Nanoparticles for generating antigen-specific T cells for immunotherapy

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Abstract

T cell therapy shows promise as an immunotherapy in both immunostimulatory and immunosuppressive applications. However, the forms of T cell-based therapy that are currently in the clinic, such as adoptive cell transfer and vaccines, are limited by cost, time-to-treatment, and patient variability. Nanoparticles offer a modular, universal platform to improve the efficacy of various T cell therapies as nanoparticle properties can be easily modified for enhanced cell targeting, organ targeting, and cell internalization. Nanoparticles can enhance or even replace endogenous cells during each step of generating an antigen-specific T cell response – from antigen presentation and T cell activation to T cell maintenance. In this review, we discuss the unique applications of nanoparticles for antigen-specific T cell therapy, focusing on nanoparticles as vaccines (to activate endogenous antigen presenting cells (APCs)), as artificial Antigen Presenting Cells (aAPCs, to directly activate T cells), and as drug delivery vehicles (to support activated T cells).

Keywords

immunoengineering; immunotherapy; nanoparticle; T cell; bioengineering; cell therapy

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1. Introduction

Advances in cellular therapies, both in efficacy and ease of manufacturing, have expanded treatment options for cancers that were previously non-responsive to traditional treatments, including tumor resection, radiation, and chemotherapy [1,2]. Adoptive T cell therapies (ACT) such as tumor infiltrating lymphocyte (TIL), T cell receptor (TCR), and chimeric antigen receptor (CAR) T cell therapy have all shown clinical efficacy in cancer patients. TIL therapy has resulted in complete remission in a subset of melanoma patients and increased patient survival [3,4]. TCR therapy has also shown to be effective in treating melanoma as well as synovial cell sarcoma [5]. Patients with B cell malignancies have been able to achieve durable remission when treated with CAR T cell therapy [6]. However, these T cell therapies require a highly expensive and specialized ex vivo culture step for expanding and preparing cytotoxic T cells for re-infusion. This greatly limits the number of facilities that are equipped to handle such work [2]. In addition, after *ex vivo* activation, T cells must reach between millions and billions in number prior to reinfusion [7–9]. This process is lengthy and often gives rise to cytotoxic T cells that are unable to persist overtime *in vivo* [10,11]. There is also variation depending on the T cell activation technique; for example activation with anti-CD3 and anti-CD28 antibodies can be less efficient than cell based artificial antigen presenting cells (aAPCs) or nanoparticle (NP) based aAPCs [12].

Clinical and academic research on the various forms of ACT has increased the overall efficacy of treatment and ability to identify ideal candidates for each therapy. However, each form of ACT still faces challenges that affect the widespread use. For TIL therapy there is a direct correlation between the number of CD8+ T cells expanded and the potency of the treatment [13]. TILs are extracted from digested patient tumors and undergo a rapid expansion protocol in order to obtain high cell counts. The heterogeneous nature of the starting population of TILs has been noted to result in an end product that contains low frequencies of tumor specific T cells [8,13]. To increase the population of reactive T cells involves a lengthy culture time that pushes T cells to become terminally differentiated effector T cells, which can affect persistence *in vivo* [8]. TCR therapy addresses this issue by circumventing the need for TILs. Rather than relying on tumor samples, TCR therapy involves isolating T cells from a patient's peripheral blood. Retroviruses are then utilized to transduce T cells with engineered TCRs specific for tumor-associated antigens. However, the therapy is currently not applicable to all patients because of HLA-restriction [8]. CAR T therapy offers a similar advantage over TIL therapy while also addressing the issue of HLA-restriction. To produce CAR T cells, viral vectors are used to induce the expression of chimeric antigen receptors [8]. But unlike TIL therapy, CAR T cell therapy does not show profound clinical efficacy in solid tumors; CAR T cells can only recognize a ligand expressed on the cell's surface, lack the ability to recognize multiple tumor associated antigens, and are impacted greatly by immunosuppressive factors within the tumor microenvironment [14,15]. Lastly, the viral transduction methods used to produce CAR T cells often result in prolonged expression of the CAR which drive adverse reactions, such as cytokine release syndrome [16]. Despite shortcomings, T cell therapies show great promise in cancer therapy and can be further improved by addressing these issues, namely complex and expensive manufacturing and limited patient accessibility. New

bioengineered nanotechnologies, through improvements to the method of production, ease of use, and performance, have the potential to enhance the accessibility and efficacy of T cell immunotherapy for patients with a variety of diseases.

Nanoparticles provide useful platforms for engineering T cells at each stage of the therapeutic T cell response. To date, they have been utilized to deliver antigen directly to T cells, activate and expand existing antigen-specific T cells, and maintain the function and presence of therapeutic T cell populations (Figure 1). Particles can likewise enhance T cell therapies by enriching *in vitro* performance, improving *ex vivo* T cell selection and activation, and facilitating translation of benchtop therapies for *in vivo* use. Nanoparticles enrich *in vitro* performance by contributing additional immunomodulatory properties [17,18], enabling specific cell and receptor targeting [19], and enhancing intracellular delivery of drugs and biologics. Nanoparticles augment the immunomodulatory capacity of T cell therapies through innate immunostimulatory effects, enabling colocalized presentation of immune synapse signals and antigen, promoting receptor crosslinking and TCR clustering, and permitting the targeting of modulators to a specific site on the cell surface or intracellularly [20]. By controlling nanoparticle size, chemistry, and flexibility it is possible to direct the route of cellular uptake along with mechanisms for endosomal escape and direction to specific intracellular compartments (Figure 1A) [21]. This is particularly useful in T cell therapies, as intracellular delivery to T cells can be difficult due to limited mechanisms of internalization compared to other immune cell types. The size of nanoparticles in relation to the size of a cell allows for multiple particles to bind to a cell at one time. This provides advantages in *ex vivo* T cell activation, through spatial control of signal presentation to T cells [22] as well as high throughput screening of T cells, through the ability to separate targeting components onto individually distinct particles (Figure 1B) [23]. In addition to improving the development of *ex vivo* techniques, nanoparticles facilitate translation of successful *ex vivo* T cell therapies by enhancing cellular manufacturing and the phenotypic properties of T cells for *in vivo* therapeutic use [24]. By improving biocompatibility, pharmacokinetics, and biodistribution, nanoparticles also enhance *in vivo* generated T cell responses (Figure 1C). Depending on the application, nanoparticles can be administered systemically and designed for specific accumulation in certain tissues of interest by the addition of targeting ligands or changing material properties such as shape, size, elasticity, surface charge, or response to dynamic stimuli [25,26]. In this review, we will discuss various approaches to generating and maintaining an antigen-specific T cell response for immunotherapy, and how nanomaterials are used to enhance these processes.

2. Generating antigen-specific T cells

The first step of initiating an antigen-specific T cell response using nanoparticles is presentation of antigen, which can be initiated by using nanoparticles that interact directly with T cells or by using nanoparticles that interact with other cells, including antigen presenting cells (APCs). APCs and other non-T cell targets offer ease of targeting as well as potential multi-faceted effects, while T cell targeting is a more direct approach to mediate cellular immunity. Each approach to the generation of antigen-specific T cell therapy carries distinct design considerations in the context of nanomedicine and are active, emerging areas of research.

2.1. Professional APC targeting

Targeting professional APCs such as macrophages or dendritic cells offers an upstream opportunity for immune modulation with great potential to manipulate T cell behavior. There are several excellent reviews discussing the specific targeting of DCs [27] versus macrophages [28] for immunotherapy applications. APCs are actively circulating phagocytotic cells that constantly surveil blood and peripheral tissues for foreign pathogens and signs of cellular damage. When a foreign or damaged cell is detected, APCs will mature and traffic to secondary lymphoid tissues where they can present antigen and other signals to direct T cell activation, movement, differentiation, and functionality. Professional APCs are attractive therapeutic targets for T cell therapy due to their abilities to home to sites of inflammation, efficiently process and present delivered antigens, potently direct T cell behavior, and be easily targeted both extracellularly and intracellularly *in vivo* compared to T cells [29]. Likewise, APCs modulate additional components of the immune response such as inflammation that may be harnessed to cooperatively benefit outcomes of T cell therapies.

APCs act as links between the innate and adaptive immune systems, and as such, have unique traits that can be exploited for nanoparticle targeting and function. As part of their function in the innate immune system, APCs have evolved to detect and process viruses. Nanoparticles exist in the same length scales as viruses and can exhibit similar properties that utilize these evolutionarily derived pathways to mount robust immune responses [30]. APCs are members of the reticuloendothelial system, which functions to surveil and engage with foreign material. As a part of this role, APCs are constantly taking up material from their extracellular environment and employ all four major endocytosis pathways to do so: phagocytosis, marcropinocytosis, clathrin-mediated endocytosis, and caveolin-mediated endocytosis [31]. Uptake via these pathways has been demonstrated to be dependent on particle size, shape, and surface chemistry, with particles > 1000 nm primarily taken up by phagocytosis, ~200-1000 nm by means of macropinocytosis, and <250 nm through clathrin and calveolae-dependent pathways [21,31]. The effects of particle shape and surface chemistry are less clear, with conflicting reports of the effects of low to moderate aspect ratios on APC uptake of nanoparticles [32–34]. Very high aspect ratio particles, however, have been shown to make internalization by phagocytosis more likely than by endocytosis [35,36]. This could improve macrophage targeting over uptake by non-APC cells.

2.1.1. Harnessing innate immune mechanisms—Professional APCs have specific receptors that have evolved as part of the innate immune system to recognize common signals of invading pathogens and cellular damage. These signals are broadly categorized into pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs). Since nanoparticles can exploit innate immune pathways in APCs by adopting pathogen-like properties, it is useful to design nanoparticles for APC targeting to receptors of PAMPs, known as pattern recognition receptors (PRRs). The most common families of PRRs utilized for therapeutic targeting to APCs are C-type lectin receptors (CLRs) and toll-like receptors (TLRs) [37]. CLRs identify a variety of pathogens by recognizing carbohydrate structures such as mannose, fucose, and glucan [38]. CLR activation initiates uptake of the pathogen, processing, and antigen presentation. Conjugation of mannose in particular has been utilized widely to target both dendritic cells and

macrophages, as well as activate CLRs [39–42]. There is likewise evidence that targeting specific sub-classes of CLRs or multiple CLRs with tunable nanoparticles may give finer control of downstream T cell responses. For example, Bandyopadhyay et al. found that targeting the CLR sub receptor DEC-205 produced an anti-inflammatory IL-10 response that was dependent on nanoparticle surface ligand density [43]. The mechanism of this response was shown to be due to DEC-205 receptor crosslinking at higher densities. To target multiple CLRs at once, Duinkerken et al. conjugated ligands for the DC-SIGN and Langerin CLRs onto a polyamidoamine dendrimer along with gp100 antigen [44]. Targeting both receptors compared to a single receptor increased in situ DC uptake 10-fold, antigen-specific cross-presentation ~ 3-fold and doubled the percentage of antigen-specific CD107^{+} CD8^{+} T cells after treatment.

Similar strategies have been employed to target TLRs. Rather than recognizing carbohydrates, TLRs recognize a variety of conserved pathogenic structures including oligonucleotides and the endotoxin lipopolysaccharide (LPS). Nanoparticles can conjugate to the surface or encapsulate DNA [45] or TLR agonists such as LPS [46,47]. Lastly, interactions between multiple PRRs have been shown to instruct T helper cell differentiation as well as increase robustness of the adaptive immune response [38]. Li et al. recently demonstrated a trivalent nanoparticle vaccine targeting CLR DC-SIGN and TLR7 [40]. Dual receptor targeting increased DC specific antigen presentation and activation of antigen (Ag)-specific T cells ~2.5-fold compared to TLR7 and antigen alone. In the future it may be helpful to consider using nanoparticles to target multiple PRRs or specific sub-receptors to modulate T cell behavior more precisely.

In addition to the ability to conjugate targeting molecules on the surface, the choice of nanoparticle material itself can directly modulate the immune behavior of a professional APC via innate immune mechanisms. Several common biomaterials used for constructing nanoparticles have reported intrinsic immunostimulatory effects [48] Nanoparticle size, shape, and surface chemistry have been shown activate immune pathways by interaction with PRRs, inflammasomes, and other aspects of the innate immune system [49–51]. Adjuvant effects of nanomaterials alone can be powerful enough to decrease the need for traditional adjuvants. Luo et al. created a simple nanoparticle vaccine by mixing an intrinsically immunogenic nanoparticle (PC7A) with antigen. PC7A generated cytotoxic T cell responses higher than both alum and LPS [50]. PC7A was found to stimulate interferon genes (STING), and delivery of the nanovaccine with PD-1 resulted in 100% survival in a mouse TC-1 tumor model along with inhibited tumor growth after rechallenge. Innate immune mechanisms of APCs are powerful modulators of downstream T cell response, but as APCs are also the link between the innate and adaptive arms of the immune system, it is useful to consider ways in which nanoparticles can harness their adaptive mechanisms to promote desirable T cell responses.

2.1.2. Harnessing adaptive immune mechanisms—Professional APCs are unique in that they can present antigen on MHC class I as well as MHC class II. Typically, endogenous antigens are expressed on MHC class I and exogenous antigens are expressed on MHC class II, which leads to downstream activation of CD8^{+} T cells and CD4^{+} T cells, respectively. Depending on the application, it may be useful to target presentation on a

specific type of MHC to control desirable T cell activation and presentation. In addition to targeting MHC I through intracellular delivery, APCs can cross-present extracellular antigens onto MHC class I to direct the CD8^{+} T cell response through a process known as cross-presentation. Cross-presentation is especially important in the context of pathogens that do not traditionally infect APCs as well as in generating a cytotoxic T cell response from vaccination [52]. Methods to induce or enhance cross-presentation include changing nanoparticle size, route of endocytosis, or surface chemistry [53], but the mechanisms of this are poorly understood and not consistent across the literature [54]. In some cases, graphene oxide [55] and super paramagnetic iron oxide (SPION) [56] particles have been shown to disrupt antigen processing and cross-presentation, although the disruption pathways appear to be more complex than material alone. In the case of the SPION particles, Blank et al. were able to decrease CD4^{+} T cell activation after treatment of DCs with ~100nm, positively charged SPION particles as a result of the inhibition of cross-presentation, providing a useful strategy to ameliorate Ag-specific autoimmunity.

Along with cross presentation, APC homing and maturation are adaptive mechanisms that influence the fate of manipulated T cells. After maturation, APCs express upregulated levels of costimulatory surface ligands CD40, CD80, and CD86, which have been shown to be necessary for the induction of a T cell immune response [57]. Maturation has been shown to be size dependent with amphiphilic poly(glycolic acid) (PGA) nanoparticles, and results indicate surface interactions as well as route of uptake is important in DC maturation [58]. However, uptake does not always result in maturation, and the increasing surface hydrophobicity regardless of uptake has been found to increase expression of MHC II and CD86 maturation markers [59]. Likewise, Chang et al. show nanoparticle size and coating influence DC maturation independent of uptake magnitude with small (270 nm) particles inducing greater anti-inflammatory response than large (560 nm) [53]. Mechanisms of nanoparticle induced APC maturation are largely unknown, but it was recently suggested that ~150nm poly(lactic-co-glycolic acid) (PLGA) NPs induce maturation through MAPK activation, with resulting DC phenotype dependent on particle zeta potential. Barillet et al. observed that the rate of DC uptake of NPs increased as surface charge increased from ~ −20mV to ~ +20mV, but neutral NPs had the highest magnitude of uptake, and uptake magnitude increased DC maturation [60]. Lastly, particle material alone has been found to induce maturation. For example, 10-60nm fullerene derived nanoparticles induced functional DC stimulation skewed toward Th1 polarized response [61], and 20-30nm cationic gold nanoparticles are able to mature DCs without additional stimulus [62]. Together with APC maturation, DC homing to the lymph node is a key component of effective APC targeted immunotherapy. Methods to influence APC homing include materials-based methods as well as applying external stimuli to dynamic particles. One example of this is directing magnetized nanoparticles taken up by DCs into the draining lymph nodes near a tumor to activate Ag-specific CTLs. Jin et al. applied this method and reported 11-fold increase in lymph nodes accumulation of DCs which corresponded to almost complete inhibition of tumor growth after inoculation [63].

2.1.3. Targeting APCs for vaccines—The most common applications for nanoparticle-mediated APC targeting for T cell therapy are APC targeting for vaccines

and APC targeting for immunotherapy. Nanoparticles are particularly useful in vaccine strategies, as they are able to stabilize and protect protein or nucleic acid antigen from rapid degradation *in vivo*. Likewise, nanoparticles can act as adjuvants themselves and actively deliver antigen to APCs through the specific targeting strategies mentioned previously. Several excellent reviews of nanoparticle vaccine technology exist with specific focuses on infectious disease [64], biomaterials [65], cancer [66], and COVID-19 [67]. Emerging areas of nanoparticle vaccine research include investigating the interplay between nanoparticle vaccines, immune modulation, the microbiome, oral vaccine delivery for the generation of mucosal immunity, and vaccines that work synergistically with other arms of T cell therapy [68,69]. For example, Reinhard et al. recently utilized a lipid nanocarrier mRNA vaccine in tandem with CART therapy to generate antigen-specific APCs that bolstered CART performance in a solid tumor [70]. Nicknamed "CARVac", this combination T cell therapy elicited remarkable reduction of tumor burdens *in vivo* in multiple cancer models, and complete tumor rejection after inoculation compared to CART therapy alone, which simply delayed tumor onset.

2.1.4. Targeting APCs for immunotherapy—While targeting APCs for vaccine applications is useful for disease prevention, nanoparticle APC targeting can also be used to direct T cell immunotherapies in active disease states such as cancer or autoimmune disorders. In cancer, it is essential to target not only circulating APCs, but also APCs within the tumor microenvironment for T cell mediated tumor treatment. Although controversial in studies with patients, it has been shown many times in preclinical models that nanoparticles preferentially accumulate in tumors after I.V. injection, including due to the enhanced permeability and retention effect (EPR), originally theorized to be due to leaky vasculature in the tumor [71]. Likewise, rather than focusing on CD4+ T helper cell mediated immunity, in cancer it is beneficial to focus on expanding and enriching cytotoxic lymphocytes and CD8+ effector and memory T cells to combat the active disease state. The ability of nanoparticles to facilitate APC cross priming of exogenous tumor-associated antigen (TAA) is especially important for TAA display on MHC I and subsequent priming of CD8+ T cells. CD103+ dendritic cells have been shown to be the primary APCs that cross prime in the tumor microenvironment [72] and are the drivers of immune checkpoint blockade success [73]. Because of this, targeting CD103+ DC cell subsets may be advantageous for solid tumor immunotherapy. To investigate this, Fromen et al. varied nanoparticle surface charge and demonstrated CD103+ DCs were preferentially targeted with cationic particles, which provides a feasible route for specific targeting of CD103+ DCs by nanoparticles for T cell therapy.

In some diseases such as autoimmune disorders, it is desirable to shift the immune response toward immunosuppression rather than immunostimulation for the purpose of inducing antigen-specific tolerance. Nanoparticles employ many of the same strategies as those discussed for tumor immunotherapy to target APCs, but strategies to modulate APC activity differ. In order to create a tolerogenic T cell response, nanoparticles harness tolerogenic environments such as the gut and liver, impair inflammatory function of APCs while simultaneously delivering antigen, and mimic apoptotic cell death [74]. Specifically, PEGylation of nanoparticles to induce tolerogenic APCs (tolAPCs) has been shown to

control traffic to the spleen dependent on length of the PEG chain [75]. Induction of tolAPCs using nanoparticles has shown significant success in treating autoimmune disorders *in vivo*, and there is a focus on inducing antigen-specific regulatory T cells (Tregs) to mediate these responses. Yeste et al. demonstrated this by encapsulating nanoparticles with an antigen for experimental autoimmune encephalomyelitis (EAE) [76]. APCs treated with the nanoparticles displayed a tolerogenic phenotype and induced 3-fold more FoxP3$^+$ CD4$^+$ T cells which mediated full suppression of EAE in the treatment group.

2.2.   Other cell as APCs

Professional APCs, namely dendritic cells and macrophages, are the most common targets of cancer vaccines due to their efficiency at performing cross-presentation to elicit a strong T cell response; however they present several limitations. First, delivery of vaccine components to the lymph node (LN), where lymphocytes are concentrated, is required for robust immune activation; however this process is currently inefficient [77]. Improvements in nanoparticle design have improved the ability of vaccines to directly traffic to the LN, increasing the efficacy of T cell-inducing vaccines. However, the efficiency of transport to secondary lymphoid organs, and to the specific zones within the lymph node needed to activate CD4+ and CD8+ T cells, is still low. Second, while nanoparticles have greatly improved the ability of a vaccine to induce cross-presentation in APCs, more mechanistic studies of cross-presentation are needed to further improve cancer vaccines [78]. Third, DCs are often dysfunctional in cancer patients, rendering DC targeting for cancer immunotherapy ineffective [79]. Finally, APCs are easily skewed by the tumor microenvironment into an immunosuppressive phenotype [80].

Beyond DCs and macrophages, other APCs, both professional and non-professional, have been targeted for eliciting an antigen-specific T cell response. These cells, including B cells, endothelial cells, and even cancer cells, have unique properties that make them valuable targets for distinct applications.

2.2.1.   B cells as APCs—Although B cells are most commonly associated with humoral immunity, B cells are professional APCs that also play an important role in shaping the endogenous T cell response through both cytokine secretion and direct T cell activation [81]. There is evidence that B cells both regulate the initial expansion of CD4+ T cells after antigen exposure as well as reactivate memory CD4+ T cells [82,83]. While it was initially unclear if B cells also directly activate CD8+ T cells, it is now known that B cells can perform cross-presentation, and that cross-presentation in B cells is integral to their role in mounting a CD8+ T cell response [84]. The expansion and differentiation of activated T cells can also be controlled by B cells, through cytokine and chemokine secretion, to enhance effector responses [85]. Nanoparticles are an ideal platform for the activation of B cells as they can provide the multivalent presentation of antigens required for B cell activation and have superior pharmacokinetics to free protein [86].

In some cases, B cells provide an advantage over DCs for the *ex vivo* activation of T cells as they are more abundant in circulation and easier to culture than DCs [87,88]. However, unlike with DCs, targeting nanoparticles to B cells require an antigen-specific

approach that must specifically trigger intracellular processing that leads to downstream T cell activation. Nanoparticles must trigger clustering of the B cell receptor (BCR), be internalized by the B cell for antigen processing and presentation, then antigen must be presented on MHC molecules to interact with T cells [89]. Importantly, this means that particles must deliver antigen that can be recognized by both B cells and T cells, which may recognize different epitopes. Bennett et al. have specifically investigated antigen features that result in effective B-T cell interaction, as opposed to previous studies which only optimized for B or T cell activation alone [89]. The authors conjugated viral antigens onto a polymer backbone and found that high epitope valency led to increased B cell uptake and antigen processing. Additionally, they found that presentation of the T cell epitope can be enhanced by designing the T cell antigen to be easily processed by the B cell; in this case it was achieved by conjugating the T cell epitope via a cathepsin D-sensitive linker which can be cleaved in the endosome [89]. While B cells are relatively abundant in circulation, antigen-specific B cells are much more rare, occurring at frequencies of less than 0.05% of all B cells [90]. Since native B cell activation is an antigen-specific process, this can greatly hinder the use of B cells for APCs. To combat this, Sicard et al. has developed 400 nm polystyrene particles conjugated with antigen and antibodies specific to the framework region of the BCR, allowing activation of noncognate B cells [88]. These particles triggered BCR clustering and subsequent uptake of particles. Upon internalization, the antigen was cleaved from the particles, processed, and presented on MHC class II for the activation of antigen-specific CD4+ T effector cells.

B cells can also be targeted for *in vivo* vaccination for the purpose of subsequent T cell activation, even without active targeting of B cells. Despite the spatial separation of B cells and T cells within the lymph node, B cells can travel from B cell follicles into T cell zones to deliver a strong, albeit temporally delayed, stimulation to CD4+ T cells [89]. In fact, it is shown that upon delivery of a virus-derived nanoparticle vaccine, B cells are the dominant APC initiating CD4+ T cell activation [91]. The virus-like particles (VLPs), approximately 20nm, were derived from E. Coli BL21 infected with bacteriophage Qβ. These particles maintain the surface properties of the virus, but no longer replicate, making them ideal delivery vehicles. The VLPs used here did not contain any active B cell targeting moieties, suggesting that other particle properties, in this case antigen presentation density, can also contribute to the shape of the APC response. As DCs uptake antigen non-specifically, they require large amounts of antigen in order to obtain a high enough dose intracellularly to initiate maturation. B cells, on the other hand, uptake antigen via high affinity receptor binding, allowing them to still be able to uptake sufficient amounts of antigen even when a low dose of antigen is present [91]. In this study, about 20-30 antigen epitopes per particle was sufficient to preferentially activate B cells; however, this density should be further explored. While this phenomenon may depend on the antigen type and largely be a virus-specific response, researchers can capitalize on this finding to create vaccine particles that preferentially activate B cells rather than other APCs. As described above, targeting B cells specifically allows therapies to bypass many of the disadvantages presented by DC targeting. Additionally, it is thought that B cells are involved with breaking immune tolerance, making them particularly valuable targets for therapeutic cancer vaccines [91].

2.2.2. Cancer cells as APCs—Cancer cells are not often grouped in the category of antigen presenting cells, but like most cells in the body, they do present antigen on MHC class I molecules. Similar to other non-professional APCs, interactions between cancer cells and T cells often results in a tolerizing response. This effect is due not only to the fact that as a non-professional APC, cancer cells express low levels of costimulatory molecules, but also due to the overall immunosuppressive tumor microenvironment in which the interaction takes place. However, cellular engineering techniques have allowed researchers to turn this tolerizing interaction into an activating one [92].

Tumors are attractive targets for APC engineering for several reasons. First, tumor cells already present tumor-specific antigens, meaning that a therapy could be designed to be antigen-agnostic, not requiring knowledge of specific cancer neoantigens *a priori*. This provides an advantage over traditional personalized cancer vaccines as it eliminates the need for tumor excision and proteome analysis prior to vaccine production. Second, unlike with professional APCs, there is no need to induce cross-presentation through the nanoparticle design; however, some tumors may require treatment to maintain or enhance MHC expression. Finally, nanoparticle targeting of tumor is much more efficient than targeting of LNs, as needs to be done for most APC targeting. The concept of engineering tumor cells to behave like APCs was introduced as early as 1990s [93] and has been advanced by the advent of nanotechnology, which can improve the intracellular delivery of gene-editing molecules as well as tumor cell targeting.

The first use of tumor cells as APCs was in tumor cell vaccines. In this therapeutic strategy, which has already reached the clinic, tumor cells from resected tumors are irradiated to prevent further proliferation, genetically modified *ex vivo* to express cytokines or costimulatory molecules, and then re-injected into patients [94]. Nanoparticles provide a highly efficient platform for gene delivery to tumor cells as they have high nucleic acid loading capacity and show efficient cell uptake [95]. The *ex vivo* engineering of tumor cells has often been carried out by viral vectors, including retroviruses, adenoviruses, and adeno-associated viruses (AAV), due to their highly efficient RNA and DNA delivery. These particles have been used to induce expression of immunostimulatory molecules in tumor cells such as IL-12, IL-2, IL-4, IL-6, TNF-α, IFN-γ, and granulocyte-macrophage colony-stimulating factor (GM-CSF) [94]. This therapy can also be augmented with the delivery of soluble costimulatory molecules like OX40, 4IBB, and CD40 [94].

Researchers have also explored the *in situ* modification of tumor cells. Because of safety concerns regarding the *in vivo* use of viral delivery vehicles, gene modification of tumor cells *in vivo* can be investigated with non-viral vectors such as lipid, polymeric, and inorganic nanoparticles [95]. This approach offers several advantages over other cancer vaccine approaches including eliminating the need for *ex vivo* cell manipulation, tumor protein expression profiling, and personalized therapy manufacturing. For example, Tzeng et al. have developed poly(beta-amino ester) (PBAE) nanoparticles to deliver IL-12 and 4-1BBL DNA to tumor cells, resulting in a highly functional antigen-specific T cell response [96]. Similarly, Huang et al. created tumor-targeting lipid-dendrimer-calcium-phosphate (TT-LDCP) nanoparticles to deliver IL-12 DNA and PD-L1 siRNA to the tumor, to both support T cell activation and suppress T cell inhibition [97]. In addition to turning

tumor cells into effective APCs from the inside out, nanoparticles can also be used to deliver costimulation molecules externally. For example, Kosmides et al. developed "immunoswitch" particles, which presented both anti-PD-L1 and anti-4-1BB molecules on an iron dextran particle such that the particle binds PD-L1 on tumor cells and 4-1BB on T cells, converting an inhibitory signal into a stimulatory signal [98]. The tumor cells present signal 1 naturally, and the immunoswitch assists the tumor cells in providing costimulation to the T cells.

2.3. T cell targeting

Vaccine platforms and APC targeting are indirect ways of activating T cells and can be useful methods for achieving antigen specificity. Alternatively, the use of nanoparticle-based artificial antigen presenting cells (aAPCs) can bypass the need to use endogenous cells while also allowing different parameters that affect T cell expansion to be altered.

2.3.1. aAPCs to directly stimulate T cells *ex vivo*—Currently, *ex vivo* T cell expansion remains a key step in adoptive T cell therapy. Nanoscale platforms that mimic natural APCs offer an attractive option for this process; they can be readily produced, easily stored, and properties of nanoparticles can be finetuned to suit expansion needs. aAPCs mimic essential functions of APCs by engaging the TCR and costimulatory molecules of the T cell [99]. While some aAPCs are constructed using anti-CD3 and anti-CD28 antibodies to non-specifically expand T cells as has been studied for treatment of HIV, a peptide-MHC, pMHC, complex is necessary for an antigen-specific response [100]. aAPCs have been readily utilized for *ex vivo* T cell expansion and various factors, such as ligand density and spacing as well as general material properties can be controlled to affect the efficiency of T cell expansions and therefore can be used to modulate the potency of the cellular therapy [101,102].

T cells that have the ability to recognize epitopes of TAAs provide a potent response against tumors; however, precursors for antigen-specific cells are rare which makes expanding them for adoptive transfer difficult [2,8]. To address this, Perica et al.[2] developed an enrichment and expansion (E+E) method using magnetic nanoparticle based aAPCs. The aAPCs had MHC class I loaded with melanoma associated antigens, a colon carcinoma antigen, or a Kb-restricted ovalbumin antigen along with anti-CD28 [2]. Naïve CD8+ T cells from a wild type B6 mouse were incubated with the aAPCs for the enrichment step then placed in a magnetic column. This allowed for the unbound nonspecific cells to be discarded while the desired T cells could be eluted from the column and cultured. For one of the melanoma associated antigens, Kb-TRP2, the cognate population expanded from roughly 0.03% to 17.6% by day 7 [2]. Ichikawa et al. [103] utilized this E+E platform with human HLA Class I molecules loaded with the MART-1 peptide, to expand cognate T cells from patient PBMCs. The study showed that the nano-aAPCs were able to expand MART 1 specific CD8^{+} T cells *ex vivo* more effectively than autologous dendritic cells and CD3/CD28 Dynabeads, a commercially available aAPC. Upon phenotypical analysis, a higher population of stem cell memory cognate T cells was seen from the group expanded with the nano-aAPCs [103]. Thus, this ability to engage specific T cells for ex vivo expansion can

have great implications for the preparation of endogenous T cells for use in adoptive T cell therapies.

In the case of cancer and chronic viral infections, CD8 T cells begin to express the inhibitory signals, commonly known as checkpoint signals, because of continuous stimulation by a cognate antigen [104]. This is one of the contributing factors to effector T cell suppression in the tumor microenvironment. Kosmides et al. [100] recapitulated this scenario *in vitro* by stimulating CD8 T cells with PLGA aAPCs multiple times to upregulate PD-1 expression. The aAPCs alone led to 30- and 20-fold expansion of PMEL and 2C transgenic CD8+ T cells, respectively. Further, the addition of PD-1 blockade led to enhanced activation of antigen-specific cells as seen by a 3.5-fold increase in IFN gamma secretion, which show promise in enhancing adoptively transferred T cells [100]. While PLGA particles are advantageous in that they are biodegradable and are suitable for *in vivo* use, they also can have certain limitations, including generally low protein conjugation efficiency [105]. This can then correspond to less efficient T cell activation due to a decrease in ligand density [101]. Biodegradable polymer blends can be used to construct aAPCs with increased ligand density and improved performance, including by blending PLGA, which is negatively charged, with PBAE, which is positively charged. Rhodes et al. [105] found a 1.5-fold increase in the efficiency of signal conjugation with the composite biomaterial (Figure 2B). When compared to PLGA only aAPCs, the PLGA/PBAE aAPCs bound to a larger number of antigen-specific T cells, resulting in a 35-fold increase in mean fluorescence intensity (Figure 2C). These particles led to a 15-fold greater expansion and were observed more efficient at stimulating CD8 T cells [105]. Related aAPCs constructed of polymer blends were also shown to be useful for inducing Foxp3+ Tregs [106].

Zhang et al [107] fabricated nanoparticles with high signal conjugation efficiency by functionalizing magnetic nanoclusters with azide-engineered leucocyte membranes. The azide functionalization was used to take advantage of click chemistry which required the peptide MHC and anti-CD28 to undergo mild modification while retaining complete function and efficacy. These aAPCs were able to achieve 78-fold expansion by day 3 [107]. A separate group also utilized an immune cell membrane as a part of their aAPC; Xiao et al [108] prepared an imiquimod-loaded aAPC from PLGA nanoparticles coated with anti-CD3 and a dendritic cell membrane. The DC membrane retained MHC and signal 2 expression allowing for antigen specificity. Using these nanoparticles, the CD8+ T cell population increased 26 % more when compared to the control (PBS) and T cell functionality was improved as depicted by the increase in TNF-α and IFN-γ by 8.7- and 8.4-fold, respectively [108].

2.3.2. aAPCs to directly stimulate T cells *in vivo*—By engineering biocompatible and biodegradable aAPCs, one could also directly target T cells *in vivo*, avoiding the need for *ex vivo* expansion. The same parameters that affect *ex vivo* T cell activation also affect *in vivo* T cell activation, and in some cases, the effects of certain parameters are magnified *in vivo*. The viscoelastic moduli of natural APCs were recorded for dendritic cells, macrophages, and monocytes and found to be 440 +100/−90 Pa, 900+110/−100 Pa, and 520 +90/−80 Pa respectively [109]. Bufi et al. [109] demonstrated that the elasticity of endogenous APCs varied in the presence or absence of inflammation; in inflammatory

conditions the stiffness of APCs were found to increase. This offers insight into properties that affect engagement between T cells and APCs in the setting of cancer, as chronic inflammation and cancer have a close relationship [110]. The stiffness of NPs affects cellular uptake and circulation within the blood. Anselmo et al. [111] evaluated the effects of particle stiffness *in vivo* by fabricating soft (10 kPa) and hard (3000 kPa) PEG-based hydrogel NPs . To compare circulation of the different NPs, each was injected intravenously into mice. Both soft and hard NPs, sized at 200 nm, persisted for long periods *in vivo*. However, the soft NPs were found to persist longer at higher concentrations in circulation; distribution half-life and elimination half-life for the soft NPs were longer than those of the hard NPs [112]. To evaluate cellular uptake of these NPs a mouse IgG antibody was conjugated to both soft and hard NPs to encourage interaction with macrophages. There was a significant difference between the soft and hard NPs, the hard NPs were phagocytosed at much higher rates, making softer NPs more desirable for T cell targeting [112]. Kong et al. [113] saw a similar stiffness related effect: softer NPs remained in circulation longer. The data also showed that the softer NPs accumulated in the spleen and liver more than the harder NPs. NP elasticity may also affect T cell activation *in vivo*. Our data has shown that when comparing CD3 cluster areas of T cells, those activated on a softer substrate (0.5 kPa) exhibited greater clustering than those activated on a harder substrate (3 kPa) [114]. The interaction between aAPCs and T cells seeks to mimic that of the endogenous interaction. The surface fluidity of a NP can be engineered to closely mimic the membrane fluidity of an APC. This enhances T cell activation by increasing movement of ligands and favoring clustering of receptors within the immune synapse[115,116]. Olden et al.[116] constructed silica particles with different lipid compositions in order to alter membrane fluidity and evaluate its effect on T cell activation. After incubating the particles with human T cells, they found that as surface fluidity increased there was a significant increase in fold expansion, thus a more robust expansion was achieved [116].

Particle shape is another key parameter that impacts T cell activation. Sunshine et al. and Meyer et al. have shown that elongated, or ellipsoidal, aAPCs provide increased contact with T cells (Figure 2A–F), which results in increased fold expansion (Figure 2G) [32,117]. When used *in vivo*, ellipsoidal aAPC were also better able to activate T cells against cancer antigens, resulting in reduced tumor growth compared to spherical particles (Figure 2H). Interestingly, the interface of the T cell-aAPC interaction is not the only force that allows for ellipsoidal particles to perform better *in vivo*. Meyer et al. [32] found an additional layer of help provided by ellipsoidal particles; spherical aAPCs were more readily engulfed by macrophages than the ellipsoidal aAPCs (Figure 2I), allowing for ellipsoidal aAPCs to remain in circulation for longer than their spherical counterparts, even with all other parameters (surface area, volume, and protein amount) remaining approximately the same. This finding is consistent with previous reports that have shown that ellipsoidal particles are taken up slower by macrophages than spherical particles, potentially because the amount of energy required for actin remodeling during the phagocytosis process is larger for particles with a greater aspect ratio [118,119]. I*n vivo*, ellipsoidal aAPCs resulted in 2-fold greater expansion of cognate CD8 T cells (Figure 2J). Alternatively, Bruns et al. [120] enhanced the biodistribution and *in vivo* functionality of their aAPCs by including CD47, a "don't eat me" signal to inhibit phagocytosis mediated by macrophages. CD47+ aAPCs outperformed

CD47- aAPCs *in vivo*; particles were able to stimulate antigen-specific T cells better as they accumulated more in the lymphoid organs. This correlated with statistically significant inhibition of tumor growth [120]. Song et al. [121] used similar approaches to Meyer et al. [32] and Bruns et al. [120]; this group combined PEGylated ellipsoidal aAPCs with a CD47-Fc conjugate (EaAPC[PEG/CD47]). These aAPCs were able to increase percentage of INF-gamma positive T cells, as well as enhance their ability to infiltrate tumors and inhibit growth [121]. Other aAPC parameters, such as ligand density and particle size, have also been shown to not only affect T cell activation *ex vivo*, by controlling the contact between a T cell and the aAPC, but also *in vivo* biodistribution [32,101]. The literature suggests that one apparent feature of nanosized aAPCs is that they can have improved performance at activating T cells if they are able to re-create a biomimetic micron length-scale of interaction between the aAPC surface and the T cell surface, mimicking a biological DC. For nanoparticles, this can be enabled through clustering, facilitated by nanoparticle ligands or an externally applied magnetic field, or by an elongated particle shape, such that at least one particle axis approaches the micron scale [22,23]. Thus, the physical and chemical properties of a nanoparticle can be engineered to improve the ability of aAPCs to stimulate T cells both *ex vivo* and *in vivo*.

2.3.3. Gene delivery to generate antigen-specific T cells—Gene delivery, which has clinical relevance for *in vivo* and *ex vivo* applications, can be achieved through viral or non-viral methods [122]. CAR and TCR engineering offer an alternative way of producing antigen-specific T cells and allow researchers to bypass the need to find existing T cells that naturally recognize their target. A variety of nanoscale delivery vehicles such as vectors and other biomaterial-based nanoparticles can be utilized to deliver genes to T cells and produce antigen specificity [8,123]. Viral vectors including, but not limited to adenoviral vectors, adeno-associated vectors, and retroviral vectors, have varying benefits and drawbacks. For example, retroviral vectors integrate into the host cell genome and work well for *ex vivo* transfections but can only transfect dividing cells and do not show promise for *in vivo* applications. Adenoviral vectors, however, can transfect nondividing and dividing cells but preexisting immunity to adenoviruses is common and in severe cases, has resulted in the death of patients [124]. Non-viral methods, although generally less efficient than viral vectors, are considered to be a safer method for gene delivery [124,125]. They serve as an attractive alternative for T cell reprogramming; constitutive CAR expression and prolonged CAR T cell activity caused by viral vector transfection can cause potentially fatal cytokine storms [123,124]. However, T cells can be difficult to transfect using non-viral systems because of their limited ability to engage in endocytosis [126]. Electroporation, a method of non-viral transfection, uses electrodes to disrupt the cell membrane to allow for large molecules to enter the cell [124]. It has been shown to have low rates of successful transfection and decrease cell viability [126]. Loaded NPs are another method by which non-viral gene delivery can be achieved. McKinlay et al. [126] found that altering combinations of lipids used for fabricating cationic lipid NPs greatly impacted mRNA delivery; cell membranes are nonpolar and the charges of the lipids impacts gene transfer. Billingsley et al. [127] created lipid NPs that could deliver mRNA to human T cells for CAR reprogramming. This was done without the need for electroporation, which

maintained the function and viability of the CAR T cells as shown by percent killing of Nalm-6 acute lymphoblastic leukemia cells [127].

Despite advances in *ex vivo* generation of TCR and CAR T cells, the process remains expensive and labor-intensive. To address the high cost of *ex vivo* CAR and TCR transfections, Parayath et al. [123] sought to target T cells *in vivo*, eliminating the need for complicated *ex vivo* expansions. Parayath et al. [123] successfully constructed *in vitro*-transcribed (IVT) mRNA-loaded polymer NPs for human T cell transfection with CD19-specific 1928z CAR or with the HBcore18-27 TCR (Figure 3A and 3B) . The particles were formulated with a biodegradable PBAE inner core and a PGA exterior. This material combination helped to decrease off target uptake of particles by reducing the overall charge of the particles [123]. The particles were also coated with anti-CD8, which increased transfection efficiency compared to particles that lacked this antibody. *In vivo*, it was found that the NPs led to successful transfection of the T cells (Figure 3C) with relatively little side effects. Adoptively transferred virally-transduced CAR T cells eradicated 60% of tumors and improved survival by an average of 32 days, while the IVT mRNA loaded NP-transfected 1928z CAR T cells eradicated 70% of tumors and improved survival by 37 days [123]. This platform also showed promise for solid tumors, specifically T cells transfected with ROR1 CAR transgene against prostate cancer. In addition, the authors found that the NPs may serve as a useful alternative to current *ex vivo* platforms as there was no significant difference in overall function of T cells transfected using the IVT mRNA NPs compared to T cells transduced using viral techniques based on cytokine measurements and target cell killing (Figure 3D and 3E) [123]. This same group has also utilized using DNA-carrying PBAE NPs for this purpose. Smith et al. [128] fabricated NPs decorated with anti-CD3e f(ab')2 to transfect T cells with the 194-1BBz CAR for leukemia. *In vivo* the particles had low off target effects as they bound preferentially to T cells and did not contribute to systemic toxicity as determined by blood work and cell counts. The NPs were able to able to transfect T cells, which led to 5.5 fold proliferation of CAR T cells and increase in memory like phenotype (CD44+CD62L+) [128].

3.   Maintaining an antigen-specific response

Generation of the antigen-specific T cell response, whether it is done *ex vivo* or *in vivo*, is only the first step in producing a successful immunotherapy. Maintenance of the T cell response *in vivo* can be equally challenging and is a barrier to success for both cancer therapies and autoimmune therapies. Some of the major challenges that must be addressed to bring T cell immunotherapy into the forefront include improving the persistence and preventing the exhaustion of T cells, which can be achieved through influencing T cell differentiation and metabolism as well as addressing the immunomodulatory properties of the tissue being treated. Nanoparticles can assist in this arena by allowing targeted and controlled delivery of immunomodulators to support *in vivo* functionality.

3.1.   Cytokine delivery

Cytokine support is critical for T cell activation, differentiation, and survival. Traditionally, ACT is accompanied by systemic IL-2 injections to achieve the prolonged *in vivo*

support required by T cells; however, cytokines are notoriously unstable, have poor pharmacokinetics, and demonstrate mixed results in patients due to cytokine pleiotropy [129]. Additionally, systemic cytokine injections have been associated with toxicity [130]. Nanoparticles can address these challenges by providing sustained, local, and temporally controlled delivery of both soluble and particle-bound cytokines [131]. Controlled release of soluble cytokines can be achieved with biodegradable nanoparticles, using materials such as PLGA [132], hydrogels, and liposomes [133]. Cytokines can also be conjugated to the surface of particles, which allows for more control over how T cells interact with the presented cytokine, as well as less potential for released soluble cytokines to act systemically instead of locally [134,135].

Cytokine-carrying nanoparticles can be functionalized to actively target specific immune cells or specific tissues. One method of this targeting is to fix the orientation of the presented cytokine, which has been shown to significantly impact which immune cell subtypes get activated. For example, IL-2 interacts differentially with T effector cells and T regulatory cells due to the slightly different forms of the IL-2 receptor expressed on these cells. Presenting IL-2 in such a way that interacts more efficiently with one receptor complex over another has allowed researchers to target immunostimulatory or immunosuppressive cells with specificity [134]. Another way to target nanoparticles to T cells is to add a targeting ligand to the particle that is specific to a marker expressed on T cells [135,136]. T cells may also be conjugated with nanoparticle "backpacks," providing local cytokine support that can be delivered in a highly-controlled, antigen-specific manner [137,138]. Finally, rather than targeting T cells directly, some research groups have taken a tissue-targeting approach, for example, using tumor-specific markers [97]. Here Huang et al. delivered cytokine DNA rather than protein, allowing for longer term expression of cytokine, highlighting another promising approach for cytokine delivery.

3.2. Vaccine boosts

In vivo activation of T cells is an emerging area, but complications such as efficient T cell targeting by APCs has hindered its translation. Even in systems in which T cells are activated *ex vivo*, continued proliferation and stimulation following T cell transfer has been correlated with treatment success. One approach to provide continued support to *ex vivo* expanded T cells is to co-deliver the activated T cells with a vaccine. It is thought the vaccine provides support to the T cells through multiple mechanisms, including direct cytokine secretion for T cell proliferation and differentiation to memory cells [139,140], stimulating other host immune cells to support the transferred cells, and even stimulating the immune system to recognize different targets that compliment those that are recognized by the transferred T cells [141]. This is especially useful for CAR T cell therapy, which classically targets only one tumor antigen at a time, often leading to tumor escape [142]. Vaccine boosts for CAR T cells have shown that they can not only boost the transferred CAR T cells, prolonging persistence of the cells, but they also provide protection against tumors which have lost the CAR-specific antigen [70,139]. Nanoparticles are an ideal platform for delivery of the vaccine boost, as their ideal pharmacokinetics allow them to support T cells both at the tumor site and in secondary lymphoid organs.

3.3. Small molecule delivery

Small molecule drugs can also be used to alter T cell phenotype and function by interfering with or enhancing signaling and metabolic pathways [143,144]. As with cytokines, delivering small molecules systemically can have off-target effects and toxicity. In addition, some small molecules have poor penetration of cells due to size or charge [145]. Nanoparticles can improve cellular uptake of small molecules as well as provide active targeting to specific cells. For example, Li et al. designed a "trident" nanogel that used a peptide agonist of PD-L1 to target the delivery of an adjuvant and IDO inhibitor to T cells [146]. The nanogel better equipped the T cells to survive the tumor microenvironment and resulted enhanced tumor treatment. TGF-β inhibition is another popular target of immunomodulating small molecule drugs. Yang et al. constructed gold nanoparticles carrying anti-CD8 antibodies and a TGF-β receptor inhibitor [147]. This system increased the amount of small molecule drug that accumulated in CD8$^+$ T cells as compared to delivering free drug and improved anti-cancer efficacy when given in conjunction with a vaccine. Ou et al. also delivered a TGF-β inhibitor, here using PLGA nanoparticles conjugated with either anti-CD8, a T cell-specific marker, or anti-PD-1, a functional marker, to further target delivery to exhausted T cells [136]. Finally, rather than just enhancing T effector cells, nanoparticles can also target and inhibit immunosuppressive cells in the tumor microenvironment, such as T regulatory cells [148].

3.4. Checkpoint blockade

Improvements in the persistence, phenotype, and function of antigen-specific T cells can still be overwhelmed by the immunosuppressive tumor microenvironment. Checkpoint blockade inhibitors (CBI) have seen great clinical success with restoring function to T cells that were previously blocked by inhibitory signals expressed by tumor cells, both as a stand-alone therapy and in conjunction with ACT [149]. Nanoparticles can offer improvements to the traditional antibody-based CBI by improving pharmacokinetics, enhancing trafficking into the tumor, and providing a sustained release of CBI molecules [150–152]. ACT and CBI can also be combined with physical therapies, such as photothermal therapy [153]. Zhang et al. used anti-PD1 iron oxide nanoparticles combined photothermal therapy to increase CD8+ T cell infiltration and efficacy in cancer treatment. Kosmides et al. used nanoparticles to not only deliver CBI, but also costimulation by conjugating both anti-PD-L1 and anti-4-1BB to the same nanoparticle, effectively switching inhibitory signals into stimulatory signals [98]. Additionally, nanoparticle-mediated siRNA delivery has also been shown to effectively knockdown checkpoint molecule expression and restore antigen-specific T cell activity [154,155].

4. Conclusion

Unlocking the ability to generate an antigen-specific T cell response has been of great interest since the advent of immunotherapy. As fundamental immunology research uncovered mechanisms of T cell activation, engineers have been better able to emulate or enhance this process using cellular engineering, protein engineering, and biomaterials design. Mounting an antigen-specific T cell response starts with inducing or engineering effective antigen presentation. This can be accomplished using nanoparticle vaccine delivery

to professional and non-professional antigen presenting cells, or by directly engaging T cells. Impressively, nanoparticle-targeted APCs or nanoparticle aAPCs can dramatically enrich natural antigen-specific T cells from an endogenous T cell repertoire, where antigen-specific cells are found at a frequency of around 1/100,000. Beyond activating endogenous cells, antigen-specificity can be engineered into the T cells through strategies such as CAR T and TCR engineering. After generating antigen-specific T cell responses, maintaining them involves providing cytokine support, skewing T cells towards a memory phenotype, and overcoming the immunomodulatory microenvironments of the tissue in which the T cell is acting. Nanoparticles have the potential to launch T cell-based therapies into the forefront of immunotherapy. Nanoparticles are a modular platform that are relatively easy to produce, modify, and personalize for each patient. Biocompatibility, and in many cases biodegradability, of these particles also allows us to modulate T cells *in vivo* rather than just *ex vivo*. Throughout this review we have discussed various nanoparticle properties that impact cell-particle interaction, immunomodulation, and biodistribution, such as size, shape, signal composition, and material choice. While progress in these fields is steadily advancing, there are still several challenges facing the translation and efficacy of nanoparticle-based T cell therapies. Many current approaches do not wholly appreciate the complexity of the immune response to immunotherapies. For example, more research has been done in recent years that has uncovered new subsets of immune cells that were previously thought to be one homogenous population. Understanding these subsets and how to target each individually will be invaluable to improving the efficacy of immunotherapy and reducing off target effects or toxicity. In general, more care needs to be taken in research to understand the mechanism of action of those therapies. Where nanoparticles have a particularly unique role to play in advancing immunotherapy is in reducing the cost and complexity of T cell therapy. The ability to create "universal" or "off-the-shelf" particles, using antigen-agnostic approaches, will reduce the manufacturing burden compared to cell-based therapies and broaden the range of patients who could be effectively treated by T cell immunotherapy.

Acknowledgements

7. References

[1]. Rosenberg SA, Restifo NP, Yang JC, Morgan RA, Dudley ME, Adoptive cell transfer: a clinical path to effective cancer immunotherapy., Nat. Rev. Cancer. 8 (2008) 299–308. 10.1038/nrc2355. [PubMed: 18354418]

[2]. Perica K, Bieler JG, Schütz C, Varela JC, Douglass J, Skora A, Chiu YL, Oelke M, Kinzler K, Zhou S, Vogelstein B, Schneck JP, Enrichment and Expansion with Nanoscale Artificial Antigen Presenting Cells for Adoptive Immunotherapy, ACS Nano. (2015). 10.1021/acsnano.5b02829.

[3]. Goff SL, Dudley ME, Citrin DE, Somerville RP, Wunderlich JR, Danforth DN, Zlott DA, Yang JC, Sherry RM, Kammula US, Klebanoff CA, Hughes MS, Restifo NP, Langhan MM, Shelton TE, Lu L, Kwong MLM, Ilyas S, Klemen ND, Payabyab EC, Morton KE, Toomey MA, Steinberg SM, White DE, Rosenberg SA, Randomized, Prospective Evaluation Comparing Intensity of Lymphodepletion Before Adoptive Transfer of Tumor-Infiltrating Lymphocytes for Patients With Metastatic Melanoma., J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol 34 (2016) 2389–2397. 10.1200/JCO.2016.66.7220.

[4]. Besser MJ, Shapira-Frommer R, Itzhaki O, Treves AJ, Zippel DB, Levy D, Kubi A, Shoshani N, Zikich D, Ohayon Y, Ohayon D, Shalmon B, Markel G, Yerushalmi R, Apter S, Ben-Nun A, Ben-Ami E, Shimoni A, Nagler A, Schachter J, Adoptive transfer of tumor-infiltrating lymphocytes in patients with metastatic melanoma: intent-to-treat analysis and efficacy after failure to prior immunotherapies., Clin. Cancer Res. an Off. J. Am. Assoc. Cancer Res 19 (2013) 4792–4800. 10.1158/1078-0432.CCR-13-0380.

[5]. Robbins PF, Kassim SH, Tran TLN, Crystal JS, Morgan RA, Feldman SA, Yang JC, Dudley ME, Wunderlich JR, Sherry RM, Kammula US, Hughes MS, Restifo NP, Raffeld M, Lee CCR, Li YF, El-Gamil M, Rosenberg SA, A pilot trial using lymphocytes genetically engineered with an NY-ESO-1-reactive T-cell receptor: Long-term follow-up and correlates with response, Clin. Cancer Res (2015). 10.1158/1078-0432.CCR-14-2708.

[6]. Shah NN, Fry TJ, Mechanisms of resistance to CAR T cell therapy, Nat. Rev. Clin. Oncol (2019). 10.1038/s41571-019-0184-6.

[7]. Hay KA, Turtle CJ, Chimeric Antigen Receptor (CAR) T Cells: Lessons Learned from Targeting of CD19 in B-Cell Malignancies, Drugs. (2017). 10.1007/s40265-017-0690-8.

[8]. Wu R, Forget MA, Chacon J, Bernatchez C, Haymaker C, Chen JQ, Hwu P, Radvanyi LG, Adoptive T-cell therapy using autologous tumor-infiltrating lymphocytes for metastatic melanoma: Current status and future outlook, Cancer J. (2012). 10.1097/PPO.0b013e31824d4465.

[9]. Dudley ME, Wunderlich JR, Shelton TE, Even J, Rosenberg SA, Generation of Tumor-Infiltrating Lymphocyte Cultures for Use in Adoptive Transfer Therapy for Melanoma Patients, J. Immunother (2003). 10.1097/00002371-200307000-00005.

[10]. Govers C, Sebestyén Z, Coccoris M, Willemsen RA, Debets R, T cell receptor gene therapy: strategies for optimizing transgenic TCR pairing, Trends Mol. Med (2010). 10.1016/j.molmed.2009.12.004.

[11]. June CH, Adoptive T cell therapy for cancer in the clinic, J. Clin. Invest (2007). 10.1172/JCI32446.

[12]. Levine BL, Miskin J, Wonnacott K, Keir C, Global Manufacturing of CAR T Cell Therapy, Mol. Ther. - Methods Clin. Dev (2017). 10.1016/j.omtm.2016.12.006.

[13]. Kelderman S, Heemskerk B, Fanchi L, Philips D, Toebes M, Kvistborg P, van Buuren MM, van Rooij N, Michels S, Germeroth L, Haanen JBAG, Schumacher NM, Antigen-specific TIL therapy for melanoma: A flexible platform for personalized cancer immunotherapy, Eur. J. Immunol (2016). 10.1002/eji.201545849.

[14]. Siriwon N, Kim YJ, Siegler E, Chen X, Rohrs JA, Liu Y, Wang P, CAR-T cells surface-engineered with drug-encapsulated nanoparticles can ameliorate intratumoral T-cell hypofunction, Cancer Immunol. Res (2018). 10.1158/2326-6066.CIR-17-0502.

[15]. Marofi F, Motavalli R, Safonov VA, Thangavelu L, Yumashev AV, Alexander M, Shomali N, Chartrand MS, Pathak Y, Jarahian M, Izadi S, Hassanzadeh A, Shirafkan N, Tahmasebi S, Khiavi FM, CAR T cells in solid tumors: challenges and opportunities, Stem Cell Res. Ther (2021). 10.1186/s13287-020-02128-1.

[16]. Bonifant CL, Jackson HJ, Brentjens RJ, Curran KJ, Toxicity and management in CAR T-cell therapy, Mol. Ther. - Oncolytics 3 (2016) 16011. 10.1038/mto.2016.11. [PubMed: 27626062]

[17]. Getts DR, Shea LD, Miller SD, King NJC, Harnessing nanoparticles for immune modulation, Trends Immunol. 36 (2015) 419–427. 10.1016/j.it.2015.05.007. [PubMed: 26088391]

[18]. Wang H, Mooney DJ, Biomaterial-assisted targeted modulation of immune cells in cancer treatment, Nat. Mater 17 (2018) 761–772. 10.1038/s41563-018-0147-9. [PubMed: 30104668]

[19]. Dunn ZS, Mac J, Wang P, T cell immunotherapy enhanced by designer biomaterials, Biomaterials. 217 (2019) 119265. 10.1016/j.biomaterials.2019.119265. [PubMed: 31271861]

[20]. Irvine DJ, Hanson MC, Rakhra K, Tokatlian T, Synthetic Nanoparticles for Vaccines and Immunotherapy, Chem. Rev 115 (2015) 11109–11146. 10.1021/acs.chemrev.5b00109. [PubMed: 26154342]

[21]. Cevaal PM, Ali A, Czuba-Wojnilowicz E, Symons J, Lewin SR, Cortez-Jugo C, Caruso F, In Vivo T Cell-Targeting Nanoparticle Drug Delivery Systems: Considerations for Rational Design, ACS Nano. (2021). 10.1021/acsnano.0c09514.

[22]. Perica K, Tu A, Richter A, Bieler JG, Edidin M, Schneck JP, Magnetic field-induced t cell receptor clustering by nanoparticles enhances t cell activation and stimulates antitumor activity, ACS Nano. 8 (2014) 2252–2260. 10.1021/nn405520d. [PubMed: 24564881]

[23]. Kosmides AK, Necochea K, Hickey JW, Schneck JP, Separating T Cell Targeting Components onto Magnetically Clustered Nanoparticles Boosts Activation, Nano Lett. 18 (2018) 1916–1924. 10.1021/acs.nanolett.7b05284. [PubMed: 29488768]

[24]. Stavis SM, Fagan JA, Stopa M, Liddle JA, Nanoparticle Manufacturing-Heterogeneity through Processes to Products, ACS Appl. Nano Mater 1 (2018) 4358–4385. 10.1021/acsanm.8b01239.

[25]. Ben-Akiva E, Witte SE, Meyer RA, Rhodes KR, Green JJ, Polymeric micro-and nanoparticles for immune modulation, Biomater. Sci (2018). 10.1039/c8bm01285g.

[26]. Sanz-Ortega L, Portilla Y, Pérez-Yagüe S, Barber DF, Magnetic targeting of adoptively transferred tumour-specific nanoparticle-loaded CD8+ T cells does not improve their tumour infiltration in a mouse model of cancer but promotes the retention of these cells in tumour-draining lymph nodes, J. Nanobiotechnology 17 (2019). 10.1186/s12951-019-0520-0.

[27]. Cruz LJ, Tacken PJ, Rueda F, Domingo JC, Albericio F, Figdor CG, Targeting nanoparticles to dendritic cells for immunotherapy, Methods Enzymol. 509 (2012) 143–163. 10.1016/B978-0-12-391858-1.00008-3. [PubMed: 22568905]

[28]. Hu G, Guo M, Xu J, Wu F, Fan J, Huang Q, Yang G, Lv Z, Wang X, Jin Y, Nanoparticles targeting macrophages as potential clinical therapeutic agents against cancer and inflammation, Front. Immunol 10 (2019) 1998. 10.3389/FIMMU.2019.01998/BIBTEX. [PubMed: 31497026]

[29]. Gaudino SJ, Kumar P, Cross-talk between antigen presenting cells and T cells impacts intestinal homeostasis, bacterial infections, and tumorigenesis, Front. Immunol 10 (2019) 360. 10.3389/fimmu.2019.00360. [PubMed: 30894857]

[30]. Chattopadhyay S, Chen JY, Chen HW, Jack Hu CM, Nanoparticle vaccines adopting virus-like features for enhanced immune potentiation, Nanotheranostics. 1 (2017) 244–260. 10.7150/ntno.19796. [PubMed: 29071191]

[31]. Jia J, Zhang Y, Xin Y, Jiang C, Yan B, Zhai S, Interactions Between Nanoparticles and Dendritic Cells: From the Perspective of Cancer Immunotherapy, Front. Oncol 8 (2018) 404. 10.3389/fonc.2018.00404. [PubMed: 30319969]

[32]. Meyer RA, Sunshine JC, Perica K, Kosmides AK, Aje K, Schneck JP, Green JJ, Biodegradable Nanoellipsoidal Artificial Antigen Presenting Cells for Antigen Specific T-Cell Activation, Small. (2015). 10.1002/smll.201402369.

[33]. Agarwal R, Singh V, Jurney P, Shi L, Sreenivasan SV, Roy K, Mammalian cells preferentially internalize hydrogel nanodiscs over nanorods and use shape-specific uptake mechanisms, Proc. Natl. Acad. Sci. U. S. A 110 (2013) 17247–17252. 10.1073/pnas.1305000110. [PubMed: 24101456]

[34]. Niikura K, Matsunaga T, Suzuki T, Kobayashi S, Yamaguchi H, Orba Y, Kawaguchi A, Hasegawa H, Kajino K, Ninomiya T, Ijiro K, Sawa H, Gold nanoparticles as a vaccine platform: Influence of size and shape on immunological responses in vitro and in vivo, ACS Nano. 7 (2013) 3926–3938. 10.1021/nn3057005. [PubMed: 23631767]

[35]. Champion JA, Katare YK, Mitragotri S, Particle shape: A new design parameter for micro- and nanoscale drug delivery carriers, J. Control. Release 121 (2007) 3–9. 10.1016/j.jconrel.2007.03.022. [PubMed: 17544538]

[36]. Agarwal R, Singh V, Jurney P, Shi L, Sreenivasan SV, Roy K, Mammalian cells preferentially internalize hydrogel nanodiscs over nanorods and use shape-specific uptake mechanisms, Proc. Natl. Acad. Sci. U. S. A 110 (2013) 17247–17252. 10.1073/pnas.1305000110. [PubMed: 24101456]

[37]. Goyvaerts C, Breckpot K, Pros and cons of antigen-presenting cell targeted tumor vaccines, J. Immunol. Res 2015 (2015). 10.1155/2015/785634.

[38]. Geijtenbeek TBH, Gringhuis SI, Signalling through C-type lectin receptors: Shaping immune responses, Nat. Rev. Immunol 9 (2009) 465–479. 10.1038/nri2569. [PubMed: 19521399]

[39]. Zhang C, Shi G, Zhang J, Song H, Niu J, Shi S, Huang P, Wang Y, Wang W, Li C, Kong D, Targeted antigen delivery to dendritic cell via functionalized alginate nanoparticles for

cancer immunotherapy, J. Control. Release 256 (2017) 170–181. 10.1016/j.jconrel.2017.04.020. [PubMed: 28414151]

[40]. Li R-JE, Hogervorst TP, Achilli S, Bruijns SC, Arnoldus T, Vivès C, Wong CC, Thépaut M, Meeuwenoord NJ, van den Elst H, Overkleeft HS, van der Marel GA, Filippov DV, van Vliet SJ, Fieschi F, Codée JDC, van Kooyk Y, Systematic Dual Targeting of Dendritic Cell C-Type Lectin Receptor DC-SIGN and TLR7 Using a Trifunctional Mannosylated Antigen, Front. Chem 7 (2019) 650. 10.3389/fchem.2019.00650. [PubMed: 31637232]

[41]. Chavez-Santoscoy AV, Roychoudhury R, Pohl NLB, Wannemuehler MJ, Narasimhan B, Ramer-Tait AE, Tailoring the immune response by targeting C-type lectin receptors on alveolar macrophages using " pathogen-like" amphiphilic polyanhydride nanoparticles, Biomaterials. 33 (2012) 4762–4772. 10.1016/j.biomaterials.2012.03.027. [PubMed: 22465338]

[42]. Pei M, Xu R, Zhang C, Wang X, Li C, Hu Y, Mannose-functionalized antigen nanoparticles for targeted dendritic cells, accelerated endosomal escape and enhanced MHC-I antigen presentation, Colloids Surfaces B Biointerfaces. 197 (2021) 111378. 10.1016/j.colsurfb.2020.111378. [PubMed: 33010719]

[43]. Bandyopadhyay A, Fine RL, Demento S, Bockenstedt LK, Fahmy TM, The impact of nanoparticle ligand density on dendritic-cell targeted vaccines, Biomaterials. 32 (2011) 3094–3105. 10.1016/j.biomaterials.2010.12.054. [PubMed: 21262534]

[44]. Duinkerken S, Horrevorts SK, Kalay H, Ambrosini M, Rutte L, de Gruijl TD, Garcia-Vallejo JJ, van Kooyk Y, Glyco-dendrimers as intradermal anti-tumor vaccine targeting multiple skin DC subsets, Theranostics. 9 (2019) 5797–5809. 10.7150/thno.35059. [PubMed: 31534520]

[45]. Molino NM, Neek M, Tucker JA, Nelson EL, Wang SW, Display of DNA on Nanoparticles for Targeting Antigen Presenting Cells, ACS Biomater. Sci. Eng 3 (2017) 496–501. 10.1021/acsbiomaterials.7b00148. [PubMed: 28989957]

[46]. De Faria PCB, Dos Santos LI, Coelho JP, Ribeiro HB, Pimenta MA, Ladeira LO, Gomes DA, Furtado CA, Gazzinelli RT, Oxidized multiwalled carbon nanotubes as antigen delivery system to promote superior CD8+ T Cell response and protection against Cancer, Nano Lett. 14 (2014) 5458–5470. 10.1021/nl502911a. [PubMed: 25115645]

[47]. Traini G, Ruiz-de-Angulo A, Blanco-Canosa JB, Zamacola Bascarán K, Molinaro A, Silipo A, Escors D, Mareque-Rivas JC, Cancer Immunotherapy of TLR4 Agonist-Antigen Constructs Enhanced with Pathogen-Mimicking Magnetite Nanoparticles and Checkpoint Blockade of PD-L1, Small. 15 (2019) 1803993. 10.1002/smll.201803993.

[48]. Bookstaver ML, Tsai SJ, Bromberg JS, Jewell CM, Improving Vaccine and Immunotherapy Design Using Biomaterials, Trends Immunol. 39 (2018) 135–150. 10.1016/j.it.2017.10.002. [PubMed: 29249461]

[49]. Uto T, Akagi T, Yoshinaga K, Toyama M, Akashi M, Baba M, The induction of innate and adaptive immunity by biodegradable poly(γ-glutamic acid) nanoparticles via a TLR4 and MyD88 signaling pathway, Biomaterials. 32 (2011) 5206–5212. 10.1016/j.biomaterials.2011.03.052. [PubMed: 21492934]

[50]. Luo M, Wang H, Wang Z, Cai H, Lu Z, Li Y, Du M, Huang G, Wang C, Chen X, Porembka MR, Lea J, Frankel AE, Fu YX, Chen ZJ, Gao J, A STING-activating nanovaccine for cancer immunotherapy, Nat. Nanotechnol 12 (2017) 648–654. 10.1038/nnano.2017.52. [PubMed: 28436963]

[51]. Mohsen MO, Gomes AC, Cabral-Miranda G, Krueger CC, Leoratti FM, Stein JV, Bachmann MFDelivering adjuvants and antigens in separate nanoparticles eliminates the need of physical linkage for effective vaccination, J. Control. Release 251 (2017) 92–100. 10.1016/j.jconrel.2017.02.031. [PubMed: 28257987]

[52]. Garg AD, Vara Perez M, Schaaf M, Agostinis P, Zitvogel L, Kroemer G, Galluzzi L, Trial watch: Dendritic cell-based anticancer immunotherapy., Oncoimmunology. 6 (2017) e1328341. 10.1080/2162402X.2017.1328341. [PubMed: 28811970]

[53]. Chang TZ, Stadmiller SS, Staskevicius E, Champion JA, Effects of ovalbumin protein nanoparticle vaccine size and coating on dendritic cell processing, Biomater. Sci 5 (2017) 223–233. 10.1039/c6bm00500d. [PubMed: 27918020]

[54]. Jia J, Zhang Y, Xin Y, Jiang C, Yan B, Zhai S, Interactions Between Nanoparticles and Dendritic Cells: From the Perspective of Cancer Immunotherapy, Front. Oncol 8 (2018) 404. 10.3389/fonc.2018.00404. [PubMed: 30319969]

[55]. Tkach AV, Yanamala N, Stanley S, Shurin MR, Shurin GV, Kisin ER, Murray AR, Pareso S, Khaliullin T, Kotchey GP, Castranova V, Mathur S, Fadeel B, Star A, Kagan VE, Shvedova AA, Graphene oxide, but not fullerenes, targets immunoproteasomes and suppresses antigen presentation by dendritic cells, Small. 9 (2013) 1686–1690. 10.1002/smll.201201546. [PubMed: 22887961]

[56]. Blank F, Gerber P, Rothen-Rutishauser B, Sakulkhu U, Salaklang J, De Peyer K, Gehr P, Nicod LP, Hofmann H, Geiser T, Petri-Fink A, Von Garnier C, Biomedical nanoparticles modulate specific CD4 + T cell stimulation by inhibition of antigen processing in dendritic cells, Nanotoxicology. 5 (2011) 606–621. 10.3109/17435390.2010.541293. [PubMed: 21231795]

[57]. Zhang C, Shi G, Zhang J, Song H, Niu J, Shi S, Huang P, Wang Y, Wang W, Li C, Kong D, Targeted antigen delivery to dendritic cell via functionalized alginate nanoparticles for cancer immunotherapy, J. Control. Release 256 (2017) 170–181. 10.1016/j.jconrel.2017.04.020. [PubMed: 28414151]

[58]. Kim H, Uto T, Akagi T, Baba M, Akashi MAmphiphilic Poly(Amino Acid) Nanoparticles Induce Size-Dependent Dendritic Cell Maturation, Adv. Funct. Mater 20 (2010) 3925–3931. 10.1002/adfm.201000021.

[59]. Goodman JT, Vela Ramirez JE, Boggiatto PM, Roychoudhury R, Pohl NLB, Wannemuehler MJ, Narasimhan B, Nanoparticle Chemistry and Functionalization Differentially Regulates Dendritic Cell-Nanoparticle Interactions and Triggers Dendritic Cell Maturation, Part. Part. Syst. Charact 31 (2014) 1269–1280. 10.1002/ppsc.201400148.

[60]. Barillet S, Fattal E, Mura S, Tsapis N, Pallardy M, Hillaireau H, Kerdine-Römer SImmunotoxicity of poly (lactic-co-glycolic acid) nanoparticles: influence of surface properties on dendritic cell activation, Nanotoxicology. 13 (2019) 606–622. 10.1080/17435390.2018.1564078. [PubMed: 30760076]

[61]. Yang D, Zhao Y, Guo H, Li Y, Tewary P, Xing G, Hou W, Oppenheim JJ, Zhang N, [Gd@C82(OH)22]n nanoparticles induce dendritic cell maturation and activate Th1 immune responses, in: ACS Nano, American Chemical Society, 2010: pp. 1178–1186. 10.1021/nn901478z.

[62]. Lee SB, Lee YJ, Cho SJ, Kim SK, Lee S-W, Lee J, Lim D-K, Jeon YH, Antigen-Free Radionuclide-Embedded Gold Nanoparticles for Dendritic Cell Maturation, Tracking, and Strong Antitumor Immunity, Adv. Healthc. Mater 7 (2018) 1701369. 10.1002/adhm.201701369.

[63]. Jin H, Qian Y, Dai Y, Qiao S, Huang C, Lu L, Luo Q, Chen J, Zhang Z, Magnetic enrichment of dendritic cell vaccine in lymph node with fluorescent-magnetic nanoparticles enhanced cancer immunotherapy, Theranostics. 6 (2016) 2000–2014. 10.7150/thno.15102. [PubMed: 27698936]

[64]. Pati R, Shevtsov M, Sonawane A, Nanoparticle vaccines against infectious diseases, Front. Immunol 9 (2018) 2224. 10.3389/fimmu.2018.02224. [PubMed: 30337923]

[65]. Bookstaver ML, Tsai SJ, Bromberg JS, Jewell CM, Improving Vaccine and Immunotherapy Design Using Biomaterials, Trends Immunol. 39 (2018) 135–150. 10.1016/j.it.2017.10.002. [PubMed: 29249461]

[66]. Aikins ME, Xu C, Moon JJ, Engineered Nanoparticles for Cancer Vaccination and Immunotherapy, Acc. Chem. Res 53 (2020) 2094–2105. 10.1021/acs.accounts.0c00456. [PubMed: 33017150]

[67]. Chauhan G, Madou MJ, Kalra S, Chopra V, Ghosh D, Martinez-Chapa SO, Nanotechnology for COVID-19: Therapeutics and Vaccine Research, ACS Nano. 14 (2020) 7760–7782. 10.1021/acsnano.0c04006. [PubMed: 32571007]

[68]. Lynn DJ, Pulendran B, The potential of the microbiota to influence vaccine responses, J. Leukoc. Biol 103 (2017) jlb.5MR0617-216R. 10.1189/jlb.5mr0617-216r.

[69]. Thakur A, Foged C, Nanoparticles for mucosal vaccine delivery, in: Nanoeng. Biomater. Adv. Drug Deliv, Elsevier, 2020: pp. 603–646. 10.1016/b978-0-08-102985-5.00025-5.

[70]. Reinhard K, Rengstl B, Oehm P, Michel K, Billmeier A, Hayduk N, Klein O, Kuna K, Ouchan Y, Wöll S, Christ E, Weber D, Suchan M, Bukur T, Birtel M, Jahndel V, Mroz K, Hobohm

K, Kranz L, Diken M, Kühlcke K, Türeci Ö, Sahin U, An RNA vaccine drives expansion and efficacy of claudin-CAR-T cells against solid tumors, Science (80-.). 367 (2020) 446–453. 10.1126/science.aay5967.

[71]. Wilhelm S, Tavares AJ, Dai Q, Ohta S, Audet J, Dvorak HF, Chan WCW, Analysis of nanoparticle delivery to tumors, Nat. Mater 1 (2016) 1–12.

[72]. Broz ML, Binnewies M, Boldajipour B, Nelson AE, Pollack JL, Erle DJ, Barczak A, Rosenblum MD, Daud A, Barber DL, Amigorena S, van'tVeer LJ, Sperling AI, Wolf DM, Krummel MF, Dissecting the Tumor Myeloid Compartment Reveals Rare Activating Antigen-Presenting Cells Critical for T Cell Immunity, Cancer Cell. 26 (2014) 638–652. 10.1016/j.ccell.2014.09.007. [PubMed: 25446897]

[73]. Williford JM, Ishihara J, Ishihara A, Mansurov A, Hosseinchi P, Marchell TM, Potin L, Swartz MA, Hubbell JA, Recruitment of CD103+ dendritic cells via tumor-targeted chemokine delivery enhances efficacy of checkpoint inhibitor immunotherapy, Sci. Adv 5 (2019) eaay1357. 10.1126/sciadv.aay1357. [PubMed: 31844672]

[74]. Hall BM, Waldmann H, Kishimoto TK, Maldonado RA, Nanoparticles for the induction of Antigen-Specific immunological Tolerance, Front. Immunol 9 (2018) 1. 10.3389/fimmu.2018.00230. [PubMed: 29403488]

[75]. Li PY, Bearoff F, Zhu P, Fan Z, Zhu Y, Fan M, Cort L, Kambayashi T, Blankenhorn EP, Cheng H, PEGylation enables subcutaneously administered nanoparticles to induce antigen-specific immune tolerance, J. Control. Release 331 (2021) 164–175. 10.1016/j.jconrel.2021.01.013. [PubMed: 33450320]

[76]. Yeste A, Nadeau M, Burns EJ, Weiner HL, Quintana FJ, Nanoparticle-mediated codelivery of myelin antigen and a tolerogenic small molecule suppresses experimental autoimmune encephalomyelitis, Proc. Natl. Acad. Sci. U. S. A 109 (2012) 11270–11275. 10.1073/pnas.1120611109. [PubMed: 22745170]

[77]. Irvine DJ, Aung A, Silva M, Controlling timing and location in vaccines, Adv. Drug Deliv. Rev (2020). 10.1016/j.addr.2020.06.019.

[78]. Ho NI, Huis In 't Veld LGM, Raaijmakers TK, Adema GJ, Adjuvants Enhancing Cross-Presentation by Dendritic Cells: The Key to More Effective Vaccines?, Front. Immunol 9 (2018) 2874. 10.3389/fimmu.2018.02874. [PubMed: 30619259]

[79]. Veglia F, Gabrilovich DI, Dendritic cells in cancer: the role revisited, Curr. Opin. Immunol 45 (2017) 43–51. 10.1016/j.coi.2017.01.002. [PubMed: 28192720]

[80]. Duan Z, Luo Y, Targeting macrophages in cancer immunotherapy, Signal Transduct. Target. Ther 6 (2021) 1–21. 10.1038/s41392-021-00506-6. [PubMed: 33384407]

[81]. Wennhold K, Shimabukuro-Vornhagen A, Von Bergwelt-Baildon M, B Cell-Based Cancer Immunotherapy, Transfus. Med. Hemotherapy 46 (2019) 36–46. 10.1159/000496166.

[82]. Lund FE, Randall TD, Effector and regulatory B cells: Modulators of CD4+ T cell immunity, Nat. Rev. Immunol 10 (2010) 236–247. 10.1038/nri2729. [PubMed: 20224569]

[83]. Popi AF, Longo-Maugéri IM, Mariano M, An overview of B-1 cells as antigen-presenting cells, Front. Immunol 7 (2016) 138. 10.3389/fimmu.2016.00138. [PubMed: 27148259]

[84]. Mariño E, Tan B, Binge L, Mackay CR, Grey ST, B-cell cross-presentation of autologous antigen precipitates diabetes, Diabetes. 61 (2012) 2893–2905. 10.2337/db12-0006. [PubMed: 22829452]

[85]. Shen P, Fillatreau S, Antibody-independent functions of B cells: A focus on cytokines, Nat. Rev. Immunol 15 (2015) 441–451. 10.1038/nri3857. [PubMed: 26065586]

[86]. Yu M, Wu J, Shi J, Farokhzad OC, Nanotechnology for protein delivery: Overview and perspectives, J. Control. Release 240 (2016) 24–37. 10.1016/j.jconrel.2015.10.012. [PubMed: 26458789]

[87]. Schultze JL, Michalak S, Seamon MJ, Dranoff G, Jung K, Daley J, Delgado JC, Gribben JG, Nadler LM, CD40-activated human B cells: An alternative source of highly efficient antigen presenting cells to generate autologous antigen-specific T cells for adoptive immunotherapy, J. Clin. Invest 100 (1997) 2757–2765. 10.1172/JCI119822. [PubMed: 9389740]

[88]. Sicard A, Koenig A, Graff-Dubois S, Dussurgey S, Rouers A, Dubois V, Blanc P, Chartoire D, Errazuriz-Cerda E, Paidassi H, Taillardet M, Morelon E, Moris A, Defrance T, Thaunat O, B Cells Loaded with Synthetic Particulate Antigens: A Versatile Platform to Generate

Antigen-Specific Helper T Cells for Cell Therapy, Nano Lett. 16 (2016) 297–308. 10.1021/acs.nanolett.5b03801. [PubMed: 26650819]

[89]. Bennett NR, Zwick DB, Courtney AH, Kiessling LL, Multivalent Antigens for Promoting B and T Cell Activation, ACS Chem. Biol 10 (2015) 1817–1824. 10.1021/acschembio.5b00239. [PubMed: 25970017]

[90]. Smith MJ, Packard TA, O'Neill SK, Hinman RM, Rihanek M, Gottlieb PA, Cambier JCDetection and enrichment of rare antigen-specific B cells for analysis of phenotype and function, J. Vis. Exp 2017 (2017) 55382. 10.3791/55382.

[91]. Hong S, Zhang Z, Liu H, Tang H, Hua Z, Correspondence BHB Cells Are the Dominant Antigen-Presenting Cells that Activate Naive CD4 + T Cells upon Immunization with a Virus-Derived Nanoparticle Antigen, Immunity. 49 (2018) 695–708.e4. 10.1016/j.immuni.2018.08.012. [PubMed: 30291027]

[92]. de Charette M, Marabelle A, Houot R, Turning tumour cells into antigen presenting cells: The next step to improve cancer immunotherapy?, Eur. J. Cancer 68 (2016) 134–147. 10.1016/j.ejca.2016.09.010. [PubMed: 27755997]

[93]. Ostrand-Rosenberg S, Tumor immunotherapy: the tumor cell as an antigen-presenting cell, Curr. Opin. Immunol 6 (1994) 722–727. 10.1016/0952-7915(94)90075-2. [PubMed: 7826527]

[94]. Keenan BP, Jaffee EM, Whole cell vaccines - Past progress and future strategies, Semin. Oncol 39 (2012) 276–286. 10.1053/j.seminoncol.2012.02.007. [PubMed: 22595050]

[95]. Neshat SY, Tzeng SY, Green JJ, Gene delivery for immunoengineering, Curr. Opin. Biotechnol 66 (2020) 1–10. 10.1016/j.copbio.2020.05.008. [PubMed: 32554325]

[96]. Tzeng SY, Patel KK, Wilson DR, Meyer RA, Rhodes KR, Green JJ, In situ genetic engineering of tumors for long-lasting and systemic immunotherapy, Proc. Natl. Acad. Sci. U. S. A 117 (2020) 4043–4052. 10.1073/pnas.1916039117. [PubMed: 32034097]

[97]. Huang KW, Hsu FF, Qiu JT, Chern GJ, Lee YA, Chang CC, Huang YT, Sung YC, Chiang CC, Huang RL, Lin CC, Dinh TK, Huang HC, Shih YC, Alson D, Lin CY, Lin YC, Chang PC, Lin SY, Chen Y, Highly efficient and tumor-selective nanoparticles for dual-targeted immunogene therapy against cancer, Sci. Adv 6 (2020) eaax5032. 10.1126/sciadv.aax5032. [PubMed: 31998834]

[98]. Kosmides AK, Sidhom JW, Fraser A, Bessell CA, Schneck JPDual Targeting Nanoparticle Stimulates the Immune System to Inhibit Tumor Growth, ACS Nano. 11 (2017) 5417–5429. 10.1021/acsnano.6b08152. [PubMed: 28589725]

[99]. Sunshine JC, Green JJ, Nanoengineering approaches to the design of artificial antigen-presenting cells., Nanomedicine (Lond). 8 (2013) 1173–1189. 10.2217/nnm.13.98. [PubMed: 23837856]

[100]. Kosmides AK, Meyer RA, Hickey JW, Aje K, Cheung KN, Green JJ, Schneck JP, Biomimetic biodegradable artificial antigen presenting cells synergize with PD-1 blockade to treat melanoma, Biomaterials. (2017). 10.1016/j.biomaterials.2016.11.038.

[101]. Hickey JW, Isser AY, Vicente FP, Warner SB, Mao HQ, Schneck JP, Efficient magnetic enrichment of antigen-specific T cells by engineering particle properties, Biomaterials. (2018). 10.1016/j.biomaterials.2018.09.029.

[102]. Delcassian D, Depoil D, Rudnicka D, Liu M, Davis DM, Dustin ML, Dunlop IE, Nanoscale ligand spacing influences receptor triggering in T cells and NK cells, Nano Lett. (2013). 10.1021/nl403252x.

[103]. Ichikawa J, Yoshida T, Isser A, Laino AS, Vassallo M, Woods D, Kim S, Oelke M, Jones K, Schneck JP, Weber JS, Rapid expansion of highly functional antigen-specific t cells from patients with melanoma by nanoscale artificial antigen-presenting cells, Clin. Cancer Res (2020). 10.1158/1078-0432.CCR-19-3487.

[104]. Ahn E, Araki K, Hashimoto M, Li W, Riley JL, Cheung J, Sharpe AH, Freeman GJ, Irving BA, Ahmed R, Role of PD-1 during effector CD8 T cell differentiation, Proc. Natl. Acad. Sci. U. S. A (2018). 10.1073/pnas.1718217115.

[105]. Rhodes KR, Isser A, Hickey JW, Ben-Akiva E, Meyer RA, Kosmides AK, Livingston NK, Tzeng SY, Schneck JP, Green JJ, Biodegradable Cationic Polymer Blends for Fabrication of Enhanced Artificial Antigen Presenting Cells to Treat Melanoma, ACS Appl. Mater. Interfaces (2021). 10.1021/acsami.0c19955.

[106]. Rhodes KR, Meyer RA, Wang J, Tzeng SY, Green JJ, Biomimetic tolerogenic artificial antigen presenting cells for regulatory T cell induction, Acta Biomater. (2020). 10.1016/j.actbio.2020.06.004.

[107]. Zhang Q, Wei W, Wang P, Zuo L, Li F, Xu J, Xi X, Gao X, Ma G, Xie HY, Biomimetic Magnetosomes as Versatile Artificial Antigen-Presenting Cells to Potentiate T-Cell-Based Anticancer Therapy, ACS Nano. (2017). 10.1021/acsnano.7b04955.

[108]. Xiao P, Wang J, Zhao Z, Liu X, Sun X, Wang D, Li Y, Engineering Nanoscale Artificial Antigen-Presenting Cells by Metabolic Dendritic Cell Labeling to Potentiate Cancer Immunotherapy, Nano Lett. (2021). 10.1021/acs.nanolett.0c04783.

[109]. Bufi N, Saitakis M, Dogniaux S, Buschinger O, Bohineust A, Richert A, Maurin M, Hivroz C, Asnacios A, Human Primary Immune Cells Exhibit Distinct Mechanical Properties that Are Modified by Inflammation, Biophys. J 108 (2015) 2181–2190. 10.1016/J.BPJ.2015.03.047. [PubMed: 25954876]

[110]. Coussens LM, Werb Z, Inflammation and cancer, Nature. 420 (2002) 860. 10.1038/NATURE01322. [PubMed: 12490959]

[111]. Anselmo AC, Zhang M, Kumar S, Vogus DR, Menegatti S, Helgeson ME, Mitragotri S, Elasticity of nanoparticles influences their blood circulation, phagocytosis, endocytosis, and targeting, ACS Nano. 9 (2015) 3169–3177. 10.1021/ACSNANO.5B00147/SUPPL_FILE/NN5B00147_SI_002.PDF. [PubMed: 25715979]

[112]. Anselmo AC, Zhang M, Kumar S, Vogus DR, Menegatti S, Helgeson ME, Mitragotri S, Elasticity of nanoparticles influences their blood circulation, phagocytosis, endocytosis, and targeting, ACS Nano. 9 (2015) 3169–3177. 10.1021/ACSNANO.5B00147/SUPPL_FILE/NN5B00147_SI_002.PDF. [PubMed: 25715979]

[113]. Kong SM, Costa DF, Jagielska A, Van Vliet KJ, Hammond PT, Stiffness of targeted layer-by-layer nanoparticles impacts elimination half-life, tumor accumulation, and tumor penetration, Proc. Natl. Acad. Sci. U. S. A 118 (2021) 2021. 10.1073/PNAS.2104826118/-/DCSUPPLEMENTAL.

[114]. Hickey JW, Dong Y, Chung JW, Salathe SF, Pruitt HC, Li X, Chang C, Fraser AK, Bessell CA, Ewald AJ, Gerecht S, Mao HQ, Schneck JP, Engineering an Artificial T-Cell Stimulating Matrix for Immunotherapy, Adv. Mater 31 (2019) 1807359. 10.1002/ADMA.201807359.

[115]. Ben-Akiva E, Meyer RA, Wilson DR, Green JJ, Surface Engineering for Lymphocyte Programming, Adv. Drug Deliv. Rev 114 (2017) 102. 10.1016/J.ADDR.2017.05.005. [PubMed: 28501510]

[116]. Olden BR, Perez CR, Wilson AL, Cardle II, Towne B, Lin Y-S, Kaehr B, Gustafson JA, Jensen MC, Pun SH, Cell-templated Supported Lipid Bilayers for T Cell Activation HHS Public Access, 8 (2019) 1801188. 10.1002/adhm.201801188.

[117]. Sunshine JC, Perica K, Schneck JP, Green JJ, Particle shape dependence of CD8+ T cell activation by artificial antigen presenting cells, Biomaterials. 35 (2014) 269–277. 10.1016/j.biomaterials.2013.09.050. [PubMed: 24099710]

[118]. Kapate N, Clegg JR, Mitragotri SNon-spherical micro- and nanoparticles for drug delivery: Progress over 15 years, Adv. Drug Deliv. Rev 177 (2021) 113807. 10.1016/J.ADDR.2021.05.017. [PubMed: 34023331]

[119]. Sharma G, Valenta DT, Altman Y, Harvey S, Xie H, Mitragotri S, Smith JW, Polymer Particle Shape Independently Influences Binding and Internalization by Macrophages, J. Control. Release 147 (2010) 408. 10.1016/J.JCONREL.2010.07.116. [PubMed: 20691741]

[120]. Bruns H, Bessell C, Varela JC, Haupt C, Fang J, Pasemann S, Mackensen A, Oelke M, Schneck JP, Schütz C, CD47 enhances in vivo functionality of artificial antigen-presenting cells, Clin. Cancer Res (2015). 10.1158/1078-0432.CCR-14-2696.

[121]. Song S, Jin X, Zhang L, Zhao C, Ding Y, Ang Q, Khaidav O, Shen C, PEGegylated and CcD47-conjugated nanoellipsoidal artificial antigen-presenting cells minimize phagocytosis and augment anti-tumor T-cell responses, Int. J. Nanomedicine (2019). 10.2147/IJN.S195828.

[122]. Keeler AM, ElMallah MK, Flotte TR, Gene Therapy 2017: Progress and Future Directions, Clin. Transl. Sci (2017). 10.1111/cts.12466.

[123]. Parayath NN, Stephan SB, Koehne AL, Nelson PS, Stephan MT, In vitro-transcribed antigen receptor mRNA nanocarriers for transient expression in circulating T cells in vivo, Nat. Commun (2020). 10.1038/s41467-020-19486-2.

[124]. Nayerossadat N, Ali P, Maedeh T, Viral and nonviral delivery systems for gene delivery, Adv. Biomed. Res (2012). 10.4103/2277-9175.98152.

[125]. Zhang Z, Qiu S, Zhang X, Chen W, Optimized DNA electroporation for primary human T cell engineering, BMC Biotechnol. (2018). 10.1186/s12896-018-0419-0.

[126]. McKinlay CJ, Benner NL, Haabeth OA, Waymouth RM, Wender PAEnhanced mRNA delivery into lymphocytes enabled by lipid-varied libraries of charge-altering releasable transporters, Proc. Natl. Acad. Sci. U. S. A (2018). 10.1073/pnas.1805358115.

[127]. Billingsley MM, Singh N, Ravikumar P, Zhang R, June CH, Mitchell MJIonizable Lipid Nanoparticle-Mediated mRNA Delivery for Human CAR T Cell Engineering, Nano Lett. (2020). 10.1021/acs.nanolett.9b04246.

[128]. Smith TT, Stephan SB, Moffett HF, McKnight LE, Ji W, Reiman D, Bonagofski E, Wohlfahrt ME, Pillai SPS, Stephan MT, In situ programming of leukaemia-specific t cells using synthetic DNA nanocarriers, Nat. Nanotechnol (2017). 10.1038/NNANO.2017.57.

[129]. Redeker A, Arens R, Improving adoptive T cell therapy: The particular role of T cell costimulation, cytokines, and post-transfer vaccination, Front. Immunol 7 (2016). 10.3389/fimmu.2016.00345.

[130]. Parisi G, Saco JD, Salazar FB, Tsoi J, Krystofinski P, Puig-Saus C, Zhang R, Zhou J, Cheung-Lau GC, Garcia AJ, Grasso CS, Tavaré R, Hu-Lieskovan S, Mackay S, Zalevsky J, Bernatchez C, Diab A, Wu AM, Comin-Anduix B, Charych D, Ribas A, Persistence of adoptively transferred T cells with a kinetically engineered IL-2 receptor agonist, Nat. Commun 660 (2020). 10.1038/s41467-019-12901-3.

[131]. Raker VK, Becker C, Landfester K, Steinbrink K, Targeted Activation of T Cells with IL-2-Coupled Nanoparticles, Cells. 9 (2020). 10.3390/cells9092063.

[132]. McHugh MD, Park J, Uhrich R, Gao W, Horwitz DA, Fahmy TMParacrine codelivery of TGF-β and IL-2 using CD4-targeted nanoparticles for induction and maintenance of regulatory T cells, Biomaterials. 59 (2015) 172–181. 10.1016/j.biomaterials.2015.04.003. [PubMed: 25974747]

[133]. Park J, Wrzesinski SH, Stern E, Look M, Criscione J, Ragheb R, Jay SM, Demento SL, Agawu A, Licona Limon P, Ferrandino AF, Gonzalez D, Habermann A, Flavell RA, Fahmy TM, Combination delivery of TGF-β inhibitor and IL-2 by nanoscale liposomal polymeric gels enhances tumour immunotherapy, Nat. Mater 11 (2012) 895–905. 10.1038/nmat3355. [PubMed: 22797827]

[134]. Frick SU, Domogalla MP, Baier G, Wurm FR, Mailä V, Landfester K, Steinbrink K, Interleukin-2 Functionalized Nanocapsules for T Cell-Based Immunotherapy, ACS Nano. 10 (2016) 25. 10.1021/acsnano.5b07973.

[135]. Zhang Y, Li N, Suh H, Irvine DJ, Nanoparticle anchoring targets immune agonists to tumors enabling anti-cancer immunity without systemic toxicity, Nat. Commun 9 (2018). 10.1038/s41467-017-02251-3.

[136]. Schmid D, Park CG, Hartl CA, Subedi N, Cartwright AN, Puerto RB, Zheng Y, Maiarana J, Freeman GJ, Wucherpfennig KW, Irvine DJ, Goldberg MS, T cell-targeting nanoparticles focus delivery of immunotherapy to improve antitumor immunity, Nat. Commun 8 (2017) 1–12. 10.1038/s41467-017-01830-8. [PubMed: 28232747]

[137]. Tang L, Zheng Y, Melo MB, Mabardi L, Castaño AP, Xie Y-Q, Li N, Kudchodkar SB, Wong HC, Jeng EK, V Maus M, Irvine DJ, Enhancing T cell therapy through TCR-signaling-responsive nanoparticle drug delivery, Nat. Biotechnol 36 (2018) 707. 10.1038/nbt.4181. [PubMed: 29985479]

[138]. Eskandari SK, Sulkaj I, Melo MB, Li N, Allos H, Alhaddad JB, Kollar B, Borges TJ, Eskandari AS, Zinter MA, Cai S, Assaker JP, Choi JY, Al Dulaijan BS, Mansouri A, Haik Y, Tannous BA, van Son WJ, Leuvenink HGD, Pomahac B, Riella LV, Tang L, Seelen MAJ, Irvine DJ, Azzi JRRegulatory T cells engineered with TCR signaling-responsive IL-2 nanogels suppress alloimmunity in sites of antigen encounter, Sci. Transl. Med 12 (2020). 10.1126/scitranslmed.aaw4744.

[139]. Ma L, Dichwalkar T, Chang JYH, Cossette B, Garafola D, Zhang AQ, Fichter M, Wang C, Liang S, Silva M, Kumari S, Mehta NK, Abraham W, Thai N, Li N, Dane Wittrup K, Irvine DJ, Enhanced CAR–T cell activity against solid tumors by vaccine boosting through the chimeric receptor, Science (80-.). 365 (2019) 162–168. 10.1126/science.aav8692.

[140]. Poschke I, Lövgren T, Adamson L, Nyström M, Andersson E, Hansson J, Tell R, Masucci GV, Kiessling R, A phase I clinical trial combining dendritic cell vaccination with adoptive T cell transfer in patients with stage IV melanoma, Cancer Immunol. Immunother 63 (2014) 1061–1071. 10.1007/s00262-014-1575-2. [PubMed: 24993563]

[141]. Smith TT, Moffett HF, Stephan SB, Opel CF, Dumigan AG, Jiang X, Pillarisetty VG, Pillai SPS, Wittrup KD, Stephan MT, Biopolymers codelivering engineered T cells and STING agonists can eliminate heterogeneous tumors, J. Clin. Invest 127 (2017) 2176–2191. 10.1172/JCI87624. [PubMed: 28436934]

[142]. Majzner RG, Mackall CL, Tumor Antigen Escape from CAR T-cell Therapy, Cancer Discov. 8 (2018) 1219 LP–1226. 10.1158/2159-8290.CD-18-0442. [PubMed: 30135176]

[143]. Marro BS, Zak J, Zavareh RB, Teijaro JR, Lairson LL, Correspondence MBAO, Discovery of Small Molecules for the Reversal of T Cell Exhaustion, Cell Reports. 29 (2019) 3293–3302.e3. 10.1016/j.celrep.2019.10.119. [PubMed: 31801090]

[144]. Waller AL, Funk CR, Ravindranathan S, Waller EK, Use of small molecule inhibitors of metabolism to enhance T cell proliferation and phenotype for adoptive T cell therapies, J. Immunol 202 (2019).

[145]. Mosquera J, García I, Liz-Marzán LM, Cellular Uptake of Nanoparticles versus Small Molecules: A Matter of Size, Acc. Chem. Res 51 (2018) 2305–2313. 10.1021/acs.accounts.8b00292. [PubMed: 30156826]

[146]. Li X, Wang Y, Zhang Y, Liang C, Zhang Z, Chen Y, Hu Z, Yang Z, A Supramolecular "Trident" for Cancer Immunotherapy, Adv. Funct. Mater (2021) 2100729. 10.1002/adfm.202100729.

[147]. Yang YSS, Moynihan KD, Bekdemir A, Dichwalkar TM, Noh MM, Watson N, Melo M, Ingram J, Suh H, Ploegh H, Stellacci FR, Irvine DJ, Targeting small molecule drugs to T cells with antibody-directed cell-penetrating gold nanoparticles, Biomater. Sci 7 (2019) 113–124. 10.1039/c8bm01208c.

[148]. Ou W, Thapa RK, Jiang L, Soe ZC, Gautam M, Chang JH, Jeong JH, Ku SK, Choi HG, Yong CS, Kim JO, Regulatory T cell-targeted hybrid nanoparticles combined with immuno-checkpoint blockage for cancer immunotherapy, J. Control. Release 281 (2018) 84–96. 10.1016/j.jconrel.2018.05.018. [PubMed: 29777794]

[149]. Kato D, Yaguchi T, Iwata T, Morii K, Nakagawa T, Nishimura R, Kawakami YProspects for personalized combination immunotherapy for solid tumors based on adoptive cell therapies and immune checkpoint blockade therapies, Nihon Rinsho Meneki. Gakkai Kaishi 40 (2017) 68–77. 10.2177/jsci.40.68. [PubMed: 28539557]

[150]. Luo L, Yang J, Zhu C, Jiang M, Guo X, Li W, Yin X, Yin H, Qin B, Yuan X, Li Q, Du Y, You J, Sustained release of anti-PD-1 peptide for perdurable immunotherapy together with photothermal ablation against primary and distant tumors, J. Control. Release 278 (2018) 87–99. 10.1016/j.jconrel.2018.04.002. [PubMed: 29626502]

[151]. Ordikhani F, Uehara M, Kasinath V, Dai L, Eskandari SK, Bahmani B, Yonar M, Azzi JR, Haik Y, Sage PT, Murphy GF, Annabi N, Schatton T, Guleria I, Abdi R, Targeting antigen-presenting cells by anti-PD-1 nanoparticles augments antitumor immunity, JCI Insight. 3 (2018). 10.1172/jci.insight.122700.

[152]. Wang C, Sun W, Wright G, Wang AZ, Gu Z, Inflammation-Triggered Cancer Immunotherapy by Programmed Delivery of CpG and Anti-PD1 Antibody, Adv. Mater 28 (2016) 8912–8920. 10.1002/adma.201506312. [PubMed: 27558441]

[153]. Zhang N, Song J, Liu Y, Liu M, Zhang L, Sheng D, Deng L, Yi H, Wu M, Zheng Y, Wang Z, Yang Z, Photothermal therapy mediated by phase-transformation nanoparticles facilitates delivery of anti-PD1 antibody and synergizes with antitumor immunotherapy for melanoma, J. Control. Release 306 (2019) 15–28. 10.1016/j.jconrel.2019.05.036. [PubMed: 31132380]

[154]. Kwak G, Kim D, Nam GH, Wang SY, Kim IS, Kim SH, Kwon IC, Yeo Y, Programmed Cell Death Protein Ligand-1 Silencing with Polyethylenimine-Dermatan Sulfate Complex for Dual

Inhibition of Melanoma Growth, ACS Nano. 11 (2017) 10135–10146. 10.1021/acsnano.7b04717. [PubMed: 28985469]

[155]. Wang D, Wang T, Liu J, Yu H, Jiao S, Feng B, Zhou F, Fu Y, Yin Q, Zhang P, Zhang Z, Zhou Z, Li Y, Acid-Activatable Versatile Micelleplexes for PD-L1 Blockade-Enhanced Cancer Photodynamic Immunotherapy, Nano Lett. 16 (2016) 5503–5513. 10.1021/acs.nanolett.6b01994. [PubMed: 27525587]

[156]. Dion ML, Sumner JL, Mitchell SMLGendered Citation Patterns across Political Science and Social Science Methodology Fields, Polit. Anal 26 (2018) 312–327. 10.1017/pan.2018.12.

[157]. Caplar N, Tacchella S, Birrer SQuantitative evaluation of gender bias in astronomical publications from citation counts, Nat. Astron 1 (2017) 141. 10.1038/s41550-017-0141.

[158]. Mitchell SM, Lange S, Brus H, Gendered Citation Patterns in International Relations Journals, Int. Stud. Perspect 14 (2013) 485–492. 10.1111/insp.12026.

[159]. Dworkin JD, Linn KA, Teich EG, Zurn P, Shinohara RT, Bassett DS, The extent and drivers of gender imbalance in neuroscience reference lists, Nat. Neurosci 23 (2020) 918–926. 10.1038/s41593-020-0658-y. [PubMed: 32561883]

[160]. Zhou D, Bertolero MA, Stiso J, Cornblath EJ, Teich EG, Blevins AS, VirtualmarioCamp C, Dworkin JD, Bassett DS, Gender diversity statement and code notebook v1.1, (n.d.). https://github.com/dalejn/cleanBib#references (accessed May 26, 2021).

[161]. Sood G, Laohaprapanon S, Predicting Race and Ethnicity From the Sequence of Characters in a Name, ArXiv Prepr (2018). http://arxiv.org/abs/1805.02109 (accessed May 26, 2021).

[162]. Ambekar A, Ward C, Mohammed J, Male S, Skiena SName-ethnicity classification from open sources, in: Proc. ACM SIGKDD Int. Conf. Knowl. Discov. Data Min, 2009: pp. 49–57. 10.1145/1557019.1557032.

[163]. Dworkin JD, Linn KA, Teich EG, Zurn P, Shinohara RT, Bassett DS, The extent and drivers of gender imbalance in neuroscience reference lists, Nat. Neurosci 23 (2020) 918–926. 10.1038/s41593-020-0658-y. [PubMed: 32561883]

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Highlights:

- T cell therapies have shown clinical success in cancer and autoimmunity

- T cell therapies are limited by cost and patient variability

- Nanoparticles (NPs) allow for enhanced delivery of immunomodulatory signals

- NPs can enhance natural APCs or act as aAPCs to create antigen-specific T cells

- NPs expand accessibility of patient-specific immunotherapy.

6.

Citation Diversity Statement

It has been recently demonstrated across multiple scientific disciplines that publications by women and other minority scholars are cited less frequently relative to the numbers of their papers in the field [156–159]. We aimed to proactively analyze the references cited in this work so as to prevent the under-citation of women and minority scholars. To do this, we utilized a codebook developed by Zhou et al. that reported the percentage breakdown of cited women and authors of color in our reference list, excluding self-citations [160]. The codebook obtained the predicted gender, race, and ethnic categories of the first and last authors of each citation from databases that store the probability of a first and last name of an author being carried by a woman and/or an author of color [161–163]. Using these methods, our references contain 8.22% woman(first)/woman(last), 11.11% man/woman, 36.53% woman/man, and 44.14% man/man. Likewise, they contain 31.66% author of color (first)/author of color(last), 13.55% white author/author of color, 22.28% author of color/white author, and 32.51% white author/white author. These methods are limited in that (1) names and databases may be imperfect predictors or gender, race, and ethnicity, (2) they are unable to account for nonbinary, intersex, or transgender identities, and (3) they are unable to account for Indigenous and mixed-race authors, or those who may face biases due to ambiguous racialization or ethnicization of their names. We hope this analysis provides transparency and can help support equitable practices in science.
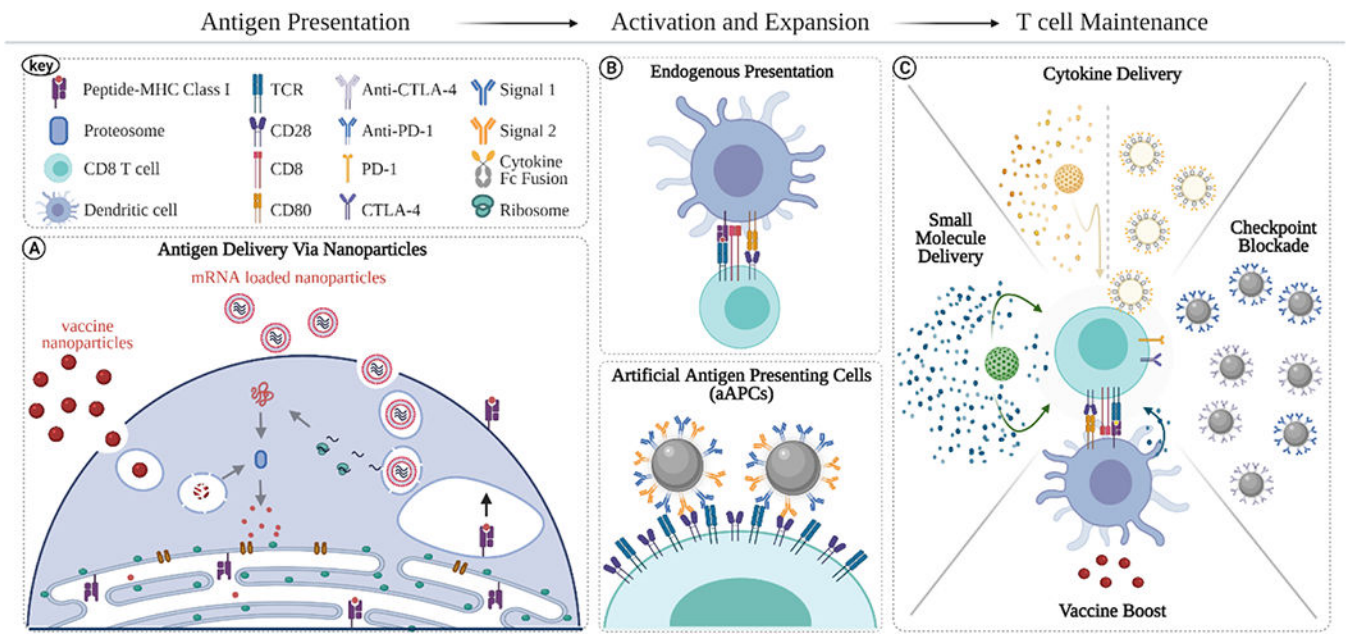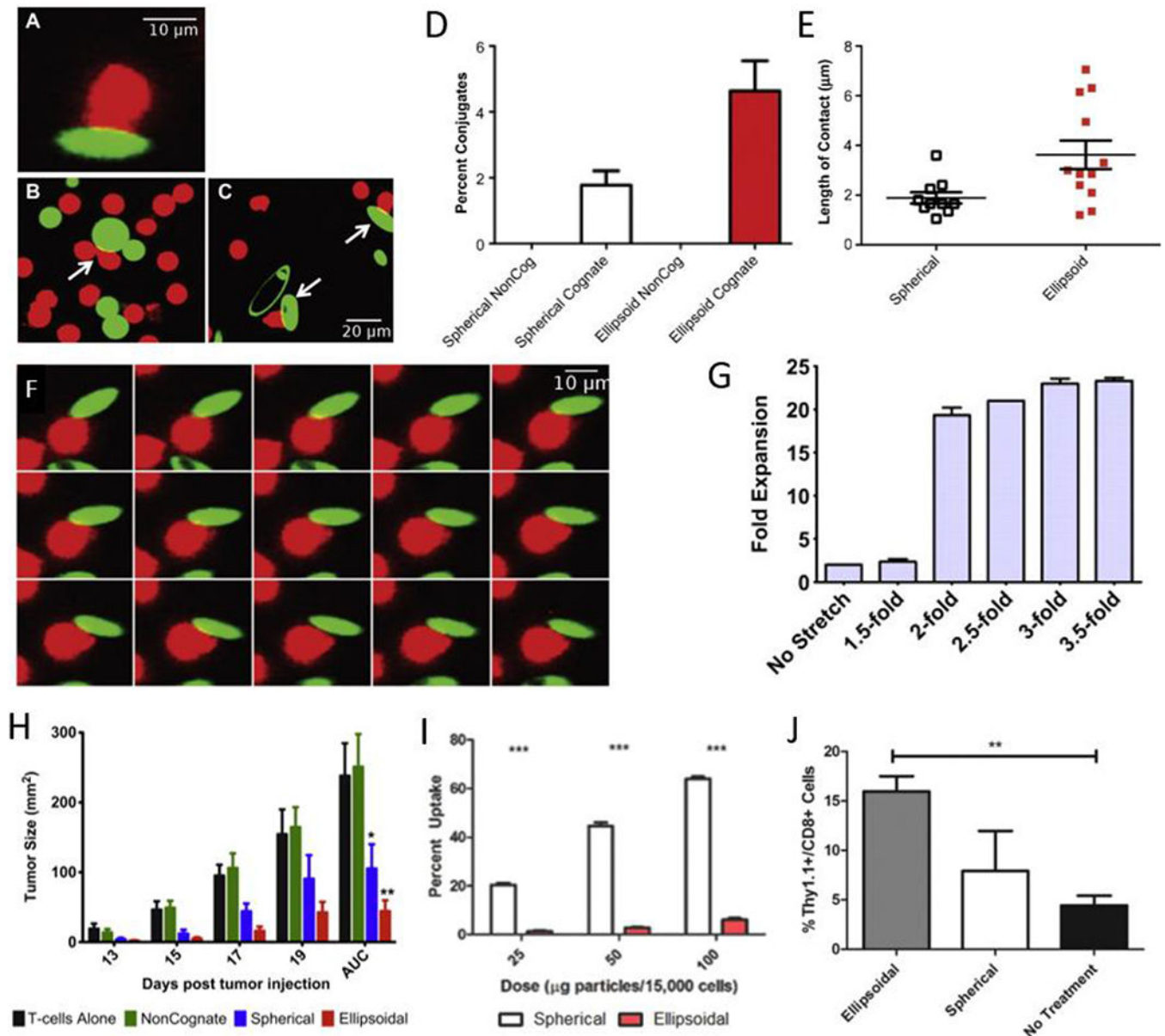
Figure 1. Use of nanoparticles for T cell therapy.
(A) Antigen delivery to a dendritic cell (DC) can be facilitated by vaccine or mRNA loaded nanoparticles. The nanoparticles enter the cell through an endocytic vesicle and the release of the loaded material is mediated through endosomal escape. Released mRNA is translated into protein and processed into peptide for MHC loading and surface presentation. The dendritic cell then activates a cognate CD8+ T cell by presenting signal 1 and signal 2. (B) Artificial antigen presenting cells (aAPCs) mimic endogenous presentation driven by APCs by presenting signals 1 and 2 on the surface to engage T cell receptors and co-stimulatory receptors. Interaction between aAPCs and T cells results in clustering on the T cell surface and engagement of multiple stimulatory receptors. (C) After expansion, nanoparticles maintain and enhance T cell activity through release of stimulatory drugs and cytokines, blocking inhibitory signals, and activation of local antigen presenting cells for restimulation. Created with Biorender.com.

Figure 2. Shape of aAPCs impact T cell activation both *in vitro* and *in vivo*.
(A) aAPC and cognate T cells form a distinctive synapse or cap upon engagement. When observing T cell binding to aAPC, ellipsoidal aAPC form conjugates with T cells more commonly than spherical aAPC (B-D), and for a longer period of time (E). (F) Time-lapse of a CD8+ T cell interacting with an ellipsoidal aAPC, depicting the formation of the synapse. (G) Increasing the stretch of the aAPC results in increased fold expansion of target T cells. (H) Ellipsoidal aAPC activate T cells *in vivo* and prevent tumor growth. (I) Spherical aAPC are taken up by macrophages more frequently than ellipsoidal aAPC. (J) PLGA ellipsoidal particles better activate target T cells *in vivo* than spherical particles [32,117]. (A-H) Reprinted from Biomaterials, 35(1), Sunshine, J. C., Perica, K., Schneck, J. P., & Green, J. J., Particle shape dependence of CD8+ T cell activation by artificial antigen presenting cells, 269–277., Copyright (2014), with permission from Elsevier.

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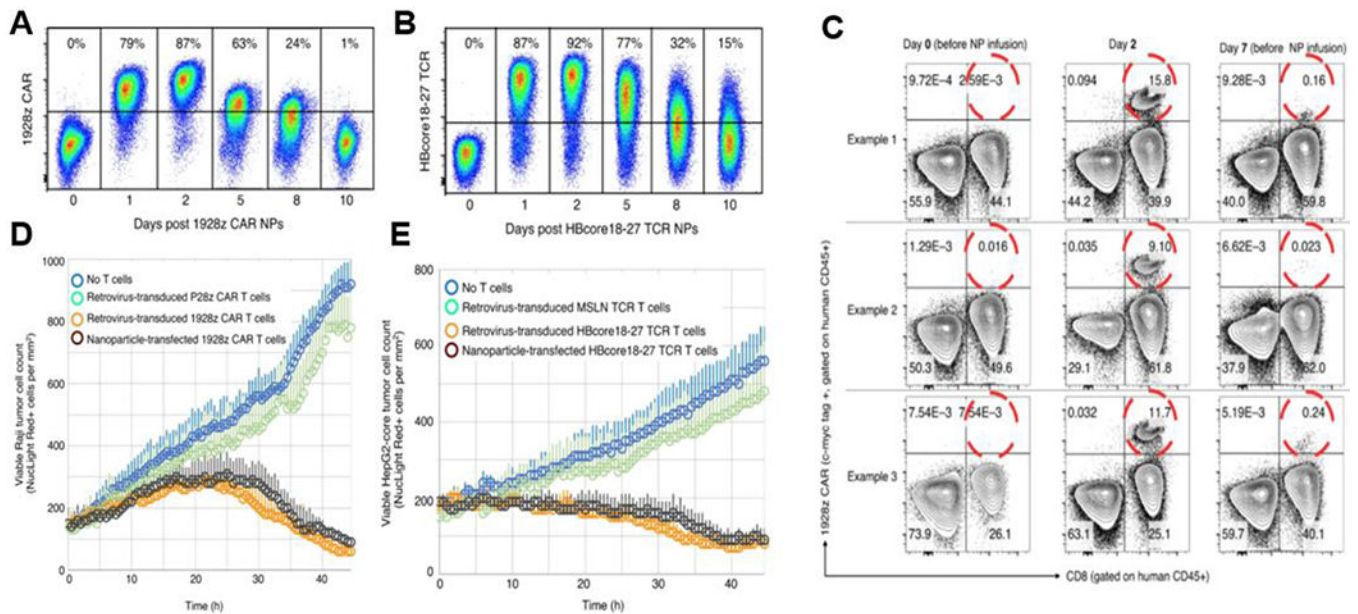
Figure 3. Nanoparticles for CAR and TCR transfection result in successful transfection of human T cells *in vitro* and *in vivo*.

A, B. Flow cytometry of transfected T cells. The cells show transient expression of CAR (**A**) or TCR (**B**) genes after transfection by mRNA loaded nanoparticles. **C.** Flow cytometry showing successful transfection of peripheral T cells with CAR genes after the nanoparticles were injected. **D, E.** T cells transfected with CAR (**D**) or TCR (**E**) genes show ability to kill target cells with similar potency to retrovirus-transduced T cells [123]

Adapted from N.N. Parayath, S.B. Stephan, A.L. Koehne, P.S. Nelson, M.T. Stephan, In vitro-transcribed antigen receptor mRNA nanocarriers for transient expression in circulating T cells *in vivo*, Nat. Commun. (2020). https://doi.org/10.1038/s41467-020-19486-2 licensed under CC BY.