. employed polymeric nanoparticles to co-deliver αPD-1 monoclonal antibodies with agonistic antibodies for the co-stimulatory receptor, αOX40. In B16F10 melanoma bearing mice, administration of this dual immunotherapy nanoparticle cured 30% of mice, whereas injection with free αOX40 and αPD-1 resulted in tumor-free survival in only 10% of mice. For the surviving mice treated with the dual immunotherapy nanoparticle, re-challenge with B16F10 cells prevented tumor development in 83% of mice. As such, this platform prompted the induction of immune memory and durable antitumoral immunity. Therefore, nanoparticle-mediated co-delivery of immune checkpoint drugs can be more efficacious than free antibodies.

The concept of linking an immune checkpoint inhibitor with an antibody agonistic to a co-stimulatory receptor can facilitate an approach similar to bispecific antibodies. This is exemplified by Kosmides et al., who developed an “immunoswitch” iron-dextran nanoparticle coated with antibodies that bound the inhibitory checkpoint PD-L1 and the co-stimulatory receptor 4-1BB. These particles increased the conjugation of CD8+ T-cells to B16F10 tumor cells in vitro tenfold, compared to iso-type control immunoswitch nanoparticles, which had minimal conjugate formation. Moreover, immunoswitch nanoparticles remained longer at the tumor upon subcutaneous injection compared to free antibodies—by 72 h post injection, there was 60% retention of the immunoswitch nanoparticles at the tumor, compared to only 8% of free antibodies. In different tumor models, including subcutaneous B16-SIY melanoma, subcutaneous B16F10 melanoma, and subcutaneous MC38-OVA+ ovarian tumor bearing mice, intratumoral treatment with immunoswitch nanoparticles resulted in delayed tumor growth and extended survival, compared to co-injection of free antibodies. Accordingly, nanoparticles can prolong retention of immune checkpoint drugs at the tumor to facilitate greater therapeutic efficacy.

An ongoing challenge for clinicians is stratifying patient response to immune checkpoint inhibitors, as the cost of these drugs is high. To address this issue, Meir et al. employed theranostic gold nanoparticles conjugated with α-PD-L1 antibodies (αPD-L1-GNPs) to stratify responders from non-responders.
After intravenous injection of αPD-L1-GNPs into mice bearing subcutaneous MC38 colon tumors, nanoparticles accumulated in the tumor, generating contrast that was detected on CT. Given the variable tumor uptake of αPD-L1-GNPs, CT signal could be used to predict response to immune checkpoint blockade. A linear correlation was reported between CT signal of αPD-L1-GNPs and tumor growth; therapeutic response to immune checkpoint blockade in mice that had high and low CT signal could be accurately predicted. As such, Meir et al. demonstrated the proof-of-concept that nanoparticles may serve as a means of non-invasive monitoring of immune checkpoint blockade response, and stratifying responders from non-responders as early as 48 h after treatment. However, more validation for other tumor types that can be treated with αPD-L1 antibodies is needed.

Nanotechnology can facilitate the development of a new delivery method. Wang et al. developed a microneedle patch, in which microneedles were coated with pH-sensitive dextran nanoparticles.[21] The nanoparticles encapsulated αPD-1 antibodies and glucose oxidase, which converted blood glucose to gluconic acid. Under acidic conditions, the nanoparticles dissociated and slowly released αPD-1 antibodies over the course of 3 days. Microneedle patch administration to mice bearing subcutaneous B16F10 melanomas resulted in the survival of 40% of mice 40 days post-treatment, whereas all mice that received free αPD-1 antibodies succumbed to disease. This finding was attributed to the sustained release of αPD-1 antibodies by the microneedles, and subsequent enhanced retention of antibodies in the tumor. While intriguing, this method of drug delivery will likely be limited to superficial tumors, such as melanomas. Overall, these...
Table 4. Functions of cytokines and chemokines involved in tumor immunity.

| Cytokine/chemokine                      | Function                                      | Targets                                                                 |
|----------------------------------------|-----------------------------------------------|------------------------------------------------------------------------|
| Interleukin-1 (IL-1)                   | Pro-inflammatory                              | T-cells, B-cells                                                      |
|                                        | Induces fever, acute phase response           |                                                                        |
| Interleukin-2 (IL-2)                   | Promotes cell growth, activation, survival    | T-cells, NK cells, B-cells, monocytes                                  |
| Interleukin-5 (IL-5)                   | Promotes cell growth, activation              | B-cells, eosinophils                                                  |
| Interleukin-6 (IL-6)                   | Pro-inflammatory                              | T-cells, B-cells, monocytes, neutrophils                               |
|                                        | Promotes neutrophil microbicidal activity    |                                                                        |
|                                        | Promotes B-cell differentiation               |                                                                        |
|                                        | Promotes Th17 cell differentiation            |                                                                        |
| Interleukin-12 (IL-12)                 | Promotes Th1 differentiation                  | T-cells                                                               |
| Interleukin-10 (IL-10)                 | Inhibits antigen-presenting cells             | T-cells, macrophages                                                  |
|                                        | Inhibits cytokine production                  |                                                                        |
| Interleukin-15 (IL-15)                 | Promotes T-cell growth, activation            | T-cells, macrophages                                                  |
|                                        | Promotes NK cell development, blocks apoptosis|                                                                        |
| Interleukin-21 (IL-21)                 | Pro-inflammatory                              | T-cells, B-cells                                                      |
|                                        | Promotes expansion of B-cells and plasma cells|                                                                        |
|                                        | Promotes Th17 cell differentiation            |                                                                        |
|                                        | Inhibits Treg differentiation                 |                                                                        |
| Interferon-gamma (IFN-γ)               | Antiviral                                     | Monocytes, macrophages, endothelial cells, tissue cells               |
|                                        | Promotes cell growth, activation              |                                                                        |
|                                        | Increases MHC expression                      |                                                                        |
| Lymphotixin (LT)                       | Pro-inflammatory                              | T-cells, B-cells, macrophages                                         |
|                                        | Promotes cytokine secretion                   |                                                                        |
| Tumor necrosis factor-alpha (TNF-α)    | Promotes cell activation                      | T-cells, B-cells, endothelial cells                                   |
|                                        | Involved in co-stimulation                    |                                                                        |
| Transforming growth factor beta (TGF-β)| Inhibits cell growth, activation              | T-cells                                                               |
| Granulocyte macrophage colony stimulating factor (GM-CSF) | Promotes antigen presentation | Macrophages, DCs                                                     |
|                                        | Involved in T-cell homeostasis                |                                                                        |
|                                        | Serves as hematopoietic cell growth factor    |                                                                        |
| Chemokine (C-C motif) ligand 4 (CCL4)  | Chemoattractant                               | Macrophages, naive T- and B-cells                                    |
| Chemokine (C-X-C motif) ligand 6 (CXCL6)| Chemoattractant                               | Neutrophils, NK cells                                                |
| Chemokine (C-X-C motif) ligand 8 (CXCL8)| Chemoattractant                               | Neutrophils, T-cells                                                |
| Chemokine (C-X-C motif) ligand 9 (CXCL9)| Chemoattractant                               | T-cells                                                             |
| Chemokine (C-X-C motif) ligand 10 (CXCL10)| Chemoattractant                            | T-cells, NK cells                                                    |
| Chemokine (C-X-C motif) ligand 12 (CXCL12)| Chemoattractant                           | T-cells, DCs                                                        |

Citations: [11, 76].

highlighted applications of nanotechnology in immune checkpoint blockade are predominantly early-stage, proof-of-concept studies. Therefore, more validation is required, as are repeat studies to confirm findings.

4. Cancer Vaccines

4.1. Introduction to Cancer Vaccines

The typical vaccine is composed of: 1) a delivery platform, 2) an antigen derived from a pathogen, and 3) an adjuvant to further boost immunogenicity. Tumor vaccines can comprise antigens from whole cell tumor lysate (tumor-associated antigens), which are purified self-antigens that are overexpressed on tumor tissue but are also present at lower levels on healthy tissue, or from tumor-specific antigens, which are mutated neo-antigens that are specific only to tumor tissues.[22] Different immune adjuvants have been explored, including TLR agonists. Various delivery vehicles have also been investigated, including liposomes, polymers, and emulsions. Cancer vaccines can be either prophylactic, aimed at preventing cancer, or therapeutic, aimed at eliminating established tumors. To-date, FDA-approved prophylactic vaccines include Gardasil, which protects against human papillomaviruses that cause cervical cancer, whereas therapeutic ones include sipuleucel-T (Provenge) and an oncolytic virus therapy, talimogene laherparepvec (T-VEC).
To elicit a robust immune response, a tumor vaccine must fulfil several requirements. First, irrespective of the mode of administration (oral or intramuscular), the vaccine must traffic to the lymph nodes. Second, at the lymph node, the vaccine needs to be taken up and processed by APCs to initiate a T-cell response. Many vaccines are endocytosed by DCs, after which they are degraded into peptide fragments within endosomal and lysosomal compartments and presented via the MHC class II pathway to CD4+ T-cells. In the presence of other T-cell priming signals, CD4+ T-cells are primed and differentiated into subsets of Th cells. However, Th cells primarily provide support to other immune cells to mount antitumoral responses. Therefore, to elicit a direct, robust cytotoxic immune response against tumor cells, cytotoxic CD8+ T-cells are needed. This immune population arises from the processing of antigen in DCs via the endogenous MHC class I antigen-presenting pathway. In this pathway, antigen is detected in the cytosol; therefore, vaccines that are endocytosed must undergo a process called cross-presentation and escape the endosome into the cytoplasm where they can be processed. Tumor vaccines should ideally be processed via both MHC class I and MHC class II antigen-presenting pathways to elicit both CD8+ and CD4+ T-cell-mediated responses, respectively. Third, vaccines should induce memory to the antigen. During the initial insult, a fraction of T-cells differentiates into memory cells, which circulate in the blood. Upon encountering the antigen of interest again, these memory T-cells differentiate into effector cells that can reject tumor cells.

For each of these requirements, there are challenges that can preclude generation of an effective immune response. Vaccines may not traffic sufficiently to the lymph nodes, and may not induce antigen presentation or processing, nor induce a CD8+ T-cell response. Given the complexity of the immune system, tolerance rather than immunogenicity may be elicited and immune memory may not form. Then, the tumor type can affect the magnitude of the immune response, as tumor immunogenicity varies. Compared to liquid tumors, therapeutic cancer vaccines are less efficacious for solid tumors due to impaired T-cell infiltration into the solid tumor mass. Furthermore, the immunosuppressive tumor microenvironment can promote T-cell exhaustion. Moreover, vaccines that target only one tumor antigen are insufficient to eliminate tumors, especially in the face of tumor heterogeneity. As such, cancer vaccines confront numerous challenges.

4.2. Nanotechnology Can Improve Efficacy of Cancer Vaccines

The aforementioned challenges of cancer vaccines can be addressed in part through nanotechnology. For example, as a delivery platform for tumor antigens and/or adjuvants, nanoparticles can protect their cargo from degradation in vivo so that it arrives at the lymph nodes. Additionally, some nanoparticles are inherently immunogenic and can simultaneously serve as an adjuvant and a delivery vehicle. After administration, the biodistribution of vaccines can be tracked by nanoparticle-mediated imaging. Upon uptake by DCs, pH-sensitive nanoparticle vaccines can promote cross-presentation to elicit an antitumoral response mediated by CD8+ T-cells. Finally, since nanoparticles can be formed from a diverse range of biomaterials, they can generate new biomimetic vaccines that may augment immunogenicity. Accordingly, the development of nanoparticle tumor vaccines is of great interest due to their ability to address many existing challenges, as will be discussed in detail below. For an overview of various nanoparticle cancer vaccines, refer to Table 5.

First, tumor antigens can be delivered via nanoparticles. For example, Gu et al. employed nanoparticles composed of hyaluronic acid and polyethylene glycol to deliver HER2-expressing tumor vaccines. Compared to free HER2 protein, there was significantly greater tumor growth delay in mice treated with HER2-expressing nanoparticles, whereas mice immunized with free HER2 protein lacked protection against tumor growth. Given this promise, the HER2-expressing nanoparticles were further developed and tested in clinical trials for patients with HER2+ tumors. The vaccine was well-tolerated and induced HER2-specific IgG antibodies in 93% of patients. As such, nanoparticle-mediated delivery of antigen can provide superior activation of the immune system and trigger antitumoral responses, compared to immunization with free antigen.

In addition to delivering tumor antigens, nanoparticles can serve as platforms for adjuvant delivery. For instance, Zhao et al. employed soluble functionalized carbon nanotubes to deliver CpG oligodeoxynucleotides (CpG-ODN) to mice with intracranial gliomas. Compared to free CpG, there was a fivefold greater CpG uptake by tumor-associated macrophages (TAMs) and microglia in vivo. Moreover, a single injection of CpG-ODN eradicated established orthotopic GL261 gliomas in 50–60% of mice and enabled the survival of >65% of mice for 90 days. Subsequently, the surviving mice treated with CpG-ODN were re-challenged with GL261 cells, but immunity to the tumor had developed as all treated mice survived. In contrast, mice treated with free CpG did not experience tumor growth delays, and died within 50 days. Accordingly, compared to free adjuvant, nanoparticles can enhance delivery of adjuvant to tumor-associated immune cells to potentiate antitumor immune responses.

Since nanoparticles can be inherently immunogenic, they can act as an adjuvant while simultaneously delivering a tumor antigen. This principle is exemplified by Wang et al. who developed a prophylactic vaccine composed of hollow mesoporous silica nanoparticles mixed with fragments of Lewis lung carcinoma (HSM-LLC). After prophylactic immunization with the vaccine, Lewis lung tumor growth in mice was inhibited, whereas mice treated with tumor antigen but no adjuvant had tumors with an average volume of 3236 ± 1329 mm³ 30 days after tumor challenge. Upon tumor re-challenge, mice vaccinated with HSM-LLC had greater tumor growth delay compared to mice injected with tumor fragments and the clinically used adjuvant, alum (Figure 2). Compared to alum + tumor fragment treatment, HSM-LLC induced greater proliferation of CD4+ memory T-cells (52.3 ± 9.8% vs 35.5 ± 2.5%) and CD8+ memory T-cells (44.6 ± 13.2% vs 33.2 ± 8.0%). As demonstrated, certain nanoparticles can serve as adjuvants; notably, they may mediate...
Table 5. Select nanoparticle vaccines investigated in vivo.

| Nanoparticle                      | Prophylactic/therapeutic | Tumor antigen               | Adjuvant             | Tumor model                        | Reference   |
|-----------------------------------|--------------------------|-----------------------------|----------------------|------------------------------------|-------------|
| Carbon nanotube                   | Therapeutic              | –                           | CpG ODN              | Orthotopic GL261 gliomas           | [28]        |
| Carbon nanotube                   | Therapeutic              | Tumor lysate                | –                    | S.C. H22 liver tumor               | [77]        |
| Cholesteryl pullulan              | Therapeutic              | HER2                        | –                    | HER2 expressing tumor patients     | [27a]       |
| Cholesteryl pullulan              | Therapeutic              | HER2                        | –                    | HER2 expressing tumor patients     | [27a]       |
| Exosome                           | Therapeutic              | MAGE3 peptides              | –                    | Stage III/IV melanoma patients     | [28]        |
| Exosomes                          | Therapeutic              | –                           | –                    | S.C. 3LL Lewis lung tumors         | [29]        |
| Gold                              | Therapeutic              | –                           | CpG ODN              | S.C. B16F10 and B16F10-RFP melanomas | [80]        |
| Gold                              | Therapeutic              | –                           | CpG ODN              | S.C. B160VA melanoma               | [80]        |
| Hyaluronic acid                   | Therapeutic              | –                           | CpG ODN              | E.G7-OVA lymphoma                  | [82]        |
| Iron oxide                        | Therapeutic              | CEA                         | –                    | S.C. MC38/CEA colon tumors         | [81]        |
| Lipid                             | Prophylactic             | –                           | –                    | S.C. E.G7-OVA lymphoma             | [81]        |
| Lipid                             | Therapeutic              | Trp2 peptide                | CpG ODN              | S.C. B16F10 melanoma               | [84]        |
| Liposome                          | Prophylactic             | Ovalbumingp70 Trp1          | MHC class II Neoepitope | Human tumor antigens (NY-ESO-1, MAGE-A3, tyrosinase and TPTE) | [33]        |
| Liposomes                         | Prophylactic             | OVA                         | CpG ODN              | S.C. E.G7-OVA lymphoma             | [85]        |
| Liposome                          | Therapeutic              | E7 peptide                  | –                    | S.C. TC-1 lung tumors              | [84]        |
| Liposome                          | Therapeutic              | Alpha-galactosylceramide    | –                    | Metastatic B16F10 lung tumors      | [87]        |
| Micelle                           | Therapeutic              | OVA                         | –                    | S.C. B16 melanoma                  | [81]        |
| Micelle                           | Prophylactic             | OVA                         | CpG                  | S.C. B16F10 melanoma               | [80]        |
| Metallofullerenol                 | Therapeutic              | –                           | –                    | S.C. Lewis lung carcinoma           | [80]        |
| Nanoring of oligonucleotides      | Therapeutic              | Tat(47–57)                  | CpG ODN              | E.G7-OVA lymphoma                  | [90]        |
| Polysaccharide                    | Prophylactic             | HER2 Oncoprotein            | –                    | S.C. CMS7HE fibrosarcoma           | [86]        |
| Polymer                           | Prophylactic             | OVA                         | Pam3CSK4 and poly(I:C) | S.C. B16F10 melanoma               | [45]        |
| Polymer coated with erythrocyte membrane | Prophylactic             | Antigenic peptide (hgp10025–13) | Monophosphoryl lipid | S.C. B16F10 melanoma model         | [41]        |
| Polymer                           | Prophylactic             | Melan-A:26, gp100:209, or gp100:44 | Poly(I:C) CpG | S.C. B16F10 melanoma               | [41]        |
| Polymer                           | Prophylactic             | Chicken egg ovalbumin       | –                    | S.C. E.G7-OVA lymphoma             | [81]        |
| Polymer                           | Prophylactic             | MART-1                      | –                    | S.C. TRAMP-C2 prostate tumors      | [94]        |
| Polymer                           | Prophylactic             | OVA                         | mSTEA                | Intradermal E.G7-OVA lymphoma      | [81]        |
| Polymer                           | Prophylactic             | OVA                         | Freund's complete adjuvant | S.C. E.G7-OVA lymphoma             | [81]        |
| Polymer                           | Prophylactic             | OVA                         | –                    | S.C. E.G7-OVA lymphoma             | [81]        |
| Polymer                           | Prophylactic             | OVA                         | –                    | E.G7-OVA lymphoma                  | [81]        |
| Polymer                           | Prophylactic             | EphA2                       | –                    | Orthotopic MC38 liver cancer       | [81]        |
| Polymer                           | Prophylactic             | OVA                         | CpG shRNA Stat3 shRNA | Metastatic MC38 lung tumors         | [81]        |

(Continued)
more potent immune responses than clinically employed adjuvants like alum. While nanoparticles can serve as delivery platforms for adjuvants or tumor antigens individually, they can also co-deliver both agents in one vehicle. Co-localization of both adjuvant and antigen to APCs can potentiate immune responses. Furthermore, the use of an adjuvant can decrease the quantity of tumor antigen needed in a vaccine. This concept of co-delivery is illustrated by Speiser et al. who designed a virus-like nanoparticle that co-delivered peptides from the melan-A tumor antigen and the adjuvant A-type CpG, an agonist for Toll-like receptor 9 (MelQbG10). This vaccine induced human DCs to cross-present and subsequently stimulate secretion of IFN-γ by CD8+ T-cells. After vaccination with MelQbG10, 64% of melanoma patients developed melan-A-specific T-cells that secreted inflammatory cytokines IFN-γ, IL-2, and TNF-α, and had upregulated lysosomal associated membrane protein 1, indicative of T-cell perforin/granzyme functionality. Evidently, co-delivery of a tumor antigen and adjuvant by a nanoparticle can elicit potent immune responses.

While adjuvants can trigger innate immunity to strengthen the immune response, cross-presentation within DCs is necessary to prompt a CD8+ T-cells response, in order to directly kill tumor cells. This challenge of cross-presentation, in which antigens are internalized into endocytic or lysosomal compartments and then escape into the cytoplasm where the MHC class I presentation machinery resides, can be addressed through pH-sensitive nanoparticles. As shown by Akita et al., incorporation of a pH-dependent fusogenic peptide, GALA, into a lipid nanoparticle can promote endosomal escape of a vaccine’s cargo. Upon uptake into endosomes, GALA facilitated endosomal fusion and release of the vaccine into the cytoplasm. In vitro fluorescence analyses indicated ≈70% endosomal escape efficiency by the nanovaccine, compared to 52% by vaccines lacking GALA. This higher endosomal escape efficiency mediated by the GALA-containing vaccine prevented EG7-OVA lymphoma growth in mice until day 20 of evaluation, compared to phosphate buffered saline (PBS)-treated mice, which all succumbed to disease by that timepoint. Through incorporation of pH-sensitive peptides, nanoparticles can facilitate endosomal escape for successful cross-presentation.

In another example of exploiting pH to promote antigen cross-presentation, Luo et al. used ultra-pH sensitive co-polymer block micelles for vaccination. Upon cellular uptake, one ultra-pH sensitive nanoparticle formulation, PC7A NP, localized to and disrupted endocytic vesicles, resulting in the delivery of its antigen cargo, redox-activatable dye labeled ovalbumin (OVA), into the cytosol. As a proxy to quantify endosomal disruption and cytosolic delivery of cargo, a hemolysis assay in red blood cells was performed, in which ≈90% of red blood cells were lysed when PC7A NP were at pH values below 7.0. In comparison, the control PD5A NP did not demonstrate any RBC hemolysis in the same pH range. Endosomal disruption presumably enabled cross-presentation. When CD8+ T-cells obtained from the spleen were primed with PC7A NP, OVA-specific CD8+ T-cells demonstrated 15-fold greater proliferation compared to T-cells treated with only OVA. Moreover, the PC7A NP activated the stimulator of interferon genes (STING) pathway, and a downstream interferon gamma type I response to further potentiate antitumoral immunity. In four different tumor models, B16-OVA and B16F10 melanomas, MC38 colon tumors, and human papilloma virus E6/7 TC-1 tumors, treatment with a therapeutic PC7A NP vaccine formulated with antigenic peptide delayed tumor growth and prolonged survival relative to controls. Accordingly, antigen

### Table 5. Continued.

| Nanoparticle | Prophylactic/therapeutic | Tumor antigen | Adjuvant | Tumor model | Reference |
|--------------|--------------------------|---------------|----------|-------------|-----------|
| Polymer      | Therapeutic              | TRP2          | TLR ligand, 7-acyl lipid A | Orthotopic B16F10 melanoma | [100]     |
| Polymer      | Therapeutic              | N/A           | Agonistic TLR5, TLR7 sRNA | Intraperitoneal ID8-luciferase | [101]     |
| Polymer      | Therapeutic              | None          | CpG ODN  | S.C. B16F10 melanoma | [102]     |
| Protein cage | Prophylactic              | OVA           | –         | S.C. B16OVA melanoma | [103]     |
| Selenium    | Prophylactic              | –             | –         | S.C. 4T1 breast tumors | [104]     |
| Silica       | Prophylactic              | –             | –         | S.C. Lewis lung carcinoma | [105]     |
| Virus-like particle | Therapeutic              | Peptide(16–35) derived from Melan-A/MART-1 | A-type CpG-ODN | Stage III/IV melanoma patients | [106]     |
| Virus-like particle | Therapeutic              | Melan-A/Mart-1 peptide | A-type CpG | Stage II–IV melanoma patients | [107]     |
| Virus-like particle | Therapeutic              | –             | –         | S.C. B16F10 melanoma | [108]     |

S.C., subcutaneous; mSTEAP, mouse six-transmembrane epithelial antigen of the prostate; CpG ODN, cytosine triphosphate deoxynucleotide phosphodiester guanine triphosphate deoxynucleotide oligodeoxynucleotide; CEA, carcinoembryonic antigen; OVA, ovalbumin; TLR, Toll-like receptor; TRP2, tyrosinase-related protein. Mice were inoculated with tumors, unless otherwise stated.
Figure 2. Nanoparticles can serve as vaccine platforms. A) Hollow mesoporous silica nanoparticles (HSM) are inherently immunogenic, enabling them to act as an adjuvant while delivering tumor antigen. HSM elicited potent antitumoral effects and mediated immune memory. After initial prophylactic immunization with 0.8 mg HSM/mouse and tumor cell fragments ($5 \times 10^5$ cells per mouse), mice did not develop Lewis lung carcinoma tumors and had 100% survival compared to mice immunized with tumor lysate only. Upon re-challenge with $5 \times 10^5$ Lewis lung carcinoma cells, and immunization with 0.8 mg of HSM and tumor lysate, there was a greater number of mice who remained tumor-free or had tumors smaller than 15 mm, compared to mice treated with 0.8 mg of alum and tumor fragment. This survival was mediated in part by a greater frequency of CD4+ memory T-cells. Reproduced with permission.[29] Copyright 2016, Wiley-VCH. B) Tumor cell-coated polymeric nanoparticles can elicit multi-antigenic antitumoral immunity against mice bearing B16F10 melanomas. Spider plots of individual mice immunized with either blank solution CpG-loaded polymers, tumor cell-coated polymeric nanoparticle (CCNP), CCNP with free CpG, whole cell lysate with free CpG, and CpG-loaded CCNP on days 0, 7, and 14, after which they were challenged subcutaneously with $2 \times 10^5$ B16F10 cells on day 21. Reproduced with permission.[36b] Copyright 2017, Wiley-VCH.

cross-presentation can be enhanced with nanoparticle vaccines that can disrupt endocytic vesicles under acidic pH.

Another advantage of using nanoparticles for vaccine delivery is their ability to protect their cargo from degradation while in circulation. In particular, this feature is advantageous for DNA and mRNA vaccines, which are sensitive to nuclelease degradation. Kranz et al. used cationic liposomes to form colloidal stable RNA complexes (RNA-LPX).[33] This complex protected the RNA from extracellular ribonucleases during incubation at 37 °C in mouse serum for 30 min. Notably,
RNA-LPX was taken up by APCs not only in the spleen, but also in various lymph nodes, and in bone marrow of the femur and tibia. This systemic antigen targeting to APCs facilitated therapeutic efficacy across multiple tumor models. In mice with established B16F10-Luc+ lung metastases, using the tumor antigen TRP-1 as the vaccine target, TRP-1-LPX eradicated all tumors. Similarly, when mice bearing HPB16 E6- and E7-expressing TC-1 tumors were treated with E6/E7-LPX, at either 7 and 10 days post tumor challenge, 100% and 90% of mice survived over the course of 100 days, respectively, whereas control mice all died within 40 days. Additionally, vaccination of mice bearing established CT26 colon tumors resulted in elimination of tumors and protection upon tumor re-challenge. Given these encouraging results across multiple tumor models, the RNA-LPX vaccine is under investigation in clinical trials. In an initial report from a phase I clinical trial, the vaccine was well-tolerated, and all three patients with advanced malignant melanoma developed de novo T-cell responses against vaccine antigens. As exemplified by LPX, nanoparticles can serve as a platform that protects the vaccine’s cargo and enhances efficacy.

Biomimetic nanoparticulate vaccines, including viral-like particle and tumor cell membrane–derived vaccines, have generated great interest due to their ability to generate potent immune responses. Virus-like particles are comprised of viral coat proteins that can assemble into virus capsids but, since they lack viral nucleic acids, they are not infectious. Virus-like particle vaccines can be engineered to present multiple, ordered epitopes to elicit enhanced humoral and cell-mediated immune responses. Lizotte et al. investigated the immunogenicity and antitumoral efficacy of a cowpea mosaic virus particle (CPMV) vaccine. Empty CPMVs were inherently immunogenic, as their addition to bone marrow DCs resulted in the secretion of pro-inflammatory cytokines including IL-6, TNF-α, and IL-1β. Moreover, inhalation of empty CPMVs without any antigen or adjuvant decreased formation of B16F10 metastases threefold compared to PBS treatment. In a B16F10 melanoma model, empty CPMV injections eliminated tumors in half of the treated mice. In mice with eradicated tumors, B16F10 re-challenge was resisted in 75% of mice, indicating generation of immune memory. Moreover, empty CPMV was superior at delaying growth of B16F10 tumors compared to adjuvants like dsRNA mimic, poly(I:C), and the STING agonist, 5,6-dimethylxanthene-4-acetic acid. Given that empty CPMVs were studied, it would be of interest to load adjuvants or other drugs into CPMVs and investigate the efficacy of combination therapies. As illustrated by Lizotte et al., viral-like particle vaccines are promising due to their inherent immunogenicity and their potential to serve as a versatile drug delivery vehicle.

Vaccines derived from tumor cell membranes have also been developed. This type of vaccine can present multiple membrane-bound tumor-associated antigens to antigen-presenting cells, and potentially circumvent immune escape. Simultaneously, they can overcome a major limitation associated with whole tumor cell lysate vaccines—namely, the generation of immunity against nontumor-related antigens, which reduces the efficacy of this vaccine class. In a prophylactic setting, Kroll et al. found that a nanovaccine consisting of cancer cell membranes coated onto CpG loaded nanoparticles suppressed tumor growth in 6/7 mice bearing B16F10 melanomas, whereas all mice treated with whole tumor cell lysate and free CpG succumbed to disease (Figure 2). In a therapeutic setting, the tumor cell membrane vaccine in combination with α-CTLA4 and α-PD1 antibodies delayed tumor growth in established B16F10 melanoma-bearing mice compared to vaccine or immune checkpoint blockade alone controls. Over the course of 50 days, 50% of mice that received combination therapy survived, whereas all mice treated with only the vaccine died by 30 days. As such, tumor cell membrane–derived vaccines and their combinations with other therapies represent an intriguing avenue of further research.

4.3. Challenges for Nanoparticle-Based Vaccines

As discussed, nanotechnology can confer many advantages to tumor vaccines. However, there are presently no nanoparticle-based tumor vaccines used in the clinic to treat cancer patients. To translate nanoparticle-based tumor vaccines into the clinic, there are many outstanding challenges to address. First, in preclinical studies, the vaccine of interest needs to be compared to better controls beyond a PBS vehicle control. Although there is no clinical gold standard tumor vaccine to compare to, controls including other tumor vaccines, the empty nanoparticle, clinical adjuvant, or tumor antigen alone should be considered when designing experiments. Second, as the immune system is immensely complex with multiple components involved in an intricate interplay, it is important to conduct more basic research to understand the mechanisms of action for nanoparticle-based vaccines. These studies will yield insights about optimal therapies to combine vaccines with, as well as facilitate predictions about safety and toxicity. Third, more toxicity studies need to be conducted, as early as in preclinical studies. Tissues beyond the tumor, lymph nodes, and spleen should be examined because vaccines can yield systemic immune responses. Nanoparticle-based vaccines hold great promise and in addressing these questions, this promise may be fulfilled.

5. Adoptive Cellular Therapy

5.1. Introduction to Adoptive Cell Therapy

Adoptive cellular therapies (ACT) involve isolation of immune cells from either an individual with cancer or from a healthy donor, followed by ex vivo cell expansion and injection into the patient to combat the tumor. Various effector cells have been explored for adoptive cell therapies, including tumor-infiltrating lymphocytes (TILs), T-cell receptor-transduced (TCR) T-cells, chimeric antigen receptor (CAR) T-cells, and innate immune cells such as NK cells, and double negative T-cells. More information, refer to Table 6, which summarizes some of the effector cells under investigation. To-date, two CAR-T therapies, axicabtagene ciloleucel (Yescarta) and tisagenlecleucel (Kymriah), have been approved by the FDA for treatment of individuals with lymphoma and leukemia, respectively.

Regardless of the effector cell type, there are common challenges underlying the success of ACT, including complex manufacturing processes, limited tumor trafficking, the immunosuppressive tumor microenvironment, and low T-cell persistence. First, the drawbacks of clinical-scale manufacturing of T-cells can
restrict their widespread application in cancer treatment. To isolate, genetically modify, and expand T-cells, specialized equipment and technical expertise are needed, but culminate in few hospitals.

Second, the lack of homing of adoptively transferred cells to the tumor remains a major challenge. In a normal response to inflammation, circulating T-cells roll along the endothelial cells of the blood vessel, bind to adhesion molecules expressed on endothelial cells, activate, and arrest prior to extravasation into the tissue.\(^{[49]}\) However, in the presence of a tumor, T-cell migration is disrupted due to downregulation of chemokines such as chemokine (C-X-C motif) ligand 9 (CXCL9) and chemokine (C-X-C motif) ligand 10 (CXCL10), reduction in expression of adhesion molecules such as intercellular adhesion molecules 1 and 2, and vascular cell adhesion molecule 1, and CD34, and the disorganized nature of the tumor vasculature with its irregular branching and abnormal blood flow. The homing problem is exemplified by the adoptive transfer of autologous HER2-specific T-cells in a metastatic HER2+ breast cancer patient, in which \(^{111}\)In-labeled T-cells trafficked to the lung, liver, and spleen, but there was \(\approx\)0.1% of infused \(^{111}\)In-labeled T-cells detected in metastases.\(^{[49]}\) Similarly, when Pockaj et al. investigated the localization of \(^{111}\)In-labeled tumor-infiltrating lymphocytes in metastatic melanoma patients, they reported a mere 0.0021% injected \(^{111}\)In per gram of tumor tissue.\(^{[41]}\) Third, at the site of the tumor, many cells and cytokines can interact with transferred immune cells to suppress their proliferation and cytotoxicity against tumor cells. Tregs produce anti-inflammatory cytokines, such as interleukin-10 (IL-10), and transforming growth factor beta (TGF-\(\beta\))\(^{[42]}\). Upon cell-mediated contact with CD4+ and CD8+ T-cells, Tregs can induce T-cell anergy and subsequent inhibition of tumor cell recognition and elimination. TAMs further promote immunosuppression by secreting tolerogenic factors, such as IL-10, TGF-\(\beta\), and prostaglandins. TAMs also block CD8+ T-cell proliferation and recruit Tregs via the secretion of the chemokine (C-C motif) ligand 22 (CCL22). Myeloid derived suppressor cells promote differentiation of Tregs and catalyze the formation of reactive oxygen and nitric oxide species, which blunt T-cell responses. Additionally, tumor cells can express PD-L1 and PD-L2 receptors on adoptively transferred T-cells to induce T-cell exhaustion and reduce the efficacy of ACT.\(^{[43]}\) Fourth, the persistence of adoptively transferred cells is another challenge due to the hostile tumor microenvironment, characterized by hypoxia, acidity, and nutrient depletion.\(^{[43]}\) Tumor-generated arginase-1 and indoleamine 2,3-dioxygenase 1 can induce cell cycle arrest.

### Table 6. Overview of immune cells explored in adoptive cellular therapies preclinically.

| Effector cell                        | Description                                                                 | Advantages                                                                 | Limitations                                                                 | Clinically approved |
|--------------------------------------|-----------------------------------------------------------------------------|----------------------------------------------------------------------------|----------------------------------------------------------------------------|---------------------|
| Tumor infiltrating lymphocytes (TILs) | Mixed population of patient-derived CD4+ and CD8+ T-cells isolated from solid tumors | Polyclonal T-cells are expanded so can recognize multiple tumor-associated antigens | - Short persistence of TILs in vivo  
- Autoimmunity  
- Largely restricted to treatment of melanomas since other solid tumors do not consistently yield tumor-reactive TILs  
- Lengthy duration for TIL expansion  
- Expertise and resources needed for transduction of T-cells  
- MHC restriction of T-cells which limit the recognized tumor-associated antigens and the tumor clones that can be killed  
- Toxicities arise from expression of tumor-associated antigen on healthy tissue (“on-target toxicity”) | No |
| T-cell receptor T-cells (TCR-T-cells) | Peripheral blood lymphocytes transduced with TCRs specific for tumor-associated antigens (i.e., MART-1, NY-ESO-1, gp100, etc.) | Potential for “off-the-shelf” therapy | - HLA-independent so does not need a TCR to recognize a specific antigen; overcomes MHC restriction associated with TCR-T-cells  
- Not as effective in solid tumors, compared to leukemias  
- Toxicities, including cytokine release syndrome which can be fatal | No |
| Chimeric antigen receptor T-cells (CAR-T) | T-cells transduced with CARs, consisting of a B-cell receptor derived, extracellular antibody single-chain variable fragment that recognizes the target antigen, a CD3ζ signalling domain for T-cell activation, and one or more co-stimulatory domains (i.e., CD28 or 4-1BB) | - HLA-independent so does not need a TCR to recognize a specific antigen; overcomes MHC restriction associated with TCR-T-cells | - Poor in vivo survival  
- Relatively unexplored for ACT compared to other effector cells; will need further investigation on identifying favorable donors, identifying tumor types most susceptible to NK cell therapy, etc.  
- Relatively unexplored for ACT compared to other effector cells; will need further investigation on identifying favorable donors, and tumor types most susceptible to NK cell therapy, etc. | Yes |
| Natural killer cells (NK cells)      | CD56+ population of innate immune cells that kills targets based on loss of MHC class I expression | Potential for “off-the-shelf” therapy | | No |
| Double negative T-cells              | CD3+CD4-CD8- population of T-cells that can be easily expanded from healthy human donors | Potential for “off-the-shelf” therapy  
Safety; does not induce graft versus host disease | | No |

Citations: [37,38b,d,106].
in effector T-cells and prevent T-cell proliferation, respectively. The low persistence of transferred cells is exemplified by the low survival of MART-1 or gp100 TCR-T-cells infused into metastatic melanoma patients. T-cells had a median survival of 6.68 days, while infusions of IL-2 boosted median T-cell survival to 16.92 days.\textsuperscript{[44]}

5.2. Nanotechnology-Based Approaches to Improve Adoptive Cell Therapy

Nanotechnology can be applied to ACT to address manufacturing challenges, derive insights on T-cell homing to better inform ACT parameter selection, and reduce the immunosuppressive effects of the tumor microenvironment on T-cell activation, expansion, and cytotoxicity.

First, nanoparticles can improve manufacturing of T-cells for ACT. During ex vivo manufacturing, nanoparticles can mediate antigen presentation to DCs and subsequent T-cell activation. This principle is exemplified by Rosalia et al., who developed polymeric nanoparticles loaded with antigen that were efficiently processed by murine DCs.\textsuperscript{[43]} B16-OVA melanoma-bearing mice treated with CD8\textsuperscript{+} T-cells activated by polymeric-ovalbumin-loaded dendritic cells demonstrated tumor regression in 100% of mice, with 67% of mice remaining tumor free for the 47 day duration of the experiment. In contrast, CD8\textsuperscript{+} T-cells stimulated by soluble OVA merely delayed tumor growth in 92% of mice. These findings were associated with a fivefold greater persistence of polymeric-ovalbumin-induced T-cells compared to soluble ovalbumin T-cells 10 days after adoptive transfer, as well as greater numbers of IFN-\(\gamma\)-secreting CD8\textsuperscript{+} T-cells. However, this method relies on the presence of dendritic cells to expand T-cells, which adds an additional layer of complexity.

To bypass the need for dendritic cells, nanoparticles can serve as artificial APCs or carry cytokines that promote T-cell activation and proliferation ex vivo. For example, Fadel et al. created a carbon nanotube-polymer composite (CNP) that acted as an artificial APC capable of expanding both murine and human T-cells.\textsuperscript{[43]} To provide signals 1 and 2 of TCR activation, peptide-tethered polymeric nanoparticles were loaded with IL-2 to stimulate T-cell proliferation, while magnetite was integrated to enable facile separation of T-cells from carbon nanotube-polymer composites. Compared to commercially available magnetic beads, Dynabeads, and exogenous IL-2 (DYNA-IL-2), soluble tetramers that present antigens and exogenous IL-2 (TET+IL-2), CNPs mediated an \(\approx 200\)-fold expansion of murine CD8\textsuperscript{+} T-cells, which was \(\approx 2\) times greater than the expansion of cells by Dynabeads and \(\approx 4\) times that by tetramers. Notably, to expand the same number of murine T-cells as DYNA-IL-2 and TET+IL-2, CNPs used \(\approx 1000\)-fold less IL-2. CNP-expanded T-cells retained their functionality in vivo, as demonstrated by their ability to delay B16 melanoma growth. As demonstrated, nanoparticle artificial APCs can yield greater T-cell expansion compared to conventional culture methods, but more validation is required for human T-cells.

Alternatively, the use of nanoparticles may circumvent the entire process of ex vivo T-cell manufacturing. Smith et al. developed polymeric nanoparticles carrying plasmids of 194-1BBz CAR-T constructs against the CD19 antigen to target leukemia cells.\textsuperscript{[47]} Nanoparticles were functionalized with: a) anti-CD3e (Fab\textsubscript{2}) fragments to target T-cells, and b) peptides with microtubule-associated sequences and nuclear localization signals for nuclear transport. Upon injection of the nanoparticles into mice with B-cell acute lymphoblastic leukemia, peripheral T-cells were transfected and reprogrammed in situ to CAR T-cells. Transfected T-cells proliferated 5.5-fold and demonstrated a CD44\textsuperscript{+}CD62L\textsuperscript{+} memory phenotype. Moreover, tumors were eradicated in 70% of mice treated with the nanoparticles carrying the CAR-T construct, which was comparable to tumor eradication in 80% of mice receiving an infusion of 194-1BBz CAR-T-cells. Compared to ex vivo manufactured CAR T-cells, nanoparticles are less expensive, easier to produce, and can have a longer shelf-life and greater stability. Given that nanoparticle-mediated reprogramming of T-cells can offer comparable therapeutic efficacy, they represent a promising and practical clinical alternative.

Second, nanoparticles can mediate tracking of T-cell migration in vivo. One of the first studies to do so employed biocompatible, inert iron oxide nanoparticles that were derivatized with the HIV TAT peptide for enhanced entry into T-cells.\textsuperscript{[48]} Subsequently, these highly derivatized cross-linked iron oxide nanoparticles (CLIO-HD) were capable of quantitative, high-resolution magnetic resonance imaging of OVA-specific CD8\textsuperscript{+} T-cell homing to B16-OVA melanomas in mice. Cell viability remained high (>95%) after a simple 4 h incubation for labeling. CLIO-HD-labeled CD8\textsuperscript{+} T-cells were detected via MRI at a threshold of two cells per voxel in vitro, and \(\approx 3\) cells per voxel in vivo in mice. In dual tumor-bearing mice, MRI revealed that OVA-specific OT-1 CD8\textsuperscript{+} T-cells were recruited to the B16-OVA tumors on one flank, but not to control B16 tumors on the opposite flank. This result agreed with findings from the assessment of biodistribution of CLIO-HD\textsuperscript{[11]}In-oxine dual-labeled OT-1 CD8\textsuperscript{+} T-cells, which showed three times greater accumulation in OVA-expressing tumors. Cells were recruited to focal areas of the tumor, whereas other areas demonstrated little infiltration. Upon serial infusions of CLIO-HD-labeled OT-1 CD8\textsuperscript{+} T-cells, cells were recruited to different regions of the tumor. However, CLIO-HD-labeled OT-1 CD8\textsuperscript{+} T-cells were only detectable in the tumor for a maximum of 48 h. These spatial and temporal insights on T-cell recruitment can better inform selection of ACT parameters, including number and timing of infusions and number of cells to inject. Since this seminal study, others have evaluated the use of nanoparticles for T-cell tracking.

Third, nanoparticles can help T-cells overcome the immunosuppressive tumor microenvironment. Upon their arrival at the tumor, adoptively transferred cells are exposed to immunosuppressive signals from tumor cells, stromal cells, and other recruited immune cells. Accordingly, T-cell activation, proliferation, and cytotoxicity are compromised. To help overcome these barriers, nanoparticles have been employed to deliver drugs that precondition the tumor microenvironment to become more favorable for ACT, or to deliver cytokines that directly promote T-cell activation, expansion, and cytotoxicity. To re-program the tumor microenvironment to enhance conditions for ACT, Sasso et al. loaded the chemotherapeutic and
A. Gemcitabine Loaded Nanoparticles can Pre-Condition the Tumor Microenvironment to Increase ACT Efficacy

B. Immunoliposomes can Target T-cells In Vivo and Enable Repeated Expansion
immunomodulatory drug gemcitabine into lipid nanoparticle-bound T-cells to improve efficacy. In EG7-OVA lymphoma-bearing mice, treatment with 11 mg kg$^{-1}$ of gemcitabine-loaded nanoparticles prior to adoptive cell therapy significantly prolonged survival compared to no pretreatment ($p = 0.015$). In B16 melanoma-bearing mice, gemcitabine pretreatment and adoptive cell therapy enhanced survival compared to mice that were not treated ($p < 0.05$). Reproduced with permission. Copyright 2013, Elsevier B.V. B) Immunoliposomes can target and stimulate T-cells in vivo to facilitate their repeated expansion to combat B16F10 metastatic lung tumors in mice. Lung metastases were established in mice (D-8), followed by lymphodepletion (D-1) and intravenous injection of 12 $\times$ 10$^6$ luciferase-expressing pMel-1 CD8$^+$ T-cells and bioluminescence imaging (D-0). Mice received intravenous injections of immunoliposomes on D-0 and D-6. Bioluminescence imaging of T-cells revealed expansion of T-cells after immunoliposome injections. Reproduced with permission. Copyright 2011, Elsevier B.V.
bearing MC38 colorectal tumors or B16 melanomas, targeted delivery of R848 resulted in the recruitment of CD8+ T-cells to the tumor. Furthermore, α-PD-1 nanoparticle-mediated R848 delivery delayed tumor growth of MC38 colorectal tumors and enhanced survival relative to treatment with α-PD-1 antibodies + free R848 or α-PD-1 antibodies + non-targeted nanoparticles carrying R848. Accordingly, nanoparticles can target endogenous T-cells in vivo, which raises the possibility of converting immunologically "cold" tumors into "hot" ones, thereby, helping to break immune tolerance.

6. Nanoparticles Facilitate Immune Cell Tracking

Nanoparticles can enable monitoring of immune cells on a molecular, cellular, or systems level to yield various insights. Tracking immune cells can answer many basic questions regarding their mechanisms of action for various immunotherapies and facilitate their clinical translation. For instance, tracking immune cells can aid parameter optimization for adoptive cell therapies and vaccines. In ACT, imaging transferred cells can shed real-time insights on their survival, the temporal resolution of their migration, and their biodistribution. Meanwhile, for vaccine development, labeling immune cells such as dendritic cells or T-cells can provide information about the ability of the vaccine to induce antigen presentation, and subsequent T-cell proliferation, migration, and functionality. These findings should be considered when optimizing the cell/vaccine dosage, schedule, and route of administration.

6.1. Tracking on Molecular, Cellular, and Systems Levels

On a molecular level, nanotechnology can facilitate basic research on immune cell interactions. This is exemplified by Cai et al., who employed quantum dots to investigate ligand detection by T-cells. In a technique coined “synaptic contact mapping,” wide-field imaging was performed on quantum dots spaced across lipid bilayers, serving as a molecular ruler. This mapping was integrated with total internal reflection fluorescence detection of the T-cell receptor. Upon microvilli on T-cells engaging with quantum dots, there were “holes” in the bilayer, whereby decreases in quantum-dot fluorescence intensity correlated with T-cell receptor microclusters. Using synaptic contact mapping, Cai et al. found that after TCR recognition, there is stabilization of microvilli, as indicated by longer microvilli dwell times in immune synapses containing a cognate peptide-MHC. Meanwhile, in regions without ligand recognition, microvilli continued to search opposing surfaces. Synaptic contact mapping was also employed for characterization of microvilli on B-cells, macrophages, and dendritic cells; varying patterns for the immune cell types suggested that surveillance for antigens occurred differently. While this technique is still early in development, the investigation of immune synapse topology in real-time can help answer basic questions in immunology, from which immunotherapy development can be guided.

On a cellular level, nanoparticles can serve as labeling agents for microscopy and flow cytometry. For in vitro cellular tracking, immune cells, and in particular, macrophages, can internalize metallic nanoparticles of iron oxide or gold, which enables their visualization and differentiation from different populations of cells by transmission electron microscopy. Alternatively, inherently fluorescent nanoparticles or fluorophore-tagged nanoparticles can label immune cells for flow cytometry or confocal microscopy. Recently, a high-throughput, fluorophore label-free method involving single cell mass cytometry by time of flight was explored for enumerating gold nanoparticle uptake in macrophages, while simultaneously deriving multivariate cellular phenotyping data. In this approach, mass cytometry enabled detection of ~4.2 nanoparticles per cell, and was found to be 2400-fold more sensitive for detecting gold nanoparticle uptake in macrophages compared to flow cytometry. This superior sensitivity facilitated mass cytometry screening of three gold nanoparticles with different surface ligand compositions for vaccine delivery to DCs. Of the three formulations, an amphiphilic gold nanoparticle was identified to have the greatest uptake by myeloid DCs. Amphiphilic gold nanoparticle delivery of an antigen prompted sixfold proliferation of CD8+ T-cells relative to free antigen. Consequently, when mice challenged with B16F10 melanoma cells were administered the amphiphilic gold nanoparticle vaccine, they did not develop tumors, whereas mice injected with free peptide did. As demonstrated by Yang et al., new technologies can be harnessed for the development of nanoparticle therapeutics that are more efficacious than their macromolecular counterparts. However, basic research can also benefit: the propensity of various immune cells to take up nanoparticles in tandem with the multivariate phenotyping information provided by mass cytometry can yield rich information on the populations and frequencies of immune cells in circulation, in tumor tissue, and in adjacent healthy tissue. Therefore, nanoparticles can mediate immune cell tracking that can help to answer basic research questions and guide therapy development.

On a systems level, nanoparticles can serve as contrast agents for various imaging modalities, including MRI, CT, PET, and fluorescence imaging. See Table 7 for an overview of nanoparticle-mediated imaging of immune cells. Nanoparticles have enabled imaging of immune cells in the tumor microenvironment, in addition to deriving insights on the mechanisms of action of vaccines and the efficacy of ACT.

The ability of nanoparticles to in vivo label and image immune cells endogenous to the tumor microenvironment is exemplified by Daldrup-Link et al., who investigated the applicability of the FDA-approved iron oxide nanoparticle ferumoxytrol for MRI of TAMs in MMTV-PyMT mice that spontaneously developed mammary adenocarcinomas. In vitro uptake studies revealed that ferumoxytrol was preferentially internalized by TAMs: they had an ≈eightfold greater cellular iron content compared to tumor cells. As such, ferumoxytrol may serve as an indicator of TAM infiltration into the tumor. In vivo MR tumor imaging of ferumoxytrol demonstrated robust negative signal enhancement as early as 1 h post injection and signal enhancement persisted for 24 h. This signal contrast in the tumor was correlated to the presence of TAMs, as confirmed by confocal microscopy. Furthermore, when macrophages in mice were depleted by anti-colony stimulating factor 1-monoclonal antibody treatment, ferumoxytol injection resulted in reduced MR contrast compared to untreated controls, indicating the ability of ferumoxytol to detect TAM depletion. Since ferumoxytol is already an FDA-approved nanoparticle for anemia, it may be used “off-label” to qualitatively
detect TAMs and potentially stratify patients for TAM-targeted therapies. However, MRI remains an expensive imaging modality and the use of biomarkers that rely on MRI will be restricted to small subsets of patients. Furthermore, it is difficult to accurately quantify TAMs using T2 MRI, which is dependent on negative contrast. To address this issue of TAM quantification, PET can be employed. Pérez-Medina et al. radiolabeled two different high-density lipoprotein nanoparticles with $^{89}$Zr.[58] Upon injection, the nanotracers were internalized by macrophages in subcutaneous 4T1 breast tumor-bearing mice. PET revealed that 24 h postinjection, both nanotracers accumulated in the tumor, with high tumor uptake values of $16.7 \pm 1.6\%\text{ID g}^{-1}$ and $9.9 \pm 0.5\%\text{ID g}^{-1}$ (Figure 4). Histological analysis of tumor sections established the co-localization of $^{89}$Zr within macrophage-rich areas, demonstrating the feasibility of nanoparticle-mediated TAM imaging. While this method has great potential for TAM monitoring and quantification, more work is required to determine cutoffs for TAM levels that are clinically relevant for patient stratification of TAM-targeted therapies and treatment outcome prediction.

Imaging can help predict the efficacy of cancer vaccines, as demonstrated by Ferguson et al., who loaded DCs with iron oxide nanoparticles and evaluated homing of the DCs to lymph nodes via MRI.[59] DC uptake of iron oxide nanoparticles did not affect cell viability nor function at the concentrations evaluated. Subsequently, 48 h after injection of iron oxide nanoparticles into the upper hind limbs of naive mice, negative enhancement was observed in the draining lymph nodes due to migration of the DCs. Then, mice were primed twice with DCs loaded with tumor lysate, and MR revealed a significant $\approx$ ninefold increase in the size of the draining lymph nodes 30 h post injection relative to naive mice. This increase in lymph node size was specific to mice that received two rounds of DCs loaded with the same lysate, but not in mice that received two rounds of DCs loaded with different tumor lysates, suggestive of an antigen-specific immune response. Next, mice were challenged with the vaccine three times and draining lymph node enlargement was evaluated by MRI. After the third vaccine, a greater enlargement was visualized, whereas mice vaccinated only twice experienced a reduction in lymph node area to a size similar to the unprimed, contralateral lymph node. Accordingly, this feature of draining lymph node enlargement was suggested to be a predictor of vaccination booster success. Overall, iron oxide nanoparticles loaded into DCs can visualize DC migration via negative enhancement of lymph nodes; subsequently, an initial lymph node enlargement as visualized by MRI can suggest the development of an antigen-specific immune response, and continued lymph node enlargement upon administration of vaccine boosters can indicate the success of boosting. However, while negative enhancement can be quantified, the degree to which negative enhancement correlates to an immune response can be difficult to interpret. Investigations into clinical cutoffs and, most likely, complimentary information derived from other imaging modalities will be necessary for this approach to move forward. Nonetheless, this work

| Immune cell type                        | Nanoparticle                     | Imaging modality | Tumor model                               | Reference |
|----------------------------------------|----------------------------------|------------------|-------------------------------------------|-----------|
| Mouse splenocytes                       | SPIO                             | MRI              | S.C. E.G7-OVA lymphoma                    | [105]     |
| Mouse T-cell                            | Superparamagnetic iron oxide (SPIO)| MRI              | S.C. E.G7-OVA lymphoma                    | [106]     |
| Mouse T-cells                           | Maghemite nanoparticles          | MRI              | S.C. E.G7-OVA lymphoma                    | [109]     |
| Primary human MART-1 transduced T-cells| Gold nanoparticle                | CT               | S.C. SKMEL23 melanoma                     | [61]      |
| Human genetically modified natural killer cells | Endorem                       | MRI              | Orthotopic HER2/neu-positive NIH 3T3 breast tumor | [110]     |
| Human nature killer cells               | Quantum dots                     | Fluorescence imaging | MeWo metastatic melanoma                | [111]     |
| Mouse monocytes                         | USPIO                            | MRI              | Orthotopic BT048 BTIC brain tumor         | [112]     |
| Mouse macrophages                       | SPIO                             | MRI              | S.C. 4T1 mammary breast tumor             | [113]     |
| Mouse macrophages                       | Ferromagnetic iron-oxide nanocubes | MRI              | S.C. B16F10 melanoma                      | [114]     |
| Mouse tumor-associated macrophages      | Cross-linked iron oxide          | Fluorescence tomography | S.C. CT26 colon tumor                    | [115]     |
| Mouse tumor-associated macrophages      | USPIO                            | MRI              | MMTV-PyMT transgenic mice harboring mammary carcinomas | [57]      |
| Mouse tumor-associated macrophages      | Ferumoxytol                      | MRI              | Orthotopic 4T1 breast tumor               | [58]      |
| Mouse DCs                              | High density lipoprotein         | PET              | S.C. B16-OVA melanoma                     | [116]     |
| Mouse DCs                              | SPIO (endorem)                   | MRI              | Transgenic mouse model of breast cancer (MMTV-RAS) | [117]     |
| Mouse DCs                              | SPIO (endorem)                   | MRI              | S.C. E.G7-OVA lymphoma                    | [59]      |
| Mouse DCs                              | Iron oxide                       | MRI              | S.C. E.G7-OVA lymphoma                    | [59]      |
| Mouse DCs                              | Polymeric                        | Fluorescence imaging | S.C. E.G7-OVA lymphoma                | [59]      |
| Human DCs                              | SPIO                             | MRI              | S.C. Panc02 pancreatic tumor             | [118]     |
| Human DCs                              | SPIO                             | MRI              | Stage III melanoma patients              | [60]      |

SPIO, superparamagnetic iron oxide nanoparticle; MRI, magnetic resonance imaging; S.C., subcutaneous; CT, computed tomography. Mice were inoculated with tumors, unless other stated.

Table 7. Summary of nanoparticles that mediate imaging of various immune cell types.
Figure 4. Imaging of immune cells mediated by nanoparticles. A) High density lipoprotein nanoparticles were chelated to $^{89}\text{Zr}$, which labeled tumor-associated macrophages in vivo. In mice bearing subcutaneous 4T1 breast tumors, $^{89}\text{Zr}$-HDL nanotracers accumulated in tumors 24 h post-injection. Accumulation of $^{89}\text{Zr}$-HDL nanotracers co-localized to areas of the tumor rich in TAMs as corroborated by ex vivo hematoxylin and eosin staining, immunofluorescence for the macrophage markers, CD31 and IBA-1, and autoradiography of nanotracers. Scale bar = 2 mm. Reproduced with permission.[58] Copyright 2015, The Society of Nuclear Medicine and Molecular Imaging, Inc. B) Human melanoma-specific transduced T-cells labeled with gold nanoparticles facilitated in vivo microCT imaging of SKMEL23 melanoma-bearing mice. Maximum intensity projection of scans are shown for a mouse prior to 24, 48, and 72 h post intravenous injection of 16–20 × 10⁶ gold nanoparticle-labeled T-cells, in which circles indicate T-cell accumulation at the tumor. Reproduced with permission.[61] Copyright 2015, American Chemical Society.
demonstrates the versatility of nanoparticles in predicting the efficacy of vaccines.

In addition to cancer vaccines, ACT can also capitalize on nanoparticle-mediated imaging. To monitor the efficacy of cellular therapy, De Vries et al. labeled dendritic cells with either superparamagnetic iron oxide nanoparticles (SPIONs) or 111In-oxine. Then, guided by ultrasound, SPION and 111In-oxine-labeled cells were co-injected intranodally into melanoma patients. Compared to pre-DC injection imaging, the draining lymph nodes had reduced MR signal intensity 2 days after injection, indicative of migration of the SPION-labeled DCs from the injected lymph node to regional lymph nodes. However, due to the higher spatial resolution imparted by MRI, more re-injection, indicative of migration of the SPION-labeled DCs from the injected lymph node to regional lymph nodes. However, due to the higher spatial resolution imparted by MRI, more re-injection of the SPION-labeled DCs was detected in the perinodular fat and not actually in the lymph node. In these three patients, there was a lack of DC migration to draining lymph nodes. Accordingly, SPIONs can verify accurate delivery of immune cells in ACT and track their migration to predict treatment outcomes.

While MRI is the predominant modality explored for cellular tracking, other modalities are also under investigation. In particular, CT offers advantages over MRI due to its accessibility and lower cost. Meir et al. employed gold nanoparticles to label primary human T-cells transduced with the MART-1 melanoma antigen. Twenty-four hours after injection of gold nanoparticle-labeled MART-1 T-cells into SKMEL23 melanoma-bearing mice, there was strong CT signal in the tumor, suggestive of T-cell homing to the tumor (Figure 4). Tumor CT signal peaked at 48 h post T-cell injection; by 5 days, signal was no longer observed. Through a quantitative CT ruler, it was estimated that 1.3%, 2.3%, and 0.4% of the injected dose of cells were present at the tumor 24, 48, and 72 h post injection, respectively. Despite the low frequency of T-cells migrating to the tumor, the volumes of established tumors showed no significant growth 7 days post T-cell injection, whereas in mice treated with gold nanoparticle-labeled, non-targeted T-cells or gold nanoparticles alone, tumors continued to grow. Meanwhile, in mice injected with non-targeted T-cells, there was no increase in CT signal at any timepoint. Accordingly, gold nanoparticle-mediated tracking of T-cells suggested that targeted T-cells are necessary for tumor homing and that there is a low percentage of transferred T-cells that home to the tumor, which is in part attributed to T-cell migration to other organs such as the lungs. The proportion of T-cells migrating to the tumor correlated with control of tumor growth, which raises the possibility that increasing the number of injected T-cells or increasing the frequency of injections could facilitate tumor regression. Furthermore, the lack of CT signal 5 days post-injection suggests that there is low T-cell viability. Taken together, gold nanoparticle-mediated CT of T-cells can provide insights on the efficacy of ACT and shed light on optimization of ACT parameters.

Nanoparticles can enable imaging by two or more modalities; complementary information derived from multimodal imaging can offer more insights on the outcomes of immunotherapies. For example, Noh et al. developed a multifunctional nanoparticle that served as an antigen delivery system and bimodal imaging probe. The nanoparticle incorporated the fluorophore indocyanine green onto iron oxide nanoparticles for bimodal near-infrared fluorescence (NIR) and MR imaging. DCs treated with the nanoparticle were injected into the footpad of mice and their migration to the popliteal lymph nodes via lymphatic drainage was visualized by fluorescence imaging. Simultaneously, MR provided 3D, high-resolution imaging of the DCs within the lymph nodes, which revealed heterogeneous negative contrast enhancement. Ex vivo NIR analysis verified these in vivo findings. Therefore, nanoparticles can mediate multimodal imaging of immune cells, and can serve as useful tools to corroborate imaging results for preclinical investigations.

6.2. Challenges for Nanoparticle-Mediated Imaging of Immunotherapies

While great efforts have been invested in tracking immune cells with nanoparticles, there are numerous challenges that remain to be overcome. Common to all contrast agents used to label immune cells, ex vivo labeling of immune cells by nanoparticles is hampered by dilution of the probe as immune cells proliferate. Meanwhile, in vivo labeling of endogenous immune cells is confronted by lackluster specificity of the nanoparticle in exclusively targeting the population of interest. These issues pose difficulties for longitudinal immune cell tracking studies, which are important for preclinical evaluation of various immunotherapies. Furthermore, these issues make it challenging to derive quantitative clinical assessments for treatment stratification and response monitoring. As such, more work is necessary to address these problems and to further the field of immune cell tracking.

7. Combination Therapy

To augment antitumoral effects, immunotherapies have been combined with other therapies, including chemotherapy, phototherapy, radiotherapy. Given the advantages of nanoparticles in tumor accumulation and drug delivery, nanoparticles can serve as drug delivery platforms for chemotherapy, phototherapy, and radiotherapy. In combination with immunotherapies, nanoparticle-based sensitizers for radiotherapy or phototherapies can augment antitumoral immunity. Moreover, nanoparticle-based immunotherapies can complement immune checkpoint therapy to strengthen antitumoral immunity. Additionally, nanoparticles can modulate the tumor microenvironment to improve the efficacy of immunotherapies. Lastly, nanoparticles can mediate novel therapies that complement immunotherapies.

7.1. Combination Immunotherapies Potentiate Antitumoral Immune Responses

Nanoparticle-mediated vaccines have been combined with immune checkpoint inhibitors to strengthen immune responses against tumors. In one such example, Kuai et al. designed high density lipoprotein-mimicking nanodiscs that co-delivered antigen and adjuvant (sHDL-Ag/CpG); in combination with anti-PD-1 and anti-CTLA-4 therapy, nanodiscs eradicated MC-38 colon
tumors and B16F10 melanomas.\(^{[63]}\) Immunization with sHDL-Ag/CpG in immunocompetent C57Bl/6 mice prompted the induction of >tenfold greater Ag-specific CD8\(^+\) T-cells, relative to treatment with Ag + CpG + clinical adjuvant, Montanide. This greater immune response presumably led to complete regression of tumors in 88% of mice treated with s-HDL-Ag/CpG in combination with anti-PD-1, whereas free Ag + free CpG in combination with anti-PD-1 resulted in complete remission of only 25% of mice. Furthermore, Kuai et al. demonstrated that a broader spectrum of T-cell responses could be elicited with a cocktail of nanodiscs carrying a mixture of antigens. To treat B16F10 melanomas in mice, two B16F10 melanoma neo-epitopes and one melanoma-associated antigen were loaded into nanodiscs, alongside an adjuvant, CpG. Compared to free multi-Ags + CpG + Montanide, vaccination with s-HDL/multiAgs/CpG inhibited tumor growth. Combination treatment with the vaccine + anti-PD-1 + anti-CTLA-4 led to complete regression of tumors in 90% of B16F10 melanoma bearing mice, whereas soluble multiAgs + CpG + anti-PD-1 + anti-CTLA-4 elicited tumor regression in ≈38% of mice. Accordingly, nanoparticle-based vaccines can induce an immune response against tumors, which can then be potentiated with immune checkpoint therapy.

This strategy of combination therapy with a nanoparticle-based vaccine and an immune checkpoint blockade was further explored by Zhu et al. who developed nanovaccines that self-assembled in vivo: albumin/AlbiVax nanocomplexes.\(^{[64]}\) These nanovaccines comprised antigen (i.e., CpG, Trp2) conjugated by two HEG linkers to maleimide-functionalized Evans Blue (MEB), which has a binding site for endogenous albumin that can act as a natural carrier. Upon injection of MEB-CpG into FVB mice, efficient trafficking of the nanovaccine to the lymph nodes occurred, as evidenced by gamma counting of \(^{64}\)Cu-labeled nanovaccine. Three days after injection into FVB mice, MEB-CpG had 67.4 ± 9.3% injection dose per gram (ID g\(^{-1}\)) of tissue in the draining, inguinal lymph node, which was ≈eightfold greater than free CpG (8.3 ± 4.7% ID g\(^{-1}\)). Vaccines were developed against the neoantigen Adpgk, for treatment of mice bearing subcutaneous MC38 colon tumors. Vaccination with AlbiVax, consisting of Albi-CpG and AlbiAdpgk, resulted in regression of tumors in 12.5% of mice, whereas combination treatment of AlbiVax + anti-PD-1 led to tumor regression in 60% of mice. Furthermore, tumor-free mice overcame a secondary challenge with MC38 cells, indicating the development of immune memory. AlbiVax was capable of efficient trafficking to the lymph nodes, and induction of robust immune responses; in combination with anti-PD-1 which could prevent exhaustion of CTL responses, robust antitumoral immunity was elicited. While this intriguing approach capitalizes on the excellent safety profile of Evans Blue, and circumvents issues associated with safety, scale-up, and quality control of nanomaterials, work remains to determine kinetics of endogenous vaccine formation, dosage, and clearance.

7.2. Nanoparticle-Based Therapies Can Induce the Abscopal Effect

Nanoparticles can serve as platforms for radiotherapy and PDT. When combined with immunotherapies, induction of the abscopal effect can occur, which can mediate regression of untreated distant tumors or metastases. Upon irradiation of a primary tumor, dying tumor cells release tumor-associated antigens (TAA) which are phagocytosed by APCs. Subsequently, APCs traffic to the lymph nodes and prime CD8\(^+\) T-cells to recognize and destroy the tumor cells in both primary and distant tumors.\(^{[65]}\) However, this abscopal effect is a rare phenomenon in the clinic, with only 46 identified cases induced by radiation therapy between 1969 and 2014.\(^{[66]}\) By combining radiotherapy with immunotherapies, the occurrences of the abscopal effect can be boosted.\(^{[65]}\)

This concept is illustrated preclinically by Ni et al. who employed a nanoparticle radiosensitizer (Hf\(_{12}\)-DBA) that in combination with anti-PD-L1 treatment, elicited tumor shrinkage in primary, treated tumors, as well as distant, non-irradiated tumors.\(^{[67]}\) Hafnium, an electron dense element, served as the basis of building units for nanoscale metal organic framework nanoparticles, as it could absorb X-rays preferentially over tissues; the porous structures of the nMOF enabled diffusion of generated reactive oxygen species. Hf\(_{12}\)-DBA out-performed HfO\(_2\) nanoparticles, which have been investigated in clinical trials as a radiosensitizer, in radio-enhancing effects in various tumor cell lines. In combination with anti-PD-L1 treatment, Hf\(_{12}\)-DBA-mediated radiotherapy induced complete primary tumor regression in mice bearing dual subcutaneous colorectal CT26 tumors, while also shrinking distant, non-irradiated tumors. In contrast, anti-PD-L1 treatment and radiotherapy monotherapies merely delayed tumor growth of both primary and distant tumors. This greater immune response was mediated in part by the greater proliferation of antigen-specific specific IFN-\(\gamma\) producing T-cells in CT26 colon tumor bearing mice treated with combination radiotherapy and immune checkpoint blockade, compared to monotherapy treated mice. As such, the abscopal effect was activated, and potentiated by combination radiotherapy and immune checkpoint therapy.

In addition to radiotherapy, phototherapies can elicit the abscopal effect. Phototherapies involve the administration of a photosensitizer, which accumulates at the tumor. Upon laser irradiation, the photosensitizer can generate reactive oxygen species (“photodynamic therapy; PDT”) or heat (“photothermal therapy; PTT”) to ablate tumor cells. As demonstrated by Chen et al., PTT enabled by nanoparticle-based photosensitizers activated the immune system, and in combination with anti-CTLA-4 treatment, reduced the number of metastases in 4T1 breast tumor bearing mice.\(^{[68]}\) Chen co-encapsulated a photosensitizer, indocyanine green, with TLR-7 agonist, imiquimod, into polymeric nanoparticles (PLGA-ICG-R837). After photothermal therapy, PLGA-ICG-R837 treated and laser irradiated mice induced greater maturation of dendritic cells and increased secretion of IL-12p70, IL-6, and TNF-\(\alpha\) in sera, relative to control mice with surgically resected tumors and treatment with PLGA-ICG-R837. In two different tumor models, subcutaneous 4T1 breast or CT26 colorectal tumor bearing mice, PLGA-ICG-R837-mediated PTT inhibited secondary tumor growth, whereas control mice had growth of secondary tumors. In a 4T1 breast tumor model with lung metastases, PLGA-ICG-R837-mediated PTT prevented metastases in 70% of mice, whereas mice with surgery alone, or surgery + anti-CTLA-4 treatment, or PLGA-ICG-R837-mediated PTT alone, developed metastases and succumbed to disease. Accordingly,
combination treatment of nanoparticle-mediated photothermal therapy and immune checkpoint therapy can induce the abscopal effect, reduce tumor burden, and improve survival in various preclinical models.

In a novel strategy to enhance the abscopal effect, Min et al. created antigen-capturing nanoparticles that promoted the antitumoral effects of immune checkpoint therapy. Upon incubation of polymeric nanoparticles with lethally irradiated B16F10 melanoma cell lysates, polymeric nanoparticles successfully captured various neo-antigens and danger associated molecular patterns. In mice bearing bilateral B16F10 melanomas, treatment with anti-PD-1 + radiotherapy + polymeric antigen-capturing nanoparticles yielded a 20% complete response rate. Of the surviving mice, 100% resisted tumor re-challenge 3 months later, suggesting the development of immune memory. In contrast, mice that received radiotherapy + anti-PD-1 blockade had primary and secondary tumors that continued to grow, and they succumbed to disease within 40 days. Evidently, antigen-capturing nanoparticles were critical for enhancing the abscopal effect and improving survival. Underlying these findings is the efficient trafficking of polymeric antigen-capturing nanoparticles in tumor-draining lymph nodes, uptake by DCs, macrophages, and B-cells, and subsequent priming of CD8+ T-cells. As such, nanotechnology can mediate new approaches to complement radiotherapy and immunotherapy, and augment the abscopal effect.

7.3. Nanoparticles as a Single Platform for Dual Therapies to Potentiate the Immune Response

Nanoparticles can serve as a platform to mediate dual therapy, and in combination with an immunotherapy, can further augment antitumoral immunity. This concept is exemplified by He et al., who developed a core-shell nanoscale coordination polymer (NCP@pyrolipid). This polymer delivered a photosensitizer, pyrolipid, originally incorporated into porphysomes, and a chemotherapeutic drug, oxaliplatin for dual PDT and chemotherapy. To evaluate whether immunogenic cell death occurs after PDT, CT26 colorectal cells were incubated with NCP@pyrolipid, and then injected into immunocompetent BALB/c mice. This vaccination prompted the rejection of CT26 tumor challenge, suggestive that PDT with NCP@pyrolipid induced immunogenic cell death in CT26 cells, and subsequently elicited an immune response against tumor cells. In combination with anti-PD-L1 treatment, PDT in mice bearing subcutaneous, dual MC38, or CT26 tumors induced regression of primary and distant tumors (Figure 5A). In contrast, mice treated with a combination of free oxaliplatin + anti-PD-L1 antibodies + pyrolipid liposomes or monotherapies experienced delayed tumor growth of primary and distant tumors. As such, nanoparticles can mediate dual therapies and can be combined with immunotherapies to yield stronger immune responses.

In addition to serving as a platform for dual therapies, nanoparticles can modulate the tumor microenvironment and further boost antitumoral immunity. To do so, Yang et al. devised a hollow mesoporous manganese dioxide nanoparticle (H-MnO₂) that alleviated hypoxia within the tumor. H-MnO₂ were loaded with a photosensitizer, chlorin e6, and a chemotherapeutic drug, doxorubicin. Upon accumulation in the tumor, the acidic pH of the tumor microenvironment triggered the decomposition of MnO₂ to Mn²⁺ and a reaction with H₂O₂ to form water and oxygen, thereby reducing hypoxia and releasing chlorin e6 and doxorubicin. Meanwhile, the incorporation of Mn and chlorin e6 enabled MRI and fluorescence imaging, respectively. Both MRI and fluorescence imaging showed that intravenously injected H-MnO₂ into 4T1 breast tumor-bearing mice resulted in tumor accumulation. Although dual combination PDT and chemotherapy with H-MnO₂ exerted synergistic antitumoral effects, tumors continued to grow, but at a delayed rate compared to controls. Notably, tumors treated with H-MnO₂ were ≈twofold smaller than tumors treated with a non-hypoxia modulating control, consisting of tumor irradiation of mice injected with silicon dioxide nanoparticles loaded with chlorin e6 and doxorubicin. Moreover, semi-quantitative analysis of tumor slices stained for hypoxia revealed that H-MnO₂ significantly reduced tumor hypoxia, in contrast to their silicon dioxide nanoparticle counterparts, which had large regions of hypoxia within tumors. This decrease in tumor hypoxia mediated by H-MnO₂ was associated with ≈tenfold greater macrophage infiltration in the tumor in H-MnO₂ treated mice compared to untreated controls, and a ≈fourfold reduction in anti-inflammatory M2-like macrophages in H-MnO₂ treated mice relative to untreated mice. In mice bearing dual subcutaneous bilateral 4T1 tumors, the triple combination of PDT, chemotherapy, and anti-PD-L1 treatment resulted in delayed tumor growth in primary, irradiated and distant, non-irradiated tumors relative to controls (Figure 5B). The finding was correlated to a ≈fivefold greater infiltration of CD8+ T-cells for triple combination treated mice compared to untreated mice. These findings suggest that modulation of hypoxia by H-MnO₂ can alleviate immunosuppression of the tumor microenvironment.

7.4. Challenges for Combination Therapies

Given the multi-factorial nature of tumors, combination therapies are needed to treat tumors; however, this approach has many challenges and questions that remain to be addressed. First, there needs to be strong rationale for which therapies to combine. To determine optimal combinations preclinically, the combination index can be calculated to determine if there are additive or synergistic therapeutic effects. This may help identify and propel combinations that yield synergistic therapeutic effects into clinical trials. Second, consideration needs to be given to spatiotemporal factors of administration of combination therapies. Questions regarding the half-lives of monotherapies, their accumulation at the tumor (or lack of), and their kinetics of tumor accumulation should be examined. In this regard, nanotechnology can assist: nanoparticles can combine multiple therapies on a single platform, to facilitate accumulation and co-localization within the tumor. Third, consideration for spatiotemporal factors will give rise to the need for optimization of dosing and scheduling of treatments for combination therapies. This optimization can be facilitated by the multimodal imaging capabilities of nanoparticles: complimentary information derived from different imaging modalities can shed insights on the accumulation and retention of nanoparticle-based...
A. Dual Therapy Nanoparticles in Combination with Immunotherapies can Induce the Abscopal Effect

B. Nanoparticles can Modulate the Tumor Microenvironment to Potentiate the Efficacy of Immunotherapies
radiosensitizers, photosensitizers, or immunotherapies. Alternatively, nanoparticles can be employed to label and image immune cells involved in immune responses. Nanotechnology has the potential to help address challenges associated with combination therapies, through multiple means: via a nanoparticle platform that can combine therapies, or optimization of dosage and scheduling parameters, or the investigation of underlying therapeutic mechanisms.

8. Perspective

While there are many exciting applications for nanotechnology in cancer immunotherapies, there are lessons from the more mature field of cancer nanomedicine that can be applied to immunotherapies to ease clinical translation. In cancer nanomedicine, there is an overwhelming number of “all-in-one” nanoparticles, in which multiple components are combined into one platform to achieve multifunctionality. Although multimodal imaging and combination therapies are possible with these platforms, they are difficult to translate into the clinic given difficulties in scale-up, reproducibility, and regulatory guidelines for the many components that need to undergo testing prior to FDA approval. As such, many of these “all-in-one” nanoparticles do not proceed past preclinical testing in animal models. Additionally, while these nanoparticles may have multiple functions, they are often not evaluated for one function against the gold standard clinical agent. Therefore, it is unknown if these all-in-one nanoparticles hold advantages over their clinical, gold standard counterparts. These issues associated with cancer nanomedicine may arise in applications of nanotechnology to oncoimmunology.

Similar to cancer nanomedicine, there is a trend in the development of multifunctional theranostic nanoparticles that combine immunotherapy with chemotherapy or phototherapy, alongside imaging capabilities. For instance, for nanovaccines, PUBMED search terms of “nanoparticle cancer vaccine” resulted in 46 total hits by 2008, but 681 total hits as of 2018. Despite the surge in development of nanovaccines, to date, no nanoparticle cancer vaccine has been translated into the clinic. This lack, can be attributed in part, to the current trend to outfit the nanovaccine with multifunctional capabilities, such as chemotherapy, phototherapy, multimodal imaging capabilities, specific targeting of immune cell populations, and stimuli-responsiveness. While intriguing, we can lose sight of the core goal—developing an efficacious vaccine. Nanotechnology has inherent strengths (i.e., large surface area for epitope presentation, protection of adventitious and antigen, etc.) that can be capitalized upon for this purpose. Therefore, when designing nanoparticles for applications in cancer immunotherapies, it can be sufficient to have a simple platform. This simplicity would enable nanovaccines to stand up to the test of reproducibility and to ease clinical translation.

Another challenge arising from multifunctional nanoparticle platforms lies in designing experiments with the appropriate controls. Increasingly so, given the spike in the development of nanotherapeutics for oncoimmunology, it will be important to compare the efficacy of different platforms with each other. These studies will shed light on the optimal nanoparticle platform and characteristics that are best suited for each purpose. Furthermore, as much as possible, nanoparticles should be compared against their clinical counterparts. Should nanoparticle platforms elicit comparable or better results compared to their clinical counterparts, nanotherapeutics will gain further legitimacy for clinical translation.

Other experimental design considerations include the selection of animal models to test in vivo efficacy. Many immunotherapies are evaluated in syngeneic mouse models, in which mice-derived tumor cells are implanted into mice. Therapies that are under evaluation, including antibodies, vaccines, and ACT, need to be mouse-specific to target mouse immune cells. However, intrinsic differences between the mouse and human immune systems may result in discrepancies between promising preclinical results in mouse models and negative findings in clinical trials. Many adoptive cell therapy studies employ immunodeficient mice transplanted with human-derived tumor cells. These models do not fully recapitulate interactions between human-derived adoptively transferred cells with innate and adaptive immune cells, as immunodeficient mice lack B-cells, NK cells, or Tregs. To overcome this limitation, immunodeficient mouse models can be “humanized,” in which human hematopoietic progenitor cells are transplanted into mice, which differentiate into immune subsets. Given the differences in immune systems between preclinical mouse models and humans, it would be ideal to utilize humanized mouse models; however, they are expensive and dependent on access to human samples. The development of animal models that better recapitulate tumor physiology and the human immune system is ongoing. In the meantime, conscientious selection of animal models coupled with an understanding of the weaknesses inherent to existing animal models will enable us to better evaluate the efficacy and safety of a nano-therapeutic platform.

Figure 5. Applications of nanotechnology in combination therapies to enhance antitumoral immunity. A) Core-shell nanoscale coordination polymers enable photodynamic therapy and chemotherapy via incorporation of pyrrolipid and oxaliplatin, on a single platform (NCP@pyrolipid). In combination with anti-PD-L1 treatment, NCP@pyrolipid induced the abscopal effect. Dual subcutaneous MC38 colorectal tumor-bearing mice were intraperitoneally injected with PBS, porphysome, oxaliplatin + porphysome, or NCP@pyrolipid and then tumors were irradiated (670 nm, 180 J cm$^{-2}$, 100 mW cm$^{-2}$) 24 h post injection. Immediately after irradiation, anti-PD-L1 was intraperitoneally injected at a dose of 50 μg per mouse. Injections and irradiation were performed every 3 days, for three treatments total. Shown are tumor volume growth curves of the irradiated primary tumor (left) and the non-irradiated, distant tumor (right). Black arrows indicate drug administration, whereas red arrows indicate light irradiation. (+) and (−) denotes with and without light irradiation. Adapted under the terms of Creative Commons license CC BY 4.0. Copyright 2016, The Authors. B) Hollow MnO$_2$ nanoparticles alleviate tumor hypoxia, and in combination with anti-PD-L1 treatment, can potentiate antitumoral immunity. Dual subcutaneous 4T1 breast tumor bearing mice were injected with either PBS, hollow mesoporous nanoparticle (H-MnO$_2$-PEG/C&D), H-MnO$_2$-PEG/C&D + anti-PD-L1 treatment, H-MnO$_2$-PEG/C&D + light irradiation (L), or H-MnO$_2$-PEG/C&D + light irradiation (L) + anti-PD-L1 treatment. Primary mice tumors were irradiated with 660 nm light at 5 mW cm$^{-2}$ for 1 h. PD-L1 blockade was administered intravenously at 750 μg kg$^{-1}$ at days 1, 3, 5, and 7. Tumor volume growth curves of the irradiated, primary tumor (left panel) and the non-irradiated, distant tumor (right panel) are shown. Adapted under the terms of Creative Commons license CC BY 4.0. Copyright 2017, The Authors.
Figure 6. Applications of nanotechnology to cancer immunotherapies.

- **Cancer vaccines**: Nanoparticles can deliver antigen and/or adjuvants to dendritic cells; Immunogenic nanoparticles can serve as adjuvants.

- **Cell tracking**: Nanoparticles can mediate immune cell-tracking & enable monitoring of immunotherapy efficacy.

- **Immune checkpoint blockade**: Nanoparticles can deliver 1+ immune checkpoint molecule drugs & improve tumor accumulation.

- **Combination therapies**: Nanoparticles can mediate combination therapies on one platform and potentiate the immune response.

- **Adoptive cell therapy**: Nanoparticles can improve manufacturing of T-cells for adoptive cell therapies & enhance T-cell cytotoxicity.
and prevent premature enthusiasm for its application to humans. Overall, the careful consideration of experimental design, animal models, and incorporation of better controls will facilitate the advancement of nanotherapeutics into the clinic.

While there are many challenges to be addressed, the flurry of research in nanoparticle platforms for immunotherapies gives rise to many exciting advances to look forward to. In particular, through specific targeting of immune cells, nanotechnology can facilitate the development of safer immunotherapies, which in turn could reduce the doses of corticosteroids and immunosuppressants given to patients to manage autoimmunity and improve the quality of life of these patients. [2] Furthermore, nanotechnology can enable image-guided immunotherapies to monitor efficacy in real-time. This monitoring may facilitate tailoring of therapy dosage and scheduling to each patient, as well as prediction of patient response to therapy and prognosis. Increasingly, there is a trend toward combining multiple therapies to better address the multi-faceted aspects of a tumor and to elicit improved antitumoral efficacy. Nanoparticle-based immunotherapies have been combined with more traditional pillars of cancer treatment like radiotherapy, and we may also see the rise of combinations of two nanoparticle-based immunotherapies. However, great care needs to be taken in the rational selection of therapies to combine and to yield synergistic efficacy against tumors.

While applying nanotechnology to cancer immunotherapies is a recent development, there has already been innovative work in improving the efficacy of various immunotherapies including immune checkpoint inhibitors and cancer vaccines, as summarized in Figure 6. Furthermore, nanotechnology can mediate different technological approaches that enhance the generation of T-cells for adoptive cell therapies. In addition, nanoparticle-mediated tracking of immune cells has yielded insights on both preclinical and clinical efficacy of various immunotherapies. To avoid preclinical and clinical inefficacy of various immunotherapies. To this aim, nanoparticle-mediated multimodal imaging of immune cells can provide further complementary information. While challenges remain, nanotechnology can accelerate the development and clinical translation of immunotherapies. In this manner, nanotechnology may contribute to the enhanced prognosis and survival of individuals with cancer.

9. Summary

The immune system can be harnessed by immunotherapies to treat various cancers. Immunotherapies represent a promising avenue of cancer treatments, since they can improve the prognosis and survival of patients with metastatic cancers. However, they induce long-term remission in only a fraction of patients. Nanoparticles may help to improve efficacy of various immunotherapies and increase the number of patients who achieve long-term remission.

For immune checkpoint blockade, nanotechnology can be applied to improve tumor accumulation of immune checkpoint blockade antibodies and to co-deliver two different immune checkpoint antibodies, thereby improving efficacy. Furthermore, nanoparticles conjugated to immune checkpoint blockade antibodies can enable imaging to monitor therapeutic response of therapy, and stratify responders from non-responders. Additionally, nanotechnology can underlie novel delivery methods of immune checkpoint blockade inhibitors, such as the microneedle patch technique.

In the area of cancer vaccines, nanotechnology can be employed to deliver tumor antigens and/or adjuvant, and protect their cargo from degradation in vivo. Alternatively, some nanoparticles are inherently immunogenic, and can act as an adjuvant. Furthermore, nanoparticle-mediated imaging can enable tracking of vaccines. Upon uptake of DCs, pH-sensitive nanoparticle vaccines can promote cross-presentation to elicit a robust immune response. Nanoparticles can be the basis of biomimetic vaccines that can improve immunogenicity.

For adoptive cell therapies, nanoparticles can be applied to T-cell manufacturing: nanoparticles can serve as artificial antigen-presenting cells, and deliver cytokines to T-cells to promote T-cell activation and proliferation ex vivo and in vivo. Additionally, nanoparticles can re-program T-cells in vivo to generate CAR-T cells in situ. Nanoparticles can mediate tracking of T-cell migration in vivo. Through nanoparticle-mediated modulation of the tumor microenvironment, nanoparticles can help T-cells overcome immunosuppression.

Nanoparticles can serve as labelling and contrast agents for various imaging modalities to shed insights on immune cell trafficking and mechanisms of action for various immunotherapies to facilitate their clinical translation. By far the most work has been done with iron oxide nanoparticles, which can mediate magnetic resonance imaging of tumor-associated macrophages, and dendritic cells. Positron emission tomography and CT have also been employed to image immune cells. Nanoparticles can enable imaging by two or more imaging modalities.

Nanoparticles can serve as platforms for multiple therapies, including chemotherapy, phototherapy, radiotherapy, and immunotherapies. In combination with immunotherapies, nanoparticles that mediate phototherapy, radiotherapy, or chemotherapy can potentiate anti-tumoral immunity, and induce the abscopal effect. Alternatively, nanoparticles can serve as a single platform that combines immunotherapy with another therapy to enhance the immune response.

Despite several remaining challenges, nanotechnology can accelerate the development and clinical translation of immunotherapies.

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Conflict of Interest

The authors declare no conflict of interest.
