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Nanomedicine approaches to improve cancer immunotherapy

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Abstract

Significant advances have been made in the field of cancer immunotherapy by orchestrating the body's immune system to eradicate cancer cells. However, safety and efficacy concerns stemming from the systemic delivery of immunomodulatory compounds limits cancer immunotherapies expansion and application. In this context, nanotechnology presents a number of advantages, such as targeted delivery to immune cells, enhanced clinical outcomes, and reduced adverse events, which may aid in the delivery of cancer vaccines and immunomodulatory agents. With this in mind, a diverse range of nanomaterials with different physicochemical characteristics have been developed to stimulate the immune system and battle cancer. In this review, we will focus on some recent developments and the potential advantages of utilizing nanotechnology within the field of cancer immunotherapy.

Graphical abstract

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INTRODUCTION

Extensive research has revealed the integral relationship between immunity and cancer to provide new immunotherapeutic approaches that effectively treat tumors (Fig. 1). Indeed, the clinical successes of new immunotherapies, such as monoclonal antibodies (mAb), adoptive T cell transfer, cancer vaccination, oncolytic virus therapy and immune checkpoint inhibitors, are encouraging (Table 1). The rise of these new immunotherapies represents an important inflection point in the history of cancer treatment where the body's own self-defense system is relied upon to fight disease¹. Yet, clinical challenges still remain. For example, cancer immunoediting allows tumors to evade immune surveillance via downregulation of tumor associated antigens (TAAs), major histocompatibility complexes (MHCs) or co-stimulatory molecules (Fig. 2). Thus, current research has focused on re-awakening the immune system to attack aberrant cancer cells with potent cytokines, cancer vaccines, antibodies, and immune stimulating adjuvants. However, these therapies can produce significant side effects from systemic dosing and display poor pharmacokinetic profiles *in vivo*.

Nanoparticle (NP) delivery of these immunomodulatory compounds provides a possible solution to these challenges. Nanotechnology is a relatively new area of interdisciplinary science that has made a rapid and broad impact on healthcare. NP therapeutics vary widely in structure and function from carriers of small molecule drugs or biomacromolecules, such as proteins or small interfering RNA (siRNA), to vehicles for imaging and thermal absorption. To date, a variety of NP structures have been used as vehicles to deliver a wide spectrum of molecular cargos, stabilize their cargoes' biological activity, increase cargoes' solubility in biological fluids, and reduce systemic side effects. Indeed, several NP-based formulations delivering cytotoxic drugs have proven successful in the clinic². Thus, NPs provide ideal immunotherapy delivering candidates to overcome the associated challenges. Herein, we will review the current status of NP-based immunotherapeutic strategies for cancer treatment and show the broad range of immunological applications.

NANOTECHNOLOGIES IN CANCER IMMUNOTHERAPY

Activation of dendritic cells (DCs) through NPs

Macrophages, DCs, and B cells represent the immune system's three subsets of antigen-presenting cells (APCs). These cells mediate the adaptive immune response responsible for antitumor activity. APCs function by actively phagocytosing their environment for antigens and damage associated molecular patterns (DAMPs). Upon receiving stimulation, APCs present peptide antigens on MHCs to other immune cells inducing downstream effector cytokine secretion (CD4⁺ T cell) and cytotoxic T lymphocyte (CTL) responses (CD8⁺ T cell). Because of APC's key role in promoting these responses, significant efforts have focused on targeting APCs with NPs containing immunomodulating agents.

DCs are the immune system's professional APCs capable of promulgating a host of antigen specific immune responses against pathogens. Thus, immunotherapeutic strategies utilize DCs to present antigens as a means of cell-mediated therapeutic vaccination in individuals with advanced malignancies³. In this strategy, DCs are trained *ex vivo* with antigens then adoptively transferred back into patients for vaccination. Despite demonstrating an increase in antigen-specific CTL responses after immunization at metastatic tumor sites, this method still lacks examples of clinical therapeutic effectiveness in many advanced tumors³. Furthermore, this strategy can prove technically challenging and expensive⁴⁻⁷. Therefore, *in situ* DC targeting with antigen and adjuvant laden NPs loaded with antigens and adjuvants may greatly improve the clinical applications of DC mediated immunotherapies⁸.

Delivery of antigen

Loading antigens in NPs offers distinct advantages over soluble formulations. First, NPs can protect antigens from proteolytic degradation and deliver them to DCs in a targeted and prolonged manner. Furthermore, NPs restrict the entry of encapsulated antigens and adjuvants to the systemic circulation thereby increasing the localized dosages to resident immune cells and reducing toxicity. Even more importantly, DCs cross-present particulate antigens more efficiently than soluble antigens⁹. Cross-presentation potently stimulates CTLs and promotes cytotoxic anti-tumor immunity. Therefore, to improve DC mediated immunotherapy, numerous antigens and immunostimulatory compounds have been formulated in NP vehicles to target DCs *in vivo*.

For example, Gao *et al.* developed carboxymethyl chitosan/chitosan NPs (CMCS/CS-NPs) loaded with extracellular products (ECPs) of *Vibrio anguillarum*. When compared with ECPs alone, CMCS/CS-NPs significantly enhanced both adaptive and innate immune responses¹⁰. Likewise, Maji *et al.* investigated the role of cationic liposomes on the maturation and antigen presentation capacity of DCs¹¹. They found that cationic liposomes were taken up more efficiently by DCs and transported to different cellular sites for MHC processing than anionic liposomes and neutral liposomes. Once loaded with rgp63 antigen, these liposomes led to efficient APC presentation of antigen to CD4⁺ and CD8⁺ T cells compared to soluble rgp63. Rietscher *et al.* evaluated the use of hydrophilic polyethyleneglycol (PEG)-b-PAGE-b-poly(lactic-co-glycolic acid) (PPP) as a platform for prophylactic vaccination¹². When PPPs were loaded with a model ovalbumin (OVA)

antigen, they found that T cell activation by APCs significantly increased *in vitro* compared to delivering the free and soluble OVA antigen.

Delivery of adjuvant

In immunology, vaccine adjuvants potentiate immune responses to a particular antigen. Adjuvants mimic specific sets of pathogenically conserved molecules, known as pathogen associated molecular patterns (PAMPs). These immune stimulating compounds include lipopolysaccharides (LPS), common components of bacterial cell walls (e.g. mannose), and nucleic acids located in abnormal locations¹³. Because the immune system has evolved to recognize these moieties, the presence of an adjuvant in conjunction with antigens can greatly boost the activities of DCs, lymphocytes, and macrophages. However, these immunostimulators can also lead to unintended side effects, such as toxic shock syndrome, when given systemically^{14, 15}. Delivery of chemotherapies via NP systems have greatly reduced the toxicity profiles of several drugs, such as doxorubicin and amphotericin B, by promoting tissue specific targeting and lower dosages¹⁶. Likewise, adjuvant delivery via NP vehicles may address the aforementioned adjuvant toxicity concerns and benefit the field of immunotherapy¹⁷.

As an exemplary adjuvant, CpG oligonucleotides are short, single-stranded DNA molecules that potently stimulate DCs through binding Toll-like receptor (TLR)-9 within the phagosome¹⁸. Coating antigen loaded NPs with CpG stimulates DC activation, antigen presentation, and T lymphocyte expansion. Both Bourquin C *et al.* and Sokolova *et al.* demonstrated that delivery of CpG oligodeoxynucleotides by cationic gelatin-based NPs generated an antigen-specific T cell response and a protective anti-tumoral immunity in a murine model of melanoma^{19, 20}. In contrast to free CpG, CpG delivered by NPs could also selectively target APCs in the LNs where they mediated local immune stimulation^{19, 20}.

As an additional advantage, NPs can easily co-deliver tumor-associated antigens (TAAs) and adjuvants to DCs ensuring effective activation and presentation. Schlosser *et al.* demonstrated that efficient cross-priming of CTL responses in mice after vaccination with biodegradable poly(lactide-co-glycolic acid) (PLGA) microspheres was enhanced when OVA model antigen was encapsulated with either CpG or polyI:C as compared to a combinations of OVA-bearing microspheres and soluble adjuvants²¹. Likewise, Hamdy *et al.* found that PLGA NPs co-encapsulating the poorly immunogenic melanoma antigen, tyrosinase-related protein 2 (TRP2), along with adjuvant (monophosphoryl lipid A) was able to induce a therapeutic anti-tumor effect²². In the study of Li *et al.*, immunization of mice with self-adjuncting α -Al₂O₃ NPs that were conjugated to either a model tumor antigen or autophagosomes derived from tumor cells resulted in tumour regression²³. However, delivery of the two components without conjugation was less effective indicating co-delivery is a crucial factor. In another demonstration of antigen/adjuvant co-delivery importance, Fang *et al.* reported that coating polymeric NPs with a layer of cancer membranes could promote a TAA specific immune response provided the NPs included an MPL adjuvant (Fig. 3)²⁴.

DCs targeting

Actively targeting NPs and their payloads to DCs provides a promising strategy for improving the efficacy of immunotherapy²⁵. Indeed, NPs modified with DC receptor targeting ligands have shown significantly improved NP uptake via receptor-mediated endocytosis *in vivo*^{26, 27}. Some commonly employed DC ligands include mannose, fucose N-acetyl glucosamine, anti-CD11c, and anti-DEC205. Moreover, binding of these moieties to their target receptor on the DC surface can enhance maturation and further improve the effectiveness of vaccine formulations. For example, Kempf *et al.* engineered PLGA-NPs with surface-conjugations to target DC surface molecules²⁶. Compared to bare PLGA-NPs, the targeted NPs more effectively induced DC maturation as evidenced by the increase in IL-2 secretion and up-regulation of the DC maturation markers, CD83 and CD86. In a high impact vaccination study, Kranz *et al.* demonstrated that DCs could be targeted with RNA-lipoplexes effectively *in vivo*²⁸. By simply adjusting the net charge, intravenously administered lipoplexes could efficiently target DCs and immune organs, like the spleen. These NPs have shown efficacy in several cancer models and are under phase I clinical investigation. Qian and his colleagues reported a nanovaccine functionalized with tumor antigen peptides (TAPs) that targeted mDCs via the scavenger receptor class B1 (SRB1) pathway²⁹. The self-assembly, small size (~30 nm), and SRB1-targeting properties of nanovaccine resulted in efficient TAPs loading, substantial LN accumulation, mDC targeting and enhanced antigen presentation. They also demonstrated that the nanovaccine could be either used alone or encapsulated with CpG as a prophylactic and therapeutic vaccine. Another important NP parameter for targeting DCs is size. Typically, large particles (500–2000 nm) trafficking to the LNs depends on DC uptake, while small NPs (20–200 nm) can drain freely to the LNs and target LN-resident DCs³⁰. Future targeting mechanisms may lead to innovative ways of eliciting DC manipulation using nano-carriers (Fig. 4)³¹.

Change of tumor microenvironment (TME) through NPs

The TME has several unique physiological characteristics that complicate immunotherapy, including irregular vascularization, hypoxic conditions, low extracellular pH, and increases in proteolytic activity³². Furthermore, the TME can produce an immunosuppressive environment by releasing soluble cytokine mediators and attracting immune suppressive cell types, such as tumor-associated macrophages (TAMs), regulatory T cells (Tregs), and myeloid-derived suppressive cells (MDSCs) (Fig. 5)³³. These features of TME are associated with treatment resistance and poor clinical prognosis. Therefore, new cancer immunotherapeutic approaches demand control over the TME to reverse the immunosuppressive conditions. Using NPs to target immunosuppressive cells in the TME offers a promising strategy to eliminate this tumor induced immunosuppression.

In a recent preclinical study, Park *et al.* developed nanolipogels (nLGs) composed of drugs complexed to cyclodextrins and cytokine-encapsulating biodegradable polymers³⁴. The nLGs could deliver hydrophilic IL-2 and a hydrophobic small molecular inhibitor of TGF- β to the TME in a sustained fashion. Treatment with nLGs delayed tumor growth, improved survival, and increased the activity of natural killer (NK) cells and intratumoral-activated CD8⁺ CTLs. To also target immunosuppressive cells, Sacchetti *et al.* investigated the ability of Treg-specific receptors ligands to promote selective TME Tregs' internalization of PEG-

modified single-walled carbon nanotubes (PEG-SWCNTs)³⁵. PEG-SWCNTs conjugated with glucocorticoid-induced TNFR-related receptor, which is overexpressed in intratumoral Tregs, specifically accumulated in intratumoral Tregs rather than other intratumoral cells or splenic Tregs. Zhu *et al.* developed PEG-sheddable, mannose-modified NPs to improve TAM targeted delivery³⁶. Their PEGylated particles were stealthed until reaching the TME where the acidic environment cleaved the PEG coating and exposed mannose functionalizations. After intravenous administration, they found improved accumulation of these NPs in TAMs as compared to NPs conjugated to non-sheddable PEG.

Chemotherapy application can also remodel the TME and improve the therapeutic index of a subsequent immunotherapy³⁷. Lu *et al.* demonstrated that the combination of a curcumin-PEG (CUR-PEG) micelle and a TRP2 antigen vaccine resulted in a synergistic antitumor effect in melanoma bearing mice compared to individual treatment³⁸. In the immune organs, the combination therapy significantly boosted *in vivo* CTL response and IFN- γ production, while, in the TME, the combination therapy significantly down-regulated levels of immunosuppressive factors. This decrease in immunosuppression coincided with increased levels of proinflammatory cytokines and an elevation in the CD8⁺ T-cell population. Physical manipulation can also positively regulate the TME for improving immunotherapy. In conjunction with anti-CTLA-4 antibody therapy, Wang *et al.* reported that photothermal ablation of tumors with intratumorally injected PEG-SWCNTs was able to modulate adaptive immune responses, especially cellular immunity for the treatment of metastatic cancer³⁹. The mechanism appears to involve photothermal cell death mediating DAMP and TAA secretion that primes immune cells.

Delivery of Immune Modulatory Compounds through NPs

Although its scope in humans is highly controversial, the enhanced permeation and retention (EPR) effect theorizes that the leaky vasculature of tumors and the prolonged circulation times of NP delivery vehicles enhances and targets drug uptake^{40–42}. Regardless of the patient to patient variability in leaky vasculature, an extended circulation NP half-life is absolutely critical for utilizing the EPR effect. In particular, long circulating NPs must avoid the mononuclear phagocyte system (MPS) and filtration by several organ systems. To do this, NPs should be either neutral or negatively charged, sized between 8 to 200 nm, and coated with a stealthing agent, such as PEG, that blocks opsonisation⁴³. Using these NP design principals to exploit the EPR effect, researchers have efficiently delivered immune modulatory compounds to tumors.

Delivery of antibodies

Antibody based therapies have finally come of age as a promising strategy to fight cancer. As a particular advantage to limit off-target side effects, therapeutic antibodies can bind with a high degree of specificity to target proteins involved in disease pathology. However, some functional limitations of therapeutic antibodies have come to light, including inadequate pharmacokinetics, poor tissue accessibility, and impaired interactions with the immune system. These deficiencies point to areas where novel delivery strategies are needed and NPs are being investigated as candidates⁴⁴.

Recently, Kim *et al.* tailored the structure of polyion complex micelles (PICs) to load transiently charged antibody derivatives for enhanced stability, delivery to the cytosol, and antigen recognition inside cells⁴⁵. They found that an appropriate ratio of homo- and block-cationomers in antibody loaded PIC micelles enhanced the endosomal escape efficacy of the loaded antibody and improved recognition of intracellular antigens. In another study, Chen *et al.* constructed a biodegradable PLGA-NP carrying anti-OX40 mAb; a TNF receptor expressed on T cells that transmits a potent activating signal.⁴⁶ The free anti-OX40 mAb failed to show objective clinical activity when tested in phase I clinical trials. However, loading anti-OX40 onto a PLGA-NP induced CTL proliferation, tumor antigen-specific cytotoxicity, and cytokine production more strongly than free anti-OX40 mAb.

In the clinic, the most successful immunotherapeutic antibodies inhibit cancer's immunosuppressive checkpoint pathways (Table 1). These checkpoint blockade antibodies, such as CTL-associated protein-4 (CTLA-4) and programmed death-1 (PD-1), enable CTLs to target cancer cells for destruction and produce durable clinical responses in many difficult to treat cancers. However, some patients show low response rates to immune checkpoint therapies⁴⁷. To improve responses, Lei *et al.* loaded CTLA-4 in functionalized mesoporous silica (FMS) at super high densities via non-covalent interactions to provide long-lasting and localized release.⁴⁸ The FMS-entrapped anti-CTLA4 mAb induced a much greater and extended therapeutic response than the same amount given systemically. Furthermore, changes in the FMS NPs' functionality could tune the rate and durability of mAb release.

NP co-delivery of antibodies with cytokines has also showed promise. Kwong *et al.* anchored T cell stimulatory anti-CD137 and an engineered IL-2Fc fusion protein to the surfaces of PEGylated liposomes⁴⁹. Intratumoral administration of the NPs cured a majority of established primary tumors in a murine B16F10 model; avoided the lethal inflammatory toxicities caused by the equivalent dose of soluble immunotherapy; and induced protective antitumor memory. The therapy's tumor inhibitory effects were CD8⁺ T cell-dependent and also reduced in Tregs within the TME. Li *et al.* utilized an alginate hydrogel system to locally deliver celecoxib and PD-1 mAb to treat tumor-bearing mice⁵⁰. The alginate hydrogel delivery system significantly improved the antitumor activities of celecoxib and PD-1 mAb when combined. These effects were associated with the sustained high concentrations of the drugs in peripheral circulation and tumor regions. Strikingly, the simultaneous delivery of celecoxib and PD-1 mAb from this hydrogel system synergistically enhanced the presence of CD4⁺IFN- γ ⁺ and CD8⁺IFN- γ ⁺ T cells within the tumor and immune organs. These effects were accompanied with a reduction in CD4⁺FoxP3⁺ Tregs and MDSCs in the tumor bed reflecting an increased immunogenic environment. Kosmides *et al.* developed a NP platform that combined anti-PD-L1 with a T cell co-stimulatory signal, anti-4-1BB⁵¹. This dual system redirected effector T cells to recognize target cells while simultaneously blocking inhibitory checkpoints. This caused a 6-fold increase in IFN- γ production by CD8⁺ T cells with an exhausted phenotype in the presence of tumor cells *in vitro*. Additionally, tumor growth stalled and PD-1 expression dropped (~30%) in tumor infiltrating lymphocytes.

Gene delivery

NPs delivering small interfering RNA (siRNA) may provide greater utility in NP-mediated cancer immunotherapy. In a recent study, Li *et al.* constructed cationic, lipid-assisted PEG–PLGA-based NPs to deliver CTLA-4 siRNA (NP-siCTLA-4) and showed this NP delivery system could effectively enter T cells both *in vitro* and *in vivo*⁵². NP-siCTLA-4s were internalized by about 4–6% of tumor infiltrating lymphocytes and enhanced T cell proliferation. More importantly, systemic delivery of NP-siCTLA-4 significantly increased the number of effector CD4⁺ T cells and CD8⁺ T cells, decreased the ratio of CD4⁺ FOXP3⁺ Tregs, inhibited tumor growth, and prolonged survival time in mice with melanoma. Teo *et al.* also studied the sensitization of epithelial ovarian cancer cells to CTL killing by delivering PD-L1 siRNA using folic acid (FA)-functionalized polyethylenimine (PEI) polymers⁵³. The FA-conjugated polymers increased PD-L1 siRNA uptake into SKOV-3-luc cells and decreased non-specific uptake into monocytes. Roeven *et al.* observed that transfecting PD-L1 and PD-L2 siRNAs with SAINT-RED, which consists of the cationic amphiphilic lipid SAINT-18 (1-methyl-4-(cis-9-dioleyl)methyl-pyridinium-chloride) and dioleoylphosphatidylethanolamine, resulted in efficient and long-term knockdown of the PD-1 ligands without affecting DC maturation or viability⁵⁴. The PD-L siRNA/SAINT-RED transfection system in combination with minor histocompatibility antigens mRNA or peptide loading could generate clinical-grade DC vaccines to boost antitumor immunity. In order to augment the efficacy of lipid-calcium-phosphate vaccines in an advanced tumor, Xu *et al.* delivered TGF- β siRNA using a liposome-protamine-hyaluronic acid NP⁵⁵. The resulting TGF- β down-regulation boosted vaccine efficacy and inhibited tumor growth by 52% compared with vaccine treatment alone due to increased levels of tumor infiltrating CD8⁺ T cells and decreased level of intratumoral Tregs.

Cytokines delivery

The ability of cytokines to direct the immune response has motivated their use in immunotherapy (Fig. 5). Indeed, TNF- α , IFN- α/γ and IL-2 have already been approved by the FDA for cancer treatment. However, safe and systemic delivery of cytokines results in a sub-therapeutic effect because of their rapid excretion and enzymatic degradation. Therefore, high concentrations of cytokines are required, but this results in toxic side effects⁵⁶. To overcome the problems associated with systemic delivery, several NP formulations have been developed to deliver cytokines to specific cell types and tissues. By taking advantage of stealth liposomes, inhalation delivery of liposomal IL-2 significantly reduced tumor growth versus free IL-2 in mice with advanced metastatic lung cancer⁵⁷. Hagen *et al.* demonstrated that PEGylated liposomal encapsulation may also be effective in systemic application of TNF- α combined with liposomal chemotherapy for advanced solid tumors⁵⁸. In a phase-I clinical trial, patients with follicular lymphoma receiving liposomal formulations of IL-2 and a TAA showed increases in tumor infiltrating lymphocytes and regression of tumor growth⁵⁹. Finally, work by Anderson *et al.* showed liposomes could easily deliver bioactive IL-1 α , IL-2, IL-6 or GM-CSF helping to expand the array of cytokines used in future liposome formulations⁶⁰.

NPS' STRUCTURES FOR CANCER IMMUNOTHERAPY

NPs improve the pharmacokinetics and biodistribution of their cargo which can reduce side effects. Generally, approaches for improving NP pharmacokinetics and biodistribution include maintaining the size around 100 nm, keeping the Zeta-potential within 10 mV, and grafting PEG onto the particles' surface⁴². In addition, NPs can target and stimulate the immune system, thereby inducing the production of cytokines that mediate humoral and cellular immunity. Today, a variety of NPs, including VLPs, cationic liposomes, dendrimers, micelles, gold NPs *etc.*, are used for cancer vaccination and immunomodulator delivery (Fig. 6).

Virus like particles

Virus like particles (VLPs) are highly versatile NPs (20–100 nm) derived from viruses that lack the ability to replicate⁶¹. VLPs can be easily engineered via site-directed mutagenesis or bioconjugation to load immunogenic ligands, target immune cells, or augment vaccine efficacy⁶². Their size, shape, and functionality also provides efficient uptake, processing, and presentation by DCs' MHC class II molecules to activate T cells⁶³. Moreover, unlike many soluble antigens, VLPs undergo efficient cross-presentation by APCs via the MHC class I pathway to activate CD8+ T cells^{63,64}.

Today, the majority of VLP-based vaccines are in clinical development against viral pathogens, such as HIV and HPV⁶⁵. However, Lizotte *et al.* recently demonstrated that inhalation of VLPs generated from cowpea mosaic virus (CPMV) reduced lung metastases of established B16F10 melanoma and generated potent systemic anti-tumor immunity against relatively non-immunogenic B16F10 in the skin⁶⁶. These VLPs also promoted anti-tumor immune effects in ovarian, colon, and breast cancer models located at various locations (e.g., subcutaneous, lung, mammary pad, etc.). In another study by Li *et al.*, recombinant bacteriophage MS2 VLPs, whose coat protein was engineered to bind prostate acid phosphatase antigen mRNA via a 19-nucleotide RNA aptamer, induced strong immune responses and protected mice against prostate cancer challenge⁶⁷.

Cationic liposomes

Cationic liposomes have been used extensively as immunotherapy and vaccine delivery systems, especially for nucleic acids, to enable prolonged therapeutic and antigen delivery.⁶⁸ In Zhou *et al.*'s study, cationic liposomes complexed with CpG (CpG lipoplex) prevented the proliferation of tumor cells, prolonged the survival time of tumor-bearing mice, and induced higher IFN- γ production compared to naked CpG⁶⁹. In another study, Mansourian *et al.* demonstrated that 1,2-dioleoyl-3-trimethylammonium propane (DOTAP)-cholesterol-dioleoylphosphatidylethanolamine (DOPE) liposomes loaded with p5 antigenic peptide and CpG greatly enhanced CTL responses and inhibited tumor progression compared to soluble p5 and CpG³⁰. Zaks *et al.* assessed the vaccine adjuvant effects of cationic liposomes complexed to TLR agonists in mice⁷⁰. They found that cationic liposomes complexed to nucleic acids were particularly effective adjuvants for eliciting CD4⁺ and CD8⁺ T cell responses against peptide and protein antigens compared to control treatment.

Dendrimers

Dendrimers molecules repetitively branch around a focal point, and have high degrees of monodispersity, water solubility, and functionalizable peripheral groups, which make these macromolecules appropriate candidates for delivery systems. The low cost, ease of synthesis, biocompatibility, structural control, and functionalizability of polyamidoamine (PAMAM) make it a viable candidate for a variety of applications in immunotherapy and nanotechnology. As a result, PAMAM is a widely studied dendrimer platform, especially for nucleic acid delivery. In the study of Daftarian *et al.*, PAMAM dendrimers loaded with MHC class II-targeting peptides effectively and selectively delivered DNA based vaccines to APCs *in vivo*⁷¹. Subcutaneous administration of DNA-peptide-PAMAM complexes targeted DCs in the draining LNs, expanded antigen specific T cells, and promoted a robust rejection of established tumors.

PLGA

PLGA is a FDA approved biodegradable polymer widely used in several controlled release drug delivery vehicles in clinical trials.⁷² PLGA NPs can entrap a wide range of biologically active compounds. Furthermore, variation of the lactic to glycolic acid composition ratio can manipulate PLGA NPs' release profile of the encapsulated molecules. In a recent study by Kokate *et al.*, NPs consisting of PLGA and CpG-coated tumor antigen increased the maturation of DCs, induced potent CTL responses, and attenuated tumor growth *in vivo*²⁸. In another study by Heo *et al.*, PLGA NPs containing siRNAs against the signal transducer and activator of transcription-3 (STAT3) and imiquimod (R837, a DCs activator) activated DCs and silenced the immunosuppressive STAT3 genes.⁷³ Moreover, DCs transfected with both PLGA (R837/STAT3 siRNAs) and PLGA NPs containing OVA antigen resulted in the activation of OVA-specific T cells and enhanced anti-tumor immunity.

Polymeric micelles

Polymeric micelles composed of amphiphilic block copolymers spontaneously self-assemble in water above their critical micelle concentration into spherically shaped NPs⁷⁴. The inherent and easily modifiable properties of polymeric micelles make them particularly well suited for therapeutic delivery purposes⁷⁵. In a study by Luo *et al.*, Poly I:C, STAT3 siRNA and OVA antigen were co-encapsulated by PEG-b-poly-(L-lysine)-b-poly-(L-leucine) polypeptide micelles to generate a PMP/OVA/siRNA nanovaccine⁷⁶. The PMP/OVA/siRNA vaccine elevated CD86, CD40 and IL-12 expression in tumor associated DCs indicating its potent activation of APCs. Moreover, the PMP/OVA/siRNA vaccine abrogated immunosuppression by increasing mature DCs and decreasing immunosuppressive cells in tumor-draining LNs. Together these effects led to potent anti-tumor immune responses, tumor regression, and prolonged survival. In an additional study, Jeanbart *et al.* loaded polymer micelles with 6-thioguanine (MC-TG NPs) to kill MDSCs in tumor-bearing mice and enhance downstream CLT-mediated anti-tumor responses. They found that MC-TG NPs more effectively decreased the numbers of circulating monocytic and granulocytic MDSCs than equal doses of free TG. Overall, this MDSC-depleting strategy enhanced cancer immunotherapy in the B16-F10 melanoma model⁷⁷.

Gold NPs

Gold NPs (Au NPs) have been widely explored in biomedical imaging, drug delivery, diagnostic tests and photothermal tumor treatment^{78,79}. As nanocarriers, gold nanostructures possess unique properties such as chemical inertness, tunable surface chemistry, high biocompatibility, and an easily controlled morphology. Furthermore, Au NPs can be readily phagocytosed by mononuclear cells⁸⁰. As a result, Au NPs have several favorable characteristics as nanocarriers for antigen delivery. Jon Sangyong's laboratory reported that intramuscular administration of Au NP-based cancer vaccines could enable effective cancer prevention and treatment *in vivo*⁸¹. In their study, a large proportion of injected Au NPs carrying antigens drained into the local LNs. In addition, Au NPs induced effective humoral and cellular immunity against an endogenous TAA. Thus, Au NPs immune stimulating effects could serve as vaccine platforms for cancer therapy without additional adjuvants. Almeida *et al.* demonstrated that Au NP delivery of OVA (Au NP-OVA) and of CpG (Au NP-CpG) enhanced the efficacy of both agents and induced strong antigen-specific responses⁸². In addition, Au NP-OVA delivery without CpG, was sufficient to promote significant antigen-specific responses, subsequent anti-tumor activity, and prolonged survival in *in vivo* tumor models⁸². The results again point to Au NPs as a possible self adjuvanting platform. Ma *et al.* synthesized SM5-1-conjugated Au NPs (Au-SM5-1 NPs) and investigated their anti-cancer efficacy in hepatocellular carcinoma. Compared with SM5-1 alone, Au-SM5-1 NPs significantly inhibited tumor growth of both subcutaneous and orthotopic hepatocellular carcinoma tumor models⁸³. Taken together, Au NPs-based vaccines are promising nanocarriers for the delivery of TAAs and may act as an alternative self-adjuvant.

Conclusion

Manipulation of the immune system by immune modulating therapies can produce an effective and long-lasting immune response against cancer. In recent years, NP delivery systems have allowed the development of effective vehicles for delivery of TAAs and immune stimulatory molecules to professional APCs and the TME to overcome tumor-driven immunosuppressive signals. These approaches have improved outcomes and provided efficacy compared to non-NP-based therapeutics. However, to achieve maximum immunotherapeutic efficacy, there is an urgent need to design and optimize delivery systems that guarantee low toxicities, high specificity, and long-lasting efficacy. Indeed, these challenges continue to limit the diversity of systems within clinical trials to primarily liposomal NP vaccine formulations. Therefore, future clinical translation of NP-based approaches will undoubtedly require further optimization of nanostructure parameters, such as the stability, biodistribution, pharmacokinetics, toxicity of component compounds (e.g., cationic dendrimers), and size. However, the ever increasing understanding of immunology and nanotechnology will undoubtedly engineer remarkable mechanisms to modulate immune responses in the future.

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Figure 1. Cancer immunity cycle

The progression of cancer recognition and immunity typically cycles through the release of TAAs, presentation of TAAs by APCs, priming of T cells by activated APCs, migration of T cells back to the tumor, killing of tumor cells by T cells, and release of more TAAs. NP approaches to cancer immunotherapy should focus on improving the progression of these steps via delivery of antigen and immune modulators that increase response and reduce immunosuppressive mechanisms.

Figure 2. Strategies of nanoimmunotherapy for cancer

NPs can deliver antigens and adjuvants to induce DC maturation. NP formulation can enhance the presentation of TAAs. Additionally, NPs can restore T cell anti-tumor function by delivering CTLA-4 or PD-1/PD-L1 antibodies (anti-CTLA-4 or anti-PD-1/ PD-L1) that stop anergy.

Figure 3. Cancer cell membrane-coated nanoparticles for cancer vaccination
(Figure adapted from reference²⁴)

Figure 4. The sizes of vaccine delivery systems and pathogenic agents
(Figure adapted from reference³¹)

Figure 5. Cancer Immunosuppression Mechanisms

Anti-tumor immunity can be suppressed by recruited immune cells that secrete immunosuppressive compounds within the TME. Tumor cells themselves can also suppress immunity by expressing surface molecules that cause T cell energy and exhaustion. (Figure adapted from reference⁴³)

Figure 6. Nanoparticles for Cancer Immunotherapy

Nanoparticles with different structures can be used cancer immunotherapy, which includes dendrimer, micelles, virus-like nanoparticles, liposomes, gold NPs and etc.

Table 1

Anti-cancer immunotherapy approved by the Food and Drug Administration

Drug name	Immunotherapy	Cancer type	FDA approval time
Rituximab	Monoclonal Antibody: CD20	CD20-positive B-cell Non-Hodgkin's lymphoma	26/11/1997
Trastuzumab	Monoclonal Antibody: Erb B2 (HER-2)	Metastatic breast cancer	25/09/1998
Alemtuzumab	Monoclonal Antibody: CD52	B-cell Chronic lymphocytic leukemia	07/05/2001
Ibritomomab	Monoclonal Antibody: CD20	B-cell non-Hodgkin's lymphoma	19/02/2002
Tositumomab	Monoclonal Antibody: CD20	CD20-positive B-cell Non-Hodgkin's lymphoma	27/06/2003
Cetuximab	Monoclonal Antibody: EGFR	Metastatic colorectal and head and neck carcinoma	12/02/2004
Bevacizumab	Monoclonal Antibody: VEGF-A	Metastatic colorectal and non-small cell lung carcinoma	26/02/2004
Panitumumab	Monoclonal Antibody: EGFR	Metastatic colorectal carcinoma	27/09/2006
Sipuleucel-T	Cancer vaccine	Metastatic castration-resistant prostate cancer	29/04/2010
Talimogene laherparepvec	Oncolytic virus therapy: HSV-1	Advanced melanoma	27/10/2015
Ipilimumab	Immune check-point inhibitors: anti-CTLA-4	Advanced melanoma	25/03/2011
Pembrolizumab	Immune check-point inhibitors: anti-PD-1	Advanced refractory melanoma and non-small cell lung cancer	04/09/2014
Nivolumab	Immune check-point inhibitors: anti-PD-1	Unresectable or metastatic melanoma Squamous non-small cell lung cancer	22/12/2014