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Review

# Enhancing cancer immunotherapy through nanotechnology-mediated tumor infiltration and activation of immune cells

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ABSTRACT

Cancer immunotherapy has become arguably the most promising advancement in cancer research and therapy in recent years. The efficacy of cancer immunotherapy is critically dependent on specific physiological and physical processes – collectively referred to as transport barriers – including the activation of T cells by antigen presenting cells, T cells migration to and penetration into the tumor microenvironment, and movement of nutrients and other immune cells through the tumor microenvironment. Nanotechnology-based approaches have great potential to help overcome these transport barriers. In this review, we discuss the ways that nanotechnology is being leveraged to improve the efficacy and potency of various cancer immunotherapies.

## 1. Introduction

Cancer immunotherapy has generated a paradigm shift in the way that cancer is treated. However, not only have high response rates to immunotherapy been observed only in certain cancer types, but many patients fail to mount effective antitumor immune responses [1]. Multiple lines of evidence indicate that the presence of tumor-infiltrating lymphocytes (TILs) serves as a prognostic marker and predicts antitumor immune response to different therapies, including immunotherapy and chemotherapy [2]. Tumors lacking TILs have been characterized as “non-inflamed”, and generally correlate with treatment failure and poor prognosis [3]. For example, the efficacy of one type of cancer immunotherapy, immune checkpoint blockade antibodies, in patients with breast cancer, which has relatively less TILs (mean percentage of 10%) [4], is far less effective compared to that in patients with melanoma or non-small cell lung carcinoma, characterized as “inflamed” tumor types, which are abundant with TILs [5]. Thus, how to promote the transport, activity, and persistence of TILs in the tumor microenvironment is crucial for developing effective immunotherapies, especially for the “non-inflamed” tumor types.

Intratumoral accumulation of cytotoxic immune cells (e.g., TILs) and cancer therapies are crucial for enhanced anti-tumor responses. Yet, successful transport of cancer therapies depends on their sequential negotiation of biological barriers [6,7], including non-specific distribution into non-lymphatic or non-tumor tissue compartments, limitations in hemorheological/blood vessel flow and pressure gradients within tumors, the density and composition of the tumor stroma [8], and the dynamics in intratumoral cell-cell and cell-matrix interfaces affecting tensile forces [6,9]. Although these physical spatiotemporal peculiarities and aberrations of tumors have been less studied, it is becoming clear that intratumoral processes may be highly indicative of therapeutic efficacy [10–13]. Furthermore, it is becoming clear that as the tumor progresses, intratumoral transport properties change [14]. These intratumoral transport property changes may also be heterogeneous within the tumor as well as between patients, and a greater understanding of how these changes influence therapeutic efficacy will ultimately lead to fine-tuning of the tumor microenvironment. This fine-tuning would then tip the balance towards a phenotype that is amenable to immune cells and immunotherapy transport. Thus, the impact of transport phenomena on immunotherapeutic efficacy (and therapeutic resistance) should be considered when developing strategies for new immunotherapies.

Application of nanotechnologies can facilitate the transport of therapeutics into tumors. For the purpose of this review, the “operational definition for nanotechnology involves three ingredients: 1) nanoscale sizes in the device or its crucial components; 2) the man-made nature; and 3) having properties that only arise because of the nanoscopic dimensions” [15]. However, we recognize that there are other

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acceptable definitions in the scientific literature. Applying nanotechnology to package drugs, small molecules, oligonucleotides, immunomodulatory compounds, etc. into nanometer- or micrometer-size particles allows these therapeutics to pass sequential physical and biological barriers and to accumulate in tumor tissues [16–21]. The released therapeutics can affect not only cancer cells but also immune cells, consequently modifying the tumor microenvironment [22]. Nanotechnology-based cancer vaccines promote rapid expansion of tumor-specific T cells, and various forms of nanoparticles (NPs) have been utilized in the generation of T cells for adoptive T cell therapies. Furthermore, multiple laboratories have applied nanotechnology-based approaches to unleash the activities of TILs by suppressing the activities of immune checkpoint inhibitor proteins, regulatory T (Treg) cells, and immunosuppressive myeloid cells (IMCs), by mimicking tumor-associated leukocytes, and by altering the tumor extracellular matrix (ECM). However, the development of new nanotechnologies for cancer treatment will ultimately depend on overcoming biological transport barriers to enhance cancer immunotherapy [7]. This review summarizes advances in two areas of nanotechnology-based cancer immunotherapy: 1) generation of tumor antigen-specific T cells, and 2) bypassing the transport barriers in facilitating antitumor immunity.

## 2. Promoting generation and tissue infiltration of T lymphocytes using NP-based immunotherapies

### 2.1. Nanotherapeutic cancer vaccines

Immunotherapy with cancer vaccines offers the potential for highly specific cancer cell cytotoxicity, superlative T cell memory response, and minimal systemic toxicity. Therefore, it is a very attractive approach for cancer treatment. Cancer vaccines typically include a tumor antigen and an adjuvant to enhance immune responses. Since dendritic cells (DCs) are the major antigen-presenting cells (APCs), DC vaccines have also been developed, through the use of both circulating and bone marrow-derived DCs, in order to maximize antitumor immunity. The first therapeutic DC vaccine, Sipuleucel-T (Provenge®), generated from autologous peripheral blood mononuclear cells pulsed with a prostatic acid phosphatase–GM-CSF recombinant fusion protein [23], was approved for treatment of metastatic castration-resistant prostate cancer by the U.S. Food and Drug Administration (FDA) in 2010. A once promising non-DC vaccine, nelipepimut-S (E75) vaccine (NeuVax™), for human epidermal growth factor receptor 2 $(HER2)^+$ breast cancer that contains the E75 antigen peptide mixed with the adjuvant, granulocyte-macrophage colony-stimulating factor (GM-CSF) [24,25], was recently tested in a Phase III clinical trial, sponsored by Galena Biopharma, Inc. However, the clinical trial was discontinued based on negative data from a planned safety and futility interim analysis. With the recent advancements in next-generation sequencing, therapeutic vaccines can now be tailored to target a group of patient-specific mutant neoantigen epitopes, as evidenced by the success in treatment of melanoma patients with therapeutic cancer vaccines [26–28]. More vaccines are expected to reach the clinic in the coming years.

Despite recent successes, cancer vaccine development still faces a number of challenges. One key factor in determining DC vaccine or non-DC vaccine efficacy is transport of the vaccine-internalized DCs to lymphatic tissues; more specifically, transport to the T cell-rich paracortex of the lymph nodes, where stimulation of antigen-specific T cells occurs. Animal studies have shown that the route of administration determines biodistribution and, consequently, vaccine efficacy. For example, intravenously injected DC vaccines mainly accumulate in the spleen, whereas subcutaneously injected DCs preferentially home to the T cell areas of the draining lymph nodes [29]. Clinical studies have revealed that regardless of vaccine injection site, less than 5% of the DCs can reach the lymph nodes [30]. In addition, the stimulatory signals of *ex vivo* matured DCs, used for DC vaccine generation, cannot be maintained *in vivo*. Therefore, designing strategies to transport response-eliciting DC vaccines or non-DC vaccines and overcoming the sequential physical and biological barriers for this transport are critical for the success of cancer vaccines.

NPs and microparticles have been incorporated into cancer vaccines to deliver tumor antigens. NPs can be loaded with more than a single antigen epitope, can improve antigen stability, can slow the release of antigens for sustained T cell responses, and can be targeted to specific sites. Injecting NPs that contain antigens and immunomodulatory compounds leads to the accumulation of APCs, such as DCs, at the injection site, followed by APC transport into lymph nodes for antigen presentation to T cells [31–33]. However, due to the lack of optimization for direct transport through lymphatic vessels, NP-based vaccines have fallen short [34,35]. Thus, a recent strategy to develop lymph-node targeted NP-based vaccines, included not only size-tuning and covalent and non-covalent attachment of polyethylene glycol (i.e., PEGylation) to reduce NP-mediated immunogenicity, but also the hitchhiking of NPs, specifically liposomes, onto albumin proteins, which migrate to lymph nodes [36,37]. Therefore, the ability to directly transport NPs and microparticles to the tumor will ultimately produce a more potent vaccine.

NPs and microparticles can also serve as adjuvants in order to boost antitumor immunity. Although various forms of aluminum salt precipitates (alum, 1–50 μm) have been widely used as adjuvants in prophylactic vaccines for infectious diseases, these T helper 2 (Th2) cell-biased adjuvants are not effective in activating TILs, specifically, $CD8^+$ cytotoxic T cells [38,39]. Interestingly, porous silicon microparticles (PSMs), which not only serve as adjuvants but also aide in adjuvant and tumor antigen delivery, are effective in triggering DC production of type I interferon (IFN-I; including IFN-α and β), which is essential for the cytotoxic activity of $CD8^+$ cytotoxic T cells [40]. It has been well documented that IFN-I production by host APCs serves as the bridge to connect innate and adaptive immune responses [41]. In addition, the micrometer-size particles can also serve as a reservoir for sustained release of tumor antigen peptides and can facilitate antigen processing inside the DCs. Treatment with a DC vaccine carrying PSMs, serving as an adjuvant and loaded with HER2-specific peptides (Nano-DC vaccine), modulated the tumor immune microenvironment, as indicated by elevated levels of intratumoral inflammatory cytokines and tumor-infiltrating, antigen-presenting $CD11c^+$ DCs in a murine model of $HER2^+$ breast cancer. Nano-DC vaccine treatment completely inhibited tumor growth. Importantly, antitumor immunity was $CD8^+$ cytotoxic T cell-dependent, as depletion of this subtype of T lymphocytes completely abolished inhibition of tumor growth [40]. Polymer-based nanovaccines have also been developed for cancer treatment. Gao and colleagues recently reported a STING-activating nanovaccine, consisting of a synthetic polymeric NP (PC7A NP) with an antigen. This vaccine generated a strong cytotoxic T cell response [42]. The enhancement of the transport of cancer vaccines and DCs by nanotechnologies will undoubtedly lead to improved effectiveness, but the therapeutic components of cancer vaccines are also key to this efficacy.

Nanotechnology has played a very significant role in the development of next-generation messenger (m)RNA-based therapeutic cancer vaccines. In contrast to the peptide vaccines, mRNA vaccines have the advantage of incorporating multiple antigen epitopes in one minigene construct, and thus, can be customized to fit the needs of individual patients, based on the unique mutation spectrum in their cancer genome. In addition, the mRNA molecules can serve as self-adjuvants, once in complex with selected proteins on polymers, by stimulating innate immune Toll-like receptor (TLR) 7 and 8 signaling [43–45]. The mRNA vaccines also differ from the traditional DNA plasmid vaccines in that, among other advantages, they function in both dividing and non-dividing cells, and there is no risk for genomic integration [46,47]. Still, mRNA molecules are vulnerable to degradation by plasma and tissue enzymes. In addition, they cannot enter APCs by default and need to be transfected into these cells *ex vivo* (DC vaccine) or delivered by NPs *in vivo* (non-DC vaccine). Various forms of NPs have been generated by