Advanced Drug Delivery Reviews 144 (2019) 3–15

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Optimizing Advances in Nanoparticle Delivery for Cancer Immunotherapy

Joseph M. Caster [a], Cameron Callaghan [a], Steven N. Seyedin [a], Kelly Henderson [a], Bo Sun [b], Andrew Z. Wang [b,*]

[a] Department of Radiation Oncology, University of Iowa Carver College of Medicine, Iowa City, IA, USA
[b] Laboratory of Nano- and Translational Medicine, Carolina Center for Cancer Nanotechnology Excellence, Carolina Institute of Nanomedicine, Lineberger Comprehensive Cancer Center, Department of Radiation Oncology, University of North Carolina at Chapel Hill, USA

ARTICLE INFO

Article history:
Received 17 May 2019
Received in revised form 10 July 2019
Accepted 12 July 2019
Available online 19 July 2019

Keywords:
Nanomedicine
Nanoparticle
Immunotherapy
Cancer Immunotherapy

ABSTRACT

Cancer immunotherapy is one of the fastest growing and most promising fields in clinical oncology. T-cell checkpoint inhibitors are revolutionizing the management of advanced cancers including non-small cell lung cancer and melanoma. Unfortunately, many common cancers are not responsive to these drugs and resistance remains problematic. A growing number of novel cancer immunotherapies have been discovered but their clinical translation has been limited by shortcomings of conventional drug delivery. Immune signaling is tightly-regulated and often requires simultaneous or near-simultaneous activation of multiple signals in specific subpopulations of immune cells. Nucleic acid therapies, which require intact intracellular delivery, are among the most promising approaches to modulate the tumor microenvironment to a pro-immunogenic phenotype. Advanced nanomedicines can be precisely engineered to overcome many of these limitations and appear well-poised to enable the clinical translation of promising cancer immunotherapies.

Contents

1. Introduction . . . 3
2. Overcoming barriers to NP delivery . . . 4
   2.1. Targeting specific cell populations . . . 4
   2.2. Enhancing endosomal escape . . . 6
3. Utilizing NPs to enhance cancer immunotherapy . . . 6
   3.1. Enhancing antigen presentation . . . 6
      3.1.1. Co-delivery of exogenous antigens and immune adjuvants to APCs . . . 6
      3.1.2. Enhancing antigenic presentation of endogenous tumor neoantigens . . . 7
   3.2. Activating effector cells . . . 8
      3.2.1. Enhancing T-cell checkpoint inhibition . . . 8
      3.2.2. NP delivery of T-cell activators . . . 9
   3.3. Modulating the tumor microenvironment . . . 9
      3.3.1. Targeting TAMs . . . 10
      3.3.2. Targeting Th2 T-regs . . . 11
      3.3.3. Targeting TAFs . . . 11
   3.4. Targeting tumor cells . . . 11
4. Enhancing T-cell therapy . . . 12
5. Summary and conclusions . . . 12
Acknowledgments . . . 12
References . . . 13

* Corresponding author.
E-mail address: zawang@med.unc.edu (A.Z. Wang).

https://doi.org/10.1016/j.addr.2019.07.009
0169-409X/

1. Introduction

Cancer immunotherapy is one of the most rapidly evolving fields in clinical medicine. Cancer immunotherapy includes a broad collection of therapies which aim to improve the ability of the immune system to recognize and eliminate tumors. Enthusiasm for cancer immunotherapy is well-supported by the clinical literature. T-cell checkpoint inhibitors, drugs that prevent CD8+ T-cell inactivation by blocking specific inhibitory receptors on the T-cell surface, have produced remarkable improvements in long-term survival for patients with aggressive cancers including metastatic melanoma and non-small cell lung cancer (NSCLC) not seen with traditional chemotherapies [1–4]. However, there are a number of challenges facing the field of cancer immunotherapy. Many common cancers, including microsatellite stable (MSS) colorectal cancers, are poorly responsive to T-cell checkpoint inhibitors [5]. Even among highly immunogenic histologies like melanoma, less than half of patients can expect to achieve long-term disease control. Numerous mechanisms of resistance to current cancer immunotherapies have been identified including the presence of numerous immunosuppressive cell populations in the tumor microenvironment (TME), poor tumor infiltration by activated CD8+ T-cells, and cytokine-mediated conversion of mesenchymal or immunogenic cell populations into immunosuppressive variants [6–8]. Many novel drugs (including cytokines and immune-stimulators) and nucleic acid therapies have been proposed as potential solutions to these mechanisms of resistance. However, their clinical translation with conventional drug delivery has been challenging. Immune stimulating drugs can be very toxic when administered systemically. Selectively depleting immunosuppressive cell populations without concomitantly depleting pro-immunogenic populations that are necessary for establishing sustained antitumor immunity is difficult. Nucleic acids and recombinant proteins require targeted and efficient intracellular delivery to relevant cell populations in order to be effective. Finally, many immune modulators require simultaneous or near-simultaneous activation of multiple signals in target cells to exert their maximal effect. Intensive research efforts have been devoted to finding appropriate drug delivery solutions and novel nanomedicines appear well-positioned to meet many of these challenges.

Nanomedicines are a diverse collection of nanoscale materials measuring approximately 5-150 nm in mean diameter. These include therapeutic, diagnostic, and theranostic compounds (which are simultaneously therapeutic and diagnostic). Most clinically-approved nanomedicines for oncology are macromolecular drug carriers designed to improve tumoral delivery of drugs. These include liposomes, polymeric micelles, protein-drug conjugates, polymer-drug conjugates, dendrimers, and inorganic nanoparticles (NPs) [9]. The conjugation to or encapsulation within a NP drug carrier imparts a number of pharmacokinetic and pharmacodynamic advantages including improved solubility and more favorable biodistribution. The most consistent benefit of chemotherapeutic NP drug-delivery systems is an improved therapeutic index [9–11]. Owing to their increased size, NPs are largely unable to penetrate normal tissues. However, NPs can more readily exit systemic circulation and enter the extracellular space in tumors because of their relatively disorganized and leaky vasculature. As a result, NP drug carriers are preferentially excluded from normal tissues and retained in tumors. This phenomenon is referred to as the enhanced permeability and retention (EPR) effect [12,13]. The benefits of NP delivery systems go far beyond improved biodistribution. These systems are highly tunable with respect to most physical properties including shape, size, charge, hydrophobicity, and chemical composition. This tunability enables precise control over characteristics including tissue penetrability and *in vivo* drug release rates in ways not possible with other drug delivery methods [14,15]. It is also possible to design stimulus-responsive NPs that are destabilized or release their cargo when stimulated by specific physiochemical cues using various chemical linkers or elements [16–18]. Finally, the particle surface can be decorated with various moieties including saccharides, polymers, proteins, or antibodies to avoid immune detection and improve targeting and receptor-mediated uptake within specific cell populations.

2. Overcoming barriers to NP delivery

Despite the above-highlighted advantages of NP delivery systems, a number of challenges related to *in vivo* delivery have limited them from reaching their full potential. Most clinically-utilized NPs for oncology have been untargeted NP formulations of existing chemotherapeutics that rely on the EPR and passive tumor-targeting to enhance tumoral drug concentrations. However, the clinical relevance of the EPR and passive tumor targeting in many spontaneous human tumors has been called into question [19,20]. It is now recognized that many of the vascular irregularities underlying the EPR in xenograft tumor models are more extreme than those observed in spontaneous tumors. Tumor xenografts also tend to lack well-developed tumor stroma which can also act as a barrier to diffusion [21]. As a result, spontaneous human tumors may be less susceptible to passive tumor targeting by the EPR effect than previously predicted in preclinical models. This has been experimentally confirmed in clinical studies using radiolabeled or imageable ferromagnetic NPs in spontaneous solid tumors [22–24]. Uptake and retention of NPs in spontaneous tumors is generally more heterogeneous than in xenograft models. Many particles that reach the extracellular tumor space are either retained there without entering tumor cells or are cleared by tumoral macrophages prior to payload delivery. For many small molecule drugs, particularly hydrophobic ones that can readily diffuse across cell membranes, extracellular delivery may be sufficient to exert their intended effects. However, macromolecular cargos such as peptides and nucleic acids require efficient uptake and intact cytoplasmic or nuclear delivery to be effective. Clearance by phagocytic cell populations can substantially limit their efficacy.

Some of these issues may be less problematic for immune-stimulating nanomedicines than traditional chemotherapy-delivering compounds. Heterogeneous uptake and retention may be sufficient for immune-stimulating NPs. Compared to cytotoxic chemotherapies, many potent immune adjuvants are effective at relatively low concentrations. As highlighted in Fig. 1, heterogeneous uptake of chemotherapy-loaded NPs is sub-optimal and requires high tumoral dosing because only the tumor cells in proximity to the drug-loaded NPs will be affected. In contrast, the delivery of proinflammatory cytokines to a small number of antigen presenting cells (APCs) can stimulate a chain reaction leading to the recruitment and activation of multiple populations of inflammatory cells to initiate immune-mediated tumor clearance. Additionally, many of the targets of immunotherapy are not tumor cells but rather populations of immune cells found in the TME. Novel imaging studies have demonstrated that resident and recruited populations of tumor immune cells are primarily localized to the tumor periphery and perivascular regions of the TME which are easily accessible by most NPs [25]. These include phagocytic cell populations such as APCs and tumor associated macrophages (TAMs). Whereas phagocytic clearance is problematic for tumor-targeting NPs, it can actually be advantageous for novel NPs targeting non-tumor cell populations in the TME. Most novel cancer nanoimmunotherapies are "next generation" NPs engineered to address or take advantage of limitations encountered with previous generations of NPs. Several major concepts are reviewed here to highlight advanced delivery techniques utilized by these nanomedicines.

2.1. Targeting specific cell populations

Cellular targeting has been extensively studied as a solution to improve intratumoral and cell type-specific NP delivery. This can be accomplished by the incorporation of specific molecular tags on the particle surface that can interact with extracellular proteins on endothelial, tumor, or immune cell membranes to facilitate targeted uptake.

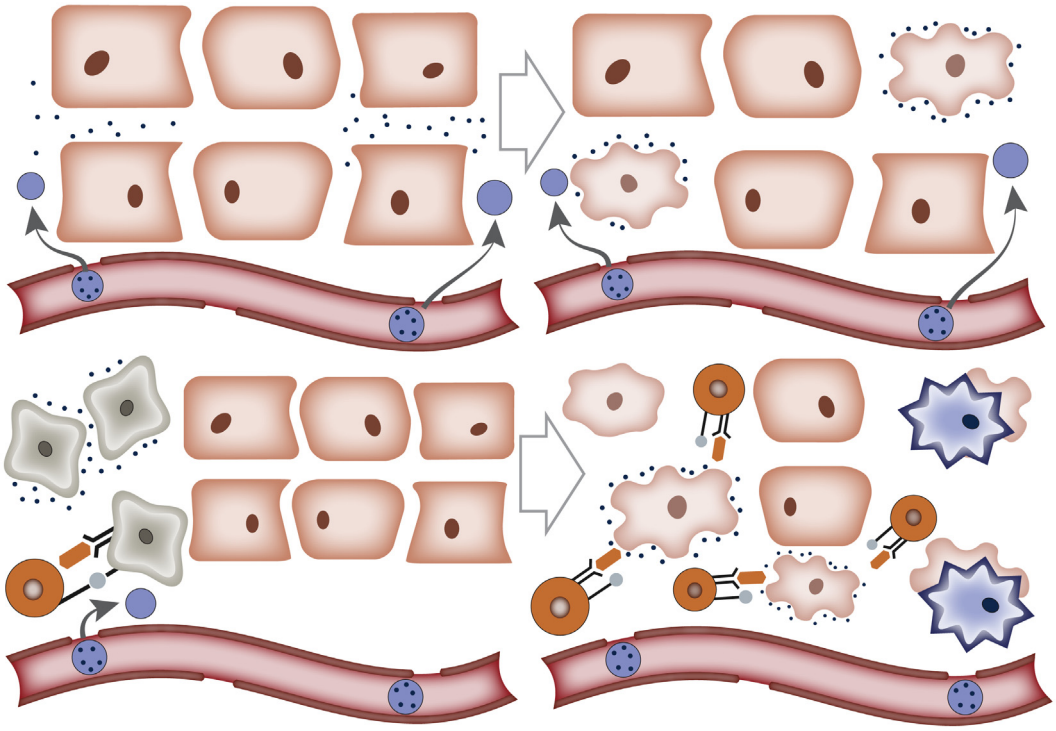

Fig 1. Immune-adjuvant NP delivery. Heterogenous accumulation of NPs within tumors is problematic for conventional chemotherapeutics as they can only exert antitumor effects on the tumor cells in their immediate vicinity (top panels). In contrast, immune adjuvants (bottom panels) only need to activate a small number of key subpopulations of cells including APCs to stimulate a coordinated antitumor response.

Numerous candidate cell-surface markers have been examined preclinically as targets for NP delivery (Table 1). Many of these are cell surface receptors that are preferentially expressed by tumors and not normal tissues to improve tumoral uptake. Targeting of well-known tumor markers including HER-2, EGFR, or PSMA can improve tumoral localization and uptake of NPs *in vivo* [26–35]. Attempts have been made to improve the efficacy of tumor-targeted NPs by targeting the most relevant cell populations capable of repopulation and tumor propagation. CD44 is a multifunctional cell surface antigen protein involved in cell function related to proliferation, migration, and signaling that is enriched in cancer initiating cells (CIC). Wei et al. recently demonstrated that the efficacy of salinomycin-encapsulated lipid-polymer NPs can be markedly enhanced against CD44+ subpopulations of prostate tumors with the incorporation of specific CD44 antibodies on the NP surface [36]. CD44 also strongly interacts with the anionic polysaccharide hyaluronic acid (HA) and surface decoration with HA has been found to improve tumor targeting in CD44 overexpressing cell lines [37–40]. The ability to selectively target specific cell populations while excluding similar cells in close proximity may be especially important for cancer immunotherapy. Several important immunogenic cell populations in the TME including regulatory T-cells (T-regs) and TAMs exist as closely related pro- and anti-immunogenic subpopulations. Concomitant eradication of pro-immunogenic populations with non-selective delivery systems could offset the potential therapeutic advantages gained by removing their immunosuppressive counterparts. Fortunately, targetable cell surface markers have been identified for many relevant populations of immune cells in the TME and the preclinical studies described below provide proof-of-principle for the selective modulation of specific subpopulations using targeted NPs.

It is worth noting that some therapeutic interventions, including ionizing radiation, can modulate the expression of cell surface receptors in ways that may be important for cancer immunotherapy. P-selectin is an endothelial integrin which is overexpressed in many human tumors. Shamay and colleagues confirmed that P-selectin-targeted fucoidan NPs improved intracellular delivery and tumor control in P-selectin overexpressing tumors [41]. They further demonstrated that focal radiotherapy (6 Gy) stimulated P-selectin overexpression in Lewis Lung xenografts that expressed very low levels at baseline. Interestingly, unilateral radiotherapy not only enhanced P-selectin expression in radiated

Table 1
Targeted nanomedicines for cell type-specific delivery.

Cell-type targeted	Targeted receptor	Targeting moiety	Examples
Tumor cells/endothelium	PSMA	Antibody	[30–32]
	EGFR	Peptide	[26–28]
		Antibody	
	Her-2	Antibody	[33–35]
	EpCAM	Peptide	[139,140]
	Folate Receptor	Folate	[141,142]
	P-selectin	Fucoidan Polysaccharide	[41]
	CD44	Antibody	[36]
		Hyaluronic Acid	[37,38,143]
M2 Tumor Associated Macrophages (TAMs)	Mannose Receptor (CD206)	Monosaccharide	[102,109]
	Sialic Acid Receptor	Sialic Acid	[111]
	SR-B1 Receptor	Apo-1 Peptide	[144]
Th2 Regulatory T-cells	GITR	Peptide	[112,113]
	Nrp1	Peptide	[145]
Tumor-Associated Fibroblasts (TAFs)	Sigma-Receptor	Anisamide	[118–120]
Dendritic Cells	SR-B1 Receptor	Peptide	[144]
T-Lymphocytes	CD3	Antibody	[146,147]
	CD4	Antibody	[148]
	CD45	Antibody	[137,149]
	CD90	Antibody	[150]
B-Lymphocytes/Plasma Cells	CD19	Antibody	[151–154]
	CD20	Antibody	[155–158]
	CD35	Gp350 Peptide	[159–161]
	CD38	Antibody	[162]

tumors, but also stimulated an abscopal-like effect in which P-selectin expression was enhanced in unirradiated tumors using bilateral flank xenografts. As predicted, focal radiotherapy significantly improved the efficacy of targeted compared to untargeted liposomes. Previous studies with folate-targeted hapten immunotherapy also observed improved tumor targeting in unirradiated tumor deposits following focal radiotherapy [42]. The ability to modulate the expression of targetable cell surface receptors in the TME of distant foci of disease using focal radiotherapy could be used to enhance abscopal responses to cancer immunotherapies. Other potential radiation-inducible targets include various integrins, the glutathione receptor, and class 1 MHCs (among others).

2.2. Enhancing endosomal escape

One major obstacle facing intracellular payload delivery is degradation in the late endosome. The interaction of NPs with cell surface receptors stimulates receptor-mediated endocytic translocation of the NP from the cell surface into the cytoplasm. Molecules taken up by this process are generally degraded in the acidic environment of the late endosome. Without additional escape mechanisms, most NP payloads are degraded in the endosome along with their NP carrier. This is particularly problematic for macromolecules such as nucleic acids that can't easily diffuse across the endosomal membrane once released from the degrading NP. Several preclinical methods of improving endosomal escape have been identified.

Perhaps the most simplistic among these is the use of amphiphilic NP carriers which are stabilized by acid-labile linkers. These carriers can take advantage of the acidic environment to stimulate rapid release of drug payload within the endosome. Hydrophobic small molecule drugs can then readily diffuse from the endosome into the cytoplasm to exert their antitumor effects. One recent example of this approach was provided by Yang and colleagues [43]. They synthesized paclitaxel-loaded PEG-BHyd-dC12 micelles stabilized by an acid-labile hydrazone bond. Using Cou-6 encapsulated NPs, they confirmed endosomal localization of the particles following receptor-mediated uptake. The labeled payload remained trapped in the endosomes of NPs lacking the acid-labile bonds whereas it quickly diffused into the cytoplasm of the functionalized particles. Acid-labile NPs were significantly more cytotoxic in several lung cancer lines than non-labile micelles or free drug. While these kinds of delivery systems may suffice for delivery of hydrophobic small-molecule drugs, more sophisticated platforms are necessary for macromolecular payloads.

NP vectors have emerged as a promising alternative to viral systems for targeted *in vivo* delivery of nucleic acid therapies. Stable *in vivo* knockdown using siRNA delivery systems has been achieved and several compounds have received FDA approval [44]. However, the efficiency of nucleic acid delivery is poor and facilitating endosomal escape remains challenging. NPs incorporating pH-sensitive cationic lipid carriers, such as DLinDMA (1,2-dilinoleyloxy-3-dimethyaminopropane), have been used to facilitate membrane fusion-mediated endosomal escape whereby the acidic environment of the late endosome triggers fusion of the NP surface with the endosomal membrane and subsequent release of the NP contents into the cytoplasm [45,46]. Newer pH-sensitive cationic lipids, such as DLin-MC3-DMA, can increase the *in vivo* ED_{50} (minimum dose of siRNA to produce the desired biological effect in 50% of subjects *in vivo*) by a factor of almost 100 [47]. However, these remain relatively inefficient with >97% of the payload being lost in the late endosome. Sato and colleagues recently evaluated the effects of modulating the hydrophobicity of the head and tail regions of the unsaturated pH-responsive lipid YSK12-C4 on intracellular delivery and endosomal escape of lipid NPs containing siRNAs targeting clotting factor VII [48]. They assessed efficiency of endosomal escape by quantifying the fraction of siRNA loaded into the RNA-induced silencing complex (RISC). Lipid NPs with CL4H6 lipids (containing bulky hydrophobic tails) were significantly more efficient carriers than traditional DLin-MC3-DMA carriers. Approximately 4.2% of the CL4H6 siRNA payload was successfully loaded to the RISC complex which translated to an approximate 50% reduction in ED_{50}. It is worth noting that a significantly higher proportion of the siRNA payload entered the cytosol than was loaded to the RISC but continued interaction between the nucleic acid payload and degraded lipid carrier limited subsequent processing. Studies such as these demonstrate that engineering of novel lipid carriers may represent a viable method to improve the efficiency and efficacy of *in vivo* NP-based nucleic acid delivery.

Another method of endosomal escape involves the use of endosmotic or proton-buffering polymers such as poly(ethylenimine) (PEI) to stimulate a proton-sponge effect [49,50]. The exact mechanisms by which proton-buffering nanocomplexes stimulate endosomal escape has not been clearly established but is believed to involve osmotic flux across the outer endosomal leaflet leading to membrane destabilization, permeability, or polymer-supported pore formation [51]. Regardless of the exact mechanism, recent preclinical studies have demonstrated that endosmotic carriers can efficiently delivery cargo to the cytoplasm intact and separate from the nanocarrier enabling efficient *in vivo* delivery of functional nucleic acid therapies. A schematic representation of endosomal escape mechanisms with engineered NPs is shown in Fig. 2.

3. Utilizing NPs to enhance cancer immunotherapy

3.1. Enhancing antigen presentation

One of the initiating events in generating tumor-specific (adaptive) immunity is the identification and uptake of tumor neoantigens by professional APCs including dendritic cells (DCs). Activated DCs must then present these antigens along with co-stimulatory molecules to effector T-cells. This process can be disrupted in several ways. First, neoantigen processing and presentation is a relatively inefficient process. Only a small fraction of the antigens presented on major histone compatibility (MHC) complex 1 by tumors are unique neoantigens that can be distinguished from normal cellular proteins and are expressed in sufficiently high concentrations to be targeted by T-cells [52]. Some immunoresistant histologies are believed to have relatively low neoantigen burdens and/or mechanisms to actively downregulate neoantigen expression or shield them from APCs and T-cells. Ablative therapies like radiation can stimulate the release of neoantigens but recognition of these antigens by APCs appears to be poor and, in the absence of co-stimulatory signals, can induce T-cell anergy. Second, many immunosuppressive cell populations in the TME secrete inhibitory cytokines and ligands to prevent DC maturation and presentation to effector cells [53]. Identifying novel ways of improving antigen processing and presentation in APCs has been a focus of intensive research in cancer immunotherapy.

3.1.1. Co-delivery of exogenous antigens and immune adjuvants to APCs

A major focus of research for immunotherapeutic nanomedicines has been the targeted delivery of engineered neoantigents to APCs in an effort to stimulate tumor vaccination. NP carriers are ideally-suited to this task for several reasons. First, they efficiently protect neoantigens for *in vivo* delivery. Second, owing to their "virus-like" physical properties, they are efficiently recognized and taken up by APC and accumulate in lymphoid tissues. Third, immunogenic stimulation requires co-activation by a second signal. Recognition of neoantigens by APCs in the absence of a stimulatory signal is not only ineffective, but can promote immune tolerance. NPs can be engineered with specific tumor peptides and immune adjuvants (like CpG-ODN) to ensure co-delivery of both signals to activated APCs [54]. Alternatively, the NP itself can be composed of potent immune adjuvants such as PC7A or PEI [55,56]. Several preclinical studies have demonstrated proof-of-principle for this approach using engineered NPs with established tumor neoantigens including OVA and gp100 [57–59]. As one example, an A1

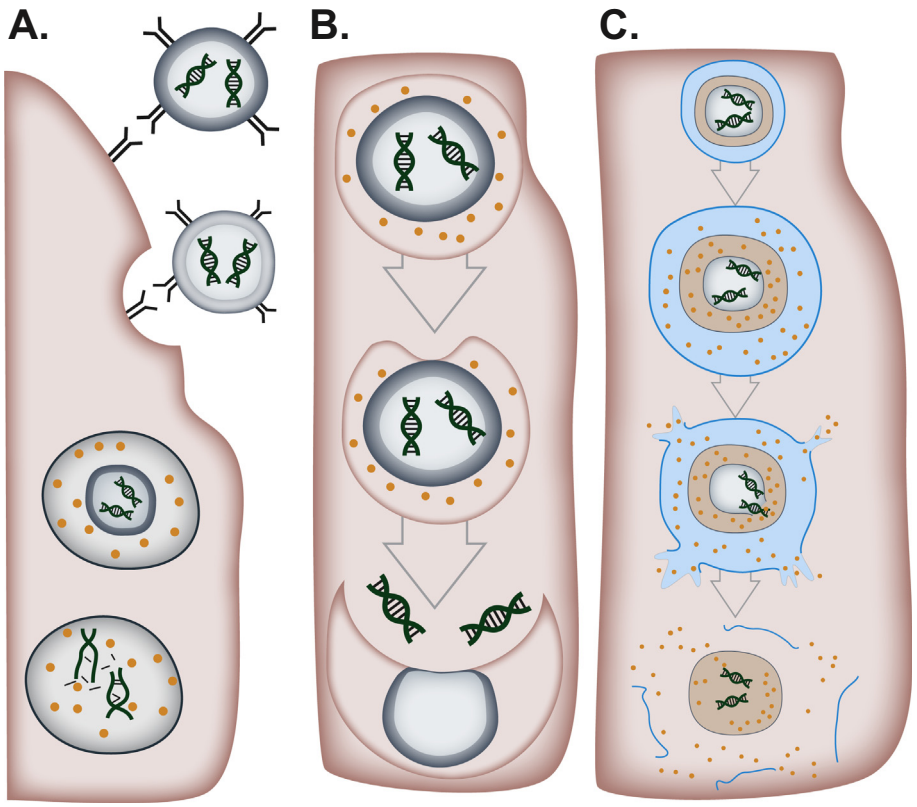

Fig 2. Mechanisms of endosomal escape. When targeted NPs interact with extracellular receptors they are internalized via receptor-mediated endocytosis. In the absence of additional escape mechanisms, macromolecular cargos (including nucleic acids) are degraded with the NPs in the acidic environment of the late endosome (A). NPs constructed for pH-sensitive cationic lipids undergo a conformational change in the acidic environment of the endosome and can fuse with the endosomal membrane to release their cargo into the cytosol intact (B). Proton-buffering nanocomplexes can also be used to absorb the incoming hydrogen ions and stimulate a "proton sponge" effect that leads to osmotic swelling and rupture of the endosomal membrane (C).

lipoprotein nanodisc used to codeliver tumor neoantigens and CpG to mice bearing MC38 or B16F10 tumors stimulated approximately 90% complete tumor regression compared to 25-38% regression with systemic delivery of CpG and tumor antigens when combined with dual checkpoint inhibition [60]. It is also possible to incorporate nucleic acid therapy into immune adjuvant and neoantigen vaccine strategies. Zhu et al. generated nucleic acid nanocarriers stabilized by PEG-grafted polypeptides for the simultaneous delivery of CpG, stat3 siRNA, and the somatic tumor neoantigen Adpgk found in syngeneic MC38 colorectal tumors [61]. Three weeks after immunization, only 1.1% of peripheral CD8+ T-cells had Adpgk-specific receptors in CpG treated mice compared to 9.5% of NP vaccine-treated mice. Inorganic, protein-based (albumin), and microparticle emulsion systems have also been engineered as cancer vaccines [62–67].

Focal therapies that modulate local immune responses may also enhance the efficacy of NP vaccines. Gao and colleagues developed a pH-sensitive endosomolytic polymeric NP that efficiently delivered OVA peptide to APCs called PC7A [55]. These NPs increased APC activation through the stimulator of interferon gene (STING) pathway. While tumor growth delay was enhanced, resistance to therapy was problematic for tumors > 100 mm^2 at the time of treatment. One of the principle activators of STING is cytosolic DNA. Radiation enhances the generation of cytosolic DNA and activation of STING is believed to be one of the central mechanisms by which radiation enhances systemic immunity following radiation [68]. Dr. Gao's group recently demonstrated that tumor rejection and systemic immunity could be enhanced in larger tumors by combining PC7A particles and focal radiotherapy and that this effect was mediated by enhanced STING activation by combination therapy [69].

RNA-based vaccine strategies delivering neoantigen mRNAs and immune adjuvants directly to APCs have also demonstrated promise. Lipid calcium phosphate NPs have been used to deliver mRNA and induce expression of tumor neoantigens such as MUC1 to successfully induce class-restricted cytotoxic T-cells and improve responses to checkpoint inhibitors in preclinical studies [70]. Liu et al. confirmed efficient delivery and translation of a modified MUC1 mRNA in 4T1 tumors and draining lymphoid tissues using mannose decorated lipid calcium phosphate NPs. The modified protein product was easily distinguished from endogenously expressed antigen. Importantly, the validity of this approach has also been demonstrated in early phase clinical trials. The systemic delivery of neoantigen mRNA using DOTMA/DOPE liposomes induced strong systemic INF responses and the generation of tumor-specific neoantigens in three patients [71].

3.1.2. Enhancing antigenic presentation of endogenous tumor neoantigens

At present, a major rate-limiting step to the clinical translation of efficient nanovaccines is the identification of specific tumor neoantigens for individual patients. A priori knowledge of targetable, specific tumor peptides in many spontaneous human tumors is rare. Additionally, clinical experience with targeted biologic therapies suggests that the stimulation of monoclonal populations of T-cells targeting a single tumor peptide may be suboptimal. Most tumor-directed biologics, including EGFR and ALK inhibitors, stimulate robust initial responses and improve progression-free survival (PFS). However, the development of resistance is inevitable and these drugs have minimal effect on overall survival (OS) [72,73]. Some mechanisms of resistance to targeted biologics, such as the upregulation of redundant signaling pathways, may not be relevant to immune vaccines. However, tumor heterogeneity and downregulation of the targeted peptides could be very problematic for monoclonal vaccines. Wang and colleagues recently demonstrated that cationic antigen-capturing NPs and ionizing

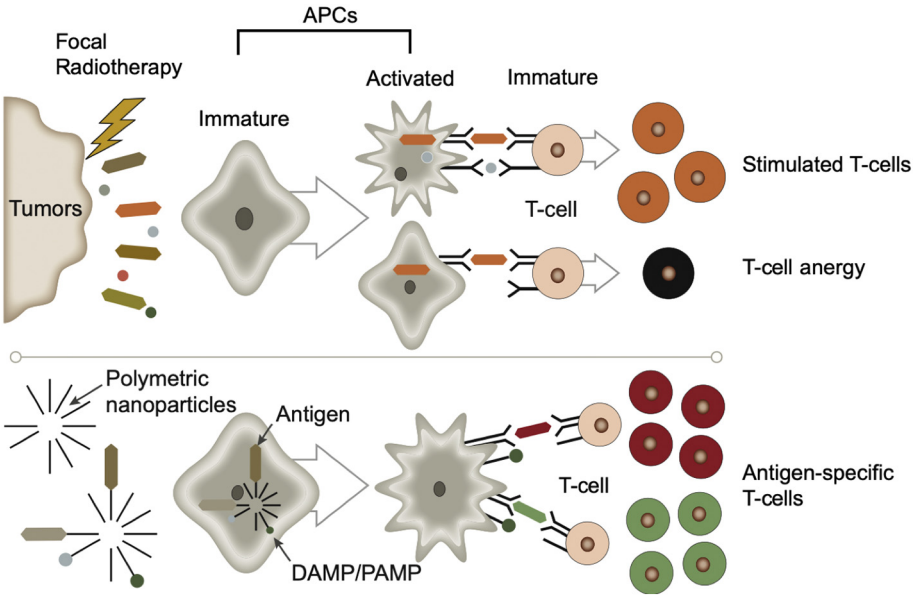

Fig 3. Using antigen-capturing NPs to enhance APC activation and generation of clonal T-cell populations. Focal radiotherapy stimulates the release of tumor antigens as well as danger signals (DAMPs and PAMPs) necessary for APC activation (top panel). Some APCs will interact with both activating signals and tumor antigens and be capable of stimulating a clonal T-cell population (orange). However, other APCs will find antigens in the absence of activating signals leading to T-cell anergy (black). Antigen-capturing NPs can improve the efficiency of clonal T-cell expansion by improving the simultaneous delivery of tumor antigens and danger signals to APCs (bottom panel).

radiation can stimulate robust polyclonal immune responses [74]. They generated polymeric PLGA NPs with various surface modifications (decoration with DOTAP, maleimide, NH2, mPEG, or unmodified PLGA) to determine the effects of NP surface chemistry on antigen capture efficiency. All of the particles except for those decorated with mPEG efficiently captured tumor neoantigens following *in silico* radiation of B16F10 tumor cells. Importantly, these particles also captured immune adjuvants including various DAMPs and alarmins that are necessary for APC co-stimulation and avoidance of immune tolerance. They then used a bilateral flank-tumor model to assess systemic immunity. Intratumoral injection of particles into the unilaterally-radiated tumor significantly enhanced abscopal responses in the unirradiated contralateral tumor compared to radiation and anti-PD-1 antibodies alone. Intratumoral injection of unmodified PLGA NPs stimulated complete regression of bilateral tumors in 20% of mice compared to 0% without any NPs. Using fluorescent-labeled NPs, they were able to confirm that the NPs were efficiently internalized by APCs and trafficked to lymphoid tissues. Fig. 3 shows how antigen-capturing NPs can improve the efficiency of T-cell activation by improving the simultaneous presentation of neoantigens and danger signals to APCs.

Cell membrane-coated NPs have also been used to improve endogenous neoantigen recognition by APCs. In this technique, NP carriers are decorated with disrupted cell membranes and their associated cell surface proteins. Kroll et al. coated CpG-loaded PLGA NPs with B16F10 surface membranes [75]. They showed that the surface of the membrane-coated NPs contained multiple established tumor neoantigens including gp100, TRP-2, and melanin A. Significantly more (86%) mice were successfully vaccinated with these particles than with whole cells and CpG. Another group demonstrated similarly encouraging results with membrane-coated PLGA NPs encapsulating the TLR-7 agonist imiquimod [76]. These novel approaches demonstrate that it may be possible to stimulate polyclonal systemic immune responses without any upfront identification of specific tumor neoantigens in individual patients using novel nanomedicines.

3.2. Activating effector cells

Activated CD8+ T-cells are the key effectors of tumor-specific (adaptive) immunity. Even if clonal antitumor T-cells that can recognize and attack tumor cells are generated, they must efficiently infiltrate the tumor stroma and remain activated in order to be effective. T-cell-mediated cytotoxicity is a tightly regulated process. Numerous receptors are expressed on the T-cell surface that can stimulate or inhibit the activity of activated T-cells [77]. Inhibitory receptors include CTLA-4 (CD152), PD-1 (CD279), TIM-2, and LAG-3 (CD223). Many tumors express ligands to these receptors, such as PDL-1, in an effort to reduce the activation and maturation of APCs and infiltrating CD8+ T-cells. Inhibitory antibodies to CTLA-4 and PD-L1 are far and away the most successful cancer immunotherapies to date. However, they are not without limitations. Among immunogenic histologies like NSCLC, they are only effective in tumors that overexpress the PD-L1 ligand [78]. Durable response rates in PD-L1 overexpressing tumors are generally less than 50%. Many non-immunogenic tumors, including MSS colorectal tumors, express high levels of PDL-1 but are not responsive to anti-PD-1 therapies [79,80]. Natural killer (NK) cells are also important innate (antigen non-specific) cytotoxic effector cells. They harbor inhibitory surface receptors such as CD47 that can be overexpressed by tumors to avoid clearance by the innate immune system [81]. Intensive research has been dedicated to identifying novel methods of improving the activation of effector cells in the TME.

3.2.1. Enhancing T-cell checkpoint inhibition

NP delivery has been used to improve the efficacy of CTLA-4 and PDL-1 inhibitors by providing sustained release in tumors and co-delivery with other immune adjuvants. One group engineered photodegradable PLA NPs co-encapsulated with hollow gold nanoshells and anti-PD-1 peptide (APP) [82]. These particles enabled sustained release of APP for up to 40 days. Accelerated release could be stimulated with a near infrared laser. In a metastatic model system, laser-irradiation of "primary" tumors stimulated partial regression and growth delay in the laser-treated and distant tumor deposits indicating the activation of a systemic immune response. Zhen Gu and his group developed a cleavable nucleic acid-based NP comprised of the potent immune-stimulant CpG-ODN to deliver anti-PDL-1 antibodies [83]. The nucleic acid shell was constructed with ssDNA and CpG repeats with cutting sites for the restriction enzyme HhaI. HhaI was caged in triglycerol monostearate NPs attached to the larger DNA NPs which could be enzymatically-cleaved by wound-responsive esterases and matrix

metalloproteinases. Wound-associated inflammation (triggered by partial tumor resection) resulted in degradation of the DNA carrier and release of anti-PDL1 antibodies and CpG-ODN fragments. Systemic NP administration and subtotal tumor resection stimulated complete tumor rejection in 40% of mice, whereas no complete rejections were observed with systemic co-administration of CpG-ODN and anti-PDL-1 antibodies.

NP-delivered nucleic acid therapies have also been used for the targeted knockdown of inhibitory receptors in effector cells or inhibitory ligands in tumors that can enhance tumoral infiltration and activity of anti-tumor CD8+ lymphocytes. Wu et al. utilized lipid-coated calcium phosphate NPs to deliver siRNA targeted against PD-1 receptor and PDL-1 ligand [84]. These NPs efficiently knocked down PD-1 receptor in tumor-infiltrating lymphocytes (isolated from patient samples) and PDL-1 in MCF-7 breast cancer cells *in vitro*. Combined knockdown significantly enhanced CD8-mediated cytotoxicity assessed using *ex vivo* cytotoxic T-cell assays. They also observed significant increases in INFγ and TNFα excretion following co-culture of PD-1$^-$ TILs and PD-L1$^-$ MCF tumor cells. Lian et al. generated cationic lipid NPs to deliver siRNA against PDL-1 and CD47 in 13 tumor cell lines *in vitro* [85]. Tumor targeting and uptake was enhanced by decorating the particle surface with an EpCAM targeting peptide. Dual knockdown significantly enhanced CD8+ and NK-mediated cytotoxicity *in vitro*. They also demonstrated that their targeted NPs efficiently improved tumoral infiltration of CD8+ and NK cells *in vivo* using a 4T1 metastatic lung cancer model. This resulted in improved local tumor control and significantly decreased frequency of lung metastases. Similar improvements in tumor regression and CD8+ infiltration were observed in B16 melanoma tumors in a similar study utilizing lymphocyte-directed NP delivery of CTLA-4 siRNA [86]. One group recently compared the efficiency of PD-1 siRNA in lymphocytes using layered double hydroxide and lipid calcium phosphate NPs [87]. While both could facilitate decreased PD-1 receptor expression, cellular uptake and gene silencing were more effective with the lipid calcium phosphate particles suggesting that it may be the preferred platform for lymphocyte-directed siRNA delivery.

Another interesting approach to enhancing anti-PD-1 therapies has been the use of PD-1 receptor-expressing nanovesicle decoys [88]. Cellular membranes can be engineered into nanoscale vesicles using a membrane extrusion method. One group generated nanovesicles with high levels of PD-1 receptor from HEK193T cells stably overexpressing PD-1. The nanovesicles remained in circulation longer than PDL-1 antibodies and induced significantly greater CD8+ tumor infiltration and tumor regression in melanoma tumor models. They could further enhance antitumor effects by co-delivering encapsulated 1 methyltrypophan (a potent inhibitor of IDO) to offset the actions of co-localized immunosuppressive cell populations.

It is also possible to enhance the effects of PD-1 blockade by increasing activation of professional APCs. Activated APCs release proinflammatory cytokines including type-1 interferons that promote the formation of antigen-specific T-cells. Cytosolic cyclic dinucleotides are used as signaling molecules by bacteria and potent immune-stimulating danger signals. Endogenous cytosolic DNA can also be converted to cyclic dinucleotide cGMP-AMP synthase (cGAS) [89]. Cytosolic dinucleotides potently stimulate type-1 INF responses in a TLR-independent mechanism by activating the endoplasmic reticulum protein STING [90]. STING agonists appear very promising in preclinical tumor models and are being investigated in clinical trials to enhance responses to checkpoint inhibitors. However, the intracellular localization of STING may be problematic even for direct tumoral injections of cyclic dinucleotides as cytosolic accumulation is poor. Wilson and colleagues generated cationic poly(beta-amino ester) (PBAE) NPs for the intracellular delivery of cyclic dinucleotide STING agonists [91]. NP formulation increased STING activation 10-fold compared to direct tumoral injection of cyclic dinucleotides and improved tumor growth delay alone or in combination with anti-PD-1 antibodies in B16F10 melanoma tumor models.

3.2.2. NP delivery of T-cell activators

Enhancing cytotoxic T-cells by activating stimulatory surface receptors has also been explored as a way to overcome resistance to checkpoint inhibitors and T-cell modulation by immunosuppressive cell-types in the TME. Agonists to OX40 have been tested in clinical trials but early results have shown modest efficacy with conventional systemic delivery [92,93]. Enrichment of these drugs in tumors using NP delivery appears to improve their efficacy. One study compared *in vivo* cytokine modulation and tumor growth delay using free and PLGA NP-delivered OX40 stimulatory antibodies [94]. NP formulation significantly enhanced CD8+ and NK tumor infiltration, proinflammatory cytokine signaling, and anti-tumor cytotoxicity compared to free antibody. Another study attempted to enhance the activation of antigen-specific clonal T-cells at the point of engagement with tumors using tumor-targeted OX40-stimulating NPs. They generated specific AFP targeted CD8+ T-cells (AFP 158-166) and PLGA polymeric NPs co-decorated with AFP binding antibodies and stimulating OX40 antibodies [95]. Dual-targeted NPs efficiently enhanced T-cell activation and cytotoxic killing of AFP-expressing HepB2 hepatocellular tumor cells but had no AFP negative SMMC-7721 cells indicating that the efficacy of OX40 stimulation is enhanced by temporal co-localization with target cells.

Simultaneous OX40 activation and PD-1 disinhibition has also been investigated to maximize CD8+ T-cell activation. Systemic co-administration of activating OX40 and inhibitory PD-1 antibodies is inefficient because only a small fraction of T-cells will be engaged by both antibodies. Mi et al. developed polymeric NPs co-decorated with OX40 and PDL-1 antibodies to enable spatialtemporal co-delivery of both signals to T-cells [96]. The dual-targeted NPs stimulated potent systemic immune responses in B16 melanoma and 4T1 breast cancer models. They observed complete tumor regression in almost 40% of animals treated with co-decorated NPs compared to only 5% in animals treated with both antibodies as systemic agents or singly decorated NPs. Durable immunity was achieved as 83% of cured mice were resistant to tumor re-challenge. In a slightly different paradigm, another group generated NPs co-decorated with inhibitory PDL-1 antibodies and stimulatory 41BB antibodies to simultaneously engage tumor and T-cells [97]. Dual-targeted NPs improved tumor growth delay and resulted in expansion of tumor-specific T-cell clonal populations. These studies provide strong evidence for the importance of simultaneous (or near simultaneous) delivery of complimentary signals to maximize the efficacy of cancer immunotherapies and nicely highlight advances not possible with conventional drug delivery.

3.3. Modulating the tumor microenvironment

Spontaneous tumors are comprised of many cell populations including tumor cells, immune cells (both immune-stimulating and immunosuppressive populations), fibroblasts, and endothelial cells in addition to acellular components. These are collectively referred to as the tumor microenvironment (TME). It is now appreciated that non-tumor cell components of the TME are critical for tumoral proliferation, migration, and therapeutic responsiveness. The TME represents both a physical and biochemical barrier to infiltrating immune cells. Immunosuppressive cell populations within the TME pose a major barrier to immune-mediated tumor clearance and cancer immunotherapies. These include Th2 CD4+ regulatory T-cells (T-regs), M2 TAMs, and tumor-associated fibroblasts (TAFs). Strategies which deplete inhibitory cell populations or convert them into immunostimulatory phenotypes have the potential to improve the efficacy of conventional cancer therapies and cancer immunotherapy. Unfortunately, many immune-modulating drugs and cytokines are poorly tolerated systemically and selective ablation of immunosuppressive but not proimmunogenic cell populations is difficult with conventional drug delivery. Extensive preclinical investigations have identified a handful of cell surface markers that are selectively overexpressed in tumoral populations of

inhibitory cell types that can potentially be targeted by stealth NPs. Nanomedicines are well-poised to enhance the feasibility of these approaches by decreasing the systemic toxicity of novel compounds and improving their delivery to relevant cellular populations.

3.3.1. Targeting TAMs

There are two principle classes of TAMs: immunostimulatory M1 macrophages, which facilitate tumor clearance, and immunosuppressive M2 macrophages that inhibit cytotoxic CD8+ T-cells and promote tumor cell proliferation/survival [98]. The basis of immune modulation by TAMs is secretion of high-levels of cytokines in the TME. M1 TAMs secrete high levels of pro-inflammatory cytokines including INF-γ, TNF-α, and IL-12 which promote maturation of APCs, Th1 Tregs, and adaptive immune responses. M2 macrophages, in contrast, secrete high levels of immunosuppressive cytokines including IL-10 and TGF-β which inhibit MHC-mediated antigen presentation and stimulate apoptosis of various lymphocyte populations. As such, the relative ratio of M2 to M1 TAMs in the TME is critical to determining sensitivity of tumors to conventional chemotherapies and immunotherapies including T-cell checkpoint inhibitors. Accordingly, high levels of M2 macrophages are associated with resistance to T-cell checkpoint inhibitors and poor long-term survival [99,100]. Fortunately, TAMs are highly plastic and several strategies to promote polarization towards the pro-immunogenic M1 phenotype have been identified. One strategy involves activation of specific toll-like receptors (TLR) on the macrophage surface. A number of TLR agonists have been identified including R848, a selective TLR7/8 agonist that potently drives macrophages towards the M1 phenotype. One group demonstrated that cyclodextran NPs efficiently deliver R848 to TAMs *in vivo* and stimulate M1 polarization [101]. This in turn sensitizes non-immunogenic tumors to T-cell checkpoint inhibitors leading to sustained tumor rejection and resistance to subsequent tumor challenge. M1 macrophages also express high levels of the mannose receptor MRc1 (CD206) that can be used for NP targeting. Mannosylated cationic albumin carriers have been used to enhance *in vivo* delivery of the TLR agonist CpG-ODN to facilitate M1 polarization. Ai et al. showed that the inclusion of mannose moieties enhanced M1 polarization (as evidenced by increased secretion of pro-inflammatory cytokines such as IL-12 and IL-6) compared to standard cationic albumin carriers [102].

Other well-characterized cytokines can also alter polarization of TAMs. Pro-inflammatory cytokines including GM-CSF, INFβ, and INFγ enhance M1 polarization primarily through activation of STAT1 and STAT5 pathways [103]. Systemically-administered GM-CSF exerts minimal effect on cell populations in the TME. NP-delivered GM-CSF, in contrast, appears to effectively polarize TAMs to the M1 phenotype and improve immune-mediated cytotoxicity. Researchers in one study generated micellar formulations of polymer-CSF conjugates linked by an acid-labile calcium carbonate linker. Very little GM-CSF was released from the micelles at physiologic pH. However, acid-mediated cleavage in the TME stimulated rapid release of active GM-CSF that enhanced M1 differentiation and improved tumor growth delay [104].

Polarization of TAMs towards the M1 phenotype can also be accomplished with NPs by targeting inflammatory signaling pathways. miR155 is an important microRNA that regulates inflammatory signaling pathways in myeloid and lymphoid pathways [105,106]. Yang et al. delivered miR155 to immune cells in the TME using layered double hydroxide NPs [107]. These particles were efficiently taken up by TAMs and demonstrated efficient endosomal escape and cytosolic delivery. miR155 markedly reduced the expression and activation of STAT-3, ERK1/2 and NFκB and polarized TAMs towards the M1 phenotype. These changes in inflammatory signaling were also associated with decreased recruitment and formation of activated myeloid derived suppressor cells (MDSCs) in the TME. miR155 treatment increased tumoral infiltration of CD8+ T-cells and enhanced responsiveness to checkpoint inhibitors.

It may also be possible to repolarize TAMs using drug-free inorganic NPs. High levels of intracellular iron can modulate cytokine signaling and cell proliferation by increasing oxidative stress, particularly in the oxidative environment of the TME. Ferumoxytol is a superparamagnetic iron-oxide NP (SPION) approved for the treatment of chronic iron deficiency anemia. These particles are readily cleared by TAMs in the TME without specific targeting moieties. *In vitro*, they have no effect on macrophage proliferation or survival [108]. However, they demonstrate clear polarization towards an M1 phenotype (as evidence by increased expression of TNF-α and CD86) and away from the M2 phenotype (decreased expression of CD206 and IL10). *In vivo*, ferumoxytol delayed local progression and significantly decreased metastatic spread in MMTV-PYMT mouse models of breast cancer.

In addition to altering the polarization of TAMs, targeted NPs have also been used to deplete M2 macrophages in the TME (Fig. 4). In one study, mannose-conjugated lipid-coated NPs encapsulating calcium zolendronate (a bisphosphonate that demonstrates selective cytotoxicity to TAMs [109]) effectively depleted established S180 tumor xenografts of inhibitory TAMs and markedly enhanced tumor growth delay. TAMs also express high levels of sialic acid receptors [110]. Zhou et al. have shown that sialic acid-coated liposomes loaded with epirubicin can enhance selective clearance of M2 TAMs and improve rejection of murine xenograft sarcoma models without significant systemic toxicity [111]. Collectively, these studies demonstrate that functionalized NPs can be engineered to efficiently repolarize or deplete inhibitory TAMs in the TME to improve responsiveness to cancer immunotherapies including T-cell checkpoint inhibitors.

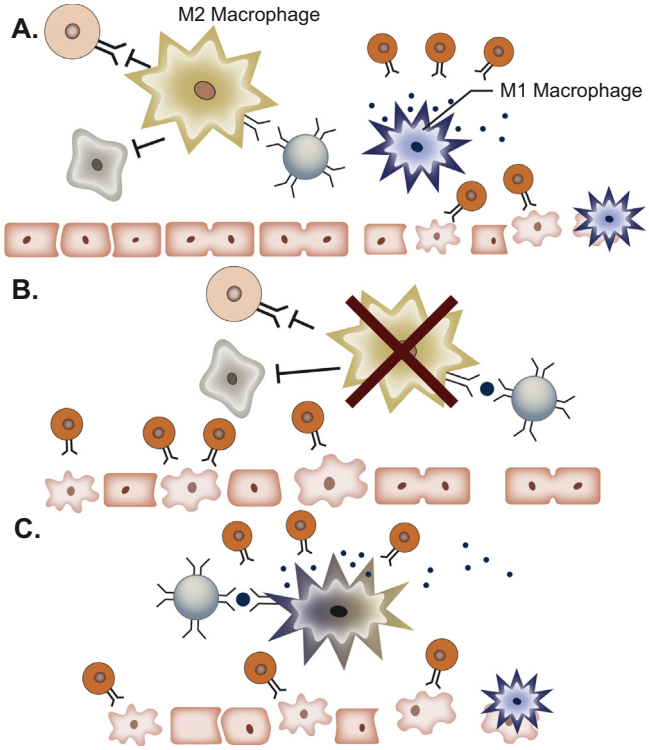

Fig 4. Modulating the TME using targeted NPs. The TME consists of many subtypes of immune cells including TAMs. M1 TAMs are a pro-immunogenic subpopulation that enhances activation and maturation of APCs and infiltrating T-cells leading to tumor clearance. In contrast, M2 TAMs are immunosuppressive cells that inhibit APC activation and stimulate anergy or apoptosis in infiltrating T-cells. Both populations exist in the TME (A) and modulating their relative composition can affect responsiveness to immunotherapies. Targeted NPs can be used to inhibit or clear M2 macrophages leading to disinhibition of infiltrating effector cells (B). Alternatively, targeted NPs can repolarize M2 macrophages to the M1 phenotype and increase activation of APCs and effector cells to improve tumor clearance (C).

3.3.2. Targeting Th2 T-regs

Th2 CD4+ regulatory T-cells are another potent immunosuppressive cell population commonly found in the TME. These cells can suppress local immune responses through inhibitory cytokine secretion (including TGF-β and IL-4,6,10) or by releasing perforin or granzymes to eliminate activated CD8+ T-cells and APCs within the TME. Several surface receptors are overexpressed on tumoral CD4+ T-regs including glucocorticoid-induced TNF receptor (GITR) and the Nrp1 receptor. Sacchetti et al. generated GITR-conjugated PEG-modified single-walled carbon nanotubes and demonstrated selective and efficient intracellular transport of targeted NPs in tumoral Th2 cells [112]. Another group more recently generated multi-layer hybrid NPs which included GITR-labeled polymeric cores to selectively deliver imatinib to Th2 T-regs [113]. The functionalized particles inhibited suppressive cytokine signaling from Th2 cells and improved activation of infiltrating CD8+ T-cells.

Preclinical work from non-oncologic studies has demonstrated that the physical characteristics of NPs can also affect the relative Th1 to Th2 response to immune adjuvants. One study using diphtheria toxins showed that whereas micro and nanosize formulations of aluminum adjuvants stimulate both Th1 and Th2 responses, calcium-phosphate nanoparticles preferentially stimulated Th1 but not Th2 responses. Antigen and adjuvant containing NPs have been used to selectively modulate the relative activity of Th1 and Th2 lymphocytes in numerous non-oncologic disease models including hepatitis B, asthma, and environmental hyperimmunities. Lessons learned from these systems can be used to improve modulation of the TME to a more immunostimulatory phenotype.

3.3.3. Targeting TAFs

TAFs are another potently immunosuppressive population of cells in the TME. TAFs are believed to result from the transformation of mesenchymal fibroblasts by cytokine signaling in tumors. TAFs can suppress tumor-specific immunity in several ways [114–116]. In some tumor types, including pancreatic cancer, TAFs are also responsible for the formation of dense, largely acellular desmoplasia that acts as a physical barrier to limit infiltration by activated CD8+ T-cells [117]. Even osmotic perfusion can be limited by a dense desmoplastic stroma. TAFs can also stimulate anergy of APCs and activated CD8+ T-cells by expressing high levels of PDL-1/2. Finally, TAFs express high levels of cytokines that inactivate pro-immunogenic Th1 T-regs and M1 TAMs in addition to polarization towards their immunosuppressive counterparts. Several strategies have been undertaken to limit or reverse the immunosuppressive actions of TAFs in the TME using targeted NPs. They primarily involve either depleting TAFs in the TME or preventing cross-talk between TAFs and other cell populations. Similar to other inhibitory cell populations in the TME, targeted NPs offer a promising approach to selectively modulating TAF-mediated immunoresistance. One hallmark that distinguishes TAFs from untransformed mesenchymal fibroblasts is the overexpression of α-SMA which is also correlated with increased surface expression of sigma receptors. Anisamides, including aminoethylanisamide, are sigma receptor agonists that can be conjugated to the surface of cationic, polymeric, or liposomal NPs for targeted intracellular uptake. TAFs also tend to be enriched around the vasculature of desmoplastic tumors and relatively accessible to NP delivery vectors. Huang and colleagues have developed several preclinical strategies to target TAFs using engineered NPs. In one study, they used anisamide-decorated lipid calcium phosphate NPs to deliver plasmid DNA to TAFs and disrupt tumoral paracrine signaling necessary for the maintenance of the TAF phenotype in a desmoplastic models of bladder (UMUC3) and pancreatic (BXPC3) cancers [118]. The targeted NPs induced high expression of the TNF-related inducing secretory ligand sTRAIL in TAFs which in turn stimulated apoptosis of adjacent tumor cells. Significant levels of apoptosis were not detected in α-SMA+ TAFs. However, apoptosis of adjacent tumor cells was associated with a reversion of TAFs to a quiescent state and significant reductions in α-SMA and FAPα (markers of fibroblast activation). Importantly, TAF inactivation led to a partial reversal of stromal desmoplasia, partial normalization of tumor vasculature, decreased tumoral hypoxia, and improved tumor growth delay. They have used similar targeted NPs to modulate TAF-mediated cytokine signaling [119]. CXCL12 is an inhibitory chemokine that suppresses CD8+ T-cell recruitment. Systemic inhibitors of the CXCL12 axis are limited by systemic toxicity and poor tumor penetration. Anisamide-NP delivery of a CXCL12-trap plasmid successfully reduced CXCL12 secretion from TAFs and improved tumoral penetration of CD8+ T-cells in orthoptic KPC pancreatic tumors. The antitumor efficacy of this approach was markedly enhanced with the co-delivery of a PDL-1-trap plasmid to prevent TAF-mediated inactivation of infiltrating T-cells.

Reversal of stromal desmoplasia and decreased inhibitory cytokine secretion can also be accomplished by targeted delivery of NPs encapsulating cytotoxic drugs to TAFs. The Huang group showed that anisamide-targeted dual delivery of gemcitabine and cisplatin can significantly reduce tumoral TAF populations, partially reverse stromal desmoplasia, and improve tumoral vascular permeability [120]. Other groups have found similar results with targeted NPs encapsulating docetaxel, doxorubicin, fraxinellone, and quercetin [121–125]. These studies support the hypothesis that it may be possible to selectively deplete TAFs to reverse tumoral desmoplasia and immunosuppressive cytokine signaling using systemically-administered, targeted NPs.

3.4. Targeting tumor cells

Tumor cells actively contribute to avoiding immune detection and can be targeted to enhance immune stimulation. Similar to inhibitory lymphocyte and macrophage populations, tumor cells excrete inhibitory cytokines to prevent APC and T-cell maturation/activation and maintain an immunosuppressive environment. The β-catenin pathway is an important mediator of this inhibitory signaling [126]. Matsuda et al. generated synthetic β-catenin-driven transgenic mouse models of HCC to demonstrate that NP-directed silencing of β-catenin signaling in tumor cells could decrease tumor growth and modulate the TME to improve responses to check point inhibitors [127]. In another study, lipid NP carriers of DRC-BCAT (an oligonucleotide targeting the CTNNB1 β-catenin gene) effectively modulated cytokine signaling to enhance CD8+ infiltration and growth arrest in multiple tumor models including B16F10, 4T1, and Neuro2A [128]. Further, the combination of the NP and dual CTLA-4/PDL-1 blockade stimulated complete or near complete regression in a majority of tumors. These same anti-tumor effects were observed in spontaneous mammary tumors using a Wnt-driven MMTV-Wnt1 transgenic mouse model.

Activation of proinflammatory signaling in tumor cells can also be achieved with nucleic acid delivery. Agonists of the retinoic acid receptor gene 1 (RIG-1) can potently stimulate type-1 interferon responses and expression of other pro-inflammatory cytokines in tumor cells as well as other cell populations in the TME (including dendritic cells and TAMs) [129]. The Wilson group generated pH-responsive endosomolytic polymeric NPs to deliver intact 5' triphosphate short double-stranded RNA (3pRNA) RIG-1 ligands [130]. The pH-responsive NPs enabled efficient endosomal escape and cytosolic delivery of the nucleic acid payload. Activation of RIG-1 triggered expression of proinflammatory cytokines, T-cell infiltration, and CD8+-mediated cytotoxicity in syngeneic CT26 models of colorectal cancer. When combined with anti-PD-1 blockade, complete regression of tumors was achieved in 30% of treated mice. It is worth noting that intratumoral delivery of 3pRNA robustly activated multiple cell populations in the TME and it is not currently clear to what extent the robust antitumor effect is mediated by effects in specific cell populations.

Immune tolerance can also result as a byproduct of abnormal tumor physiology. Dysregulated tumor metabolism maintains a relatively hypoxic and acidic environment in the TME which is known to induce T-cell anergy. It may be possible to reverse this inhibitory environment by

modulating tumor metabolism using NP nucleic acid delivery systems. A recent study engineered cationic lipid NP carriers for RNAi-mediated knock down of lactate dehydrogenase (LD) in tumor cells [131]. This significantly increased the pH of the tumor stroma and enhanced infiltration of CD8+ and NK-cells in addition to decreasing tumoral concentrations of M2 macrophages and Th2 T-regs. Silencing LD improved tumor growth delay on its own and markedly sensitized tumors to anti-PD-1 antibodies.

4. Enhancing T-cell therapy

T-cell therapies, in which patients are treated with tumor-targeted T-cells, have shown impressive efficacy in the treatment of hematogenous malignancies. T-cell therapy starts with the collection of immune cells from a cancer patient or healthy donor, followed by *ex vivo* genetic modification and expansion, and finally reinfusion to patient for cancer therapy [132]. FDA has approved two chimeric antigen receptor (CAR) T-cell therapies, Yescarta and Kymriah, for patients with lymphoma and leukemia, respectively [132]. However, several major challenges have obstructed the implementation of this paradigm as a standard-of-care in the treatment of solid tumors [133]. First, manufacturing of T-lymphocytes on a clinical scale is not easy because of the elaborate requirements for the isolation, genetic modification, and selective expansion of T cells, which entail specialized equipment and technical expertise that are not widely available [134]. Second, T-cell infiltration to solid tumors is hindered by downregulated chemokines, reduced expression of adhesion molecules on endothelial cells and disorganized tumor vasculature [135]. Lastly, transferred T-cells are facing a highly hostile TME filled with suppressive chemokines and cytokines secreted by T-regs or TAM, and PD-L1 expressed by tumor cells as well. In addition to the surrounding cells, the survival of T-cells is likely to be compromised by the low oxygen, low nutrient, and acidic conditions of the TME [136].

Nanoparticles can be exploited to address some of the aforementioned challenges. To simplify the production of CAR T-cells, Stephan et al. developed T-cell-targeting nanoparticles delivering plasmid DNA specifically constructed against CD19 antigens that were ubiquitously expressed on B lineage leukemia cells. The surface of these nanoparticles were decorated with T-cell markers—anti-CD3e f(ab')2 fragments on the surface, and further incorporated with peptides containing microtubule-associated sequences and nuclear localization signals to facilitate nuclear transport [134]. These nanoparticles were further incorporated with peptides containing microtubule-associated sequences and nuclear localization signals to facilitate nuclear transport. Upon systemic administration of these CD3-targeted nanoparticles to mice, circulating T-cells were successfully reprogrammed to leukemia-specific CAR T-cells *in situ* and proliferated 5.5-fold with high expression of CAR transgene by day 12. Antitumor efficacy was evaluated in mice with B-cell acute lymphoblastic leukemia. In the group treated with lymphocyte-targeting nanoparticles, seven out of ten mice survived tumor-free until the end of the study, which was 58 days longer than untreated control. This result was comparable with mice infused with *ex vivo*-produced CAR T-cells. This study provided proof-of-principle for the ability to reprogram the antigen-recognizing capabilities of lymphocytes with synthetic nanoparticles while achieving comparable therapeutic effect with conventional CAR-T and circumventing the complication of T-cell manufacturing. Despite the low cost, simple production, and good stability of nanoparticles, validation is needed on human T-cells before this approach can proceed to clinical translation.

To modulate the unfavorable TME, tumor-specific T-cells were equipped with nanogels containing cytokines and targeting ligands [137]. Human IL-15 super-agonist complex (IL-15Sa) was crosslinked with disulfide-containing linkers to form nanogels (NGs) and anti-CD45 antibodies (<10 mol%) were further incorporated, aiming to retain NGs on the surface of T-cells without compromising TCR signaling. A small amount of poly (ethylene glyco)-b-poly(L-lysine) was adsorbed to NGs to enhance NGs loading on T-cells. These NGs could promote T-cell expansion *in vivo* by releasing cytokines liberated when linkers were reduced during T-cell activation. When treated with transferred T-cells tagged with NGs, tumor growth was significantly inhibited compared to those treated with T-cells and free IL-15Sa. The dose of IL-15Sa could be increased up to 8-fold without inducing dose-limited toxicity when delivered within NGs, allowing multiple administrations of this T-cell therapy. Antitumor efficacy was further evaluated in a human glioblastoma mouse model. NG-tagged EGFR-targeted CAR-T cells could eradicate tumors in 80% of mice, while CAR-T cells alone or CAR-T cells plus free IL-15Sa only achieved a marginal therapeutic effect. In another effort to attenuate immunosuppression in the TME, a selective inhibitor of PI3K kinase (PI-3065) and immunostimulant-invariant natural killer T-cell (iNKT) agonist (7DW8-5) were co-encapsulated in liposomes decorated with tumor targeting iRGD peptides [138]. Mice bearing 4T1 tumors were treated with these dual-drug liposomes and much less (>4-fold) immune suppressing cells, such as TAMs, monocytic MDSCs and T-regs, were found at tumor site comparing to those treated with empty liposomes. The concentration of antitumor immune effector cells, such as $CD8^+$ T cells and iNKT cells, was significantly increased due to the treatment. Therapeutic studies have shown that liposome pretreatments could augment T-cell accumulation in tumors and thus boost the effectiveness of cell therapy. The maximized outcomes could be obtained by multiple dosing of dual-drug liposomes several weeks in advance of T-cell infusion and implementation of combined therapies pivots on their safety evaluation in clinical trials.

5. Summary and conclusions

Nanomedicines are a diverse class of engineered nanoscale compounds that can be finely tuned for the targeted *in vivo* delivery of drugs and macromolecules to specific cell populations. Actively targeting the immune system is uniquely challenging. The process of generating signal-specific immunity is tightly regulated, often requiring the simultaneous stimulation of multiple signals. Failure to simultaneously deliver both signals is not only inefficient, but can be counterproductive by facilitating immune tolerance. Many immune-modulating cell populations also exist as competing proinflammatory and immunosuppressive cell populations and efficient stimulation of class-restricted immune responses often requires specific targeting of one population without directly affecting the other. As highlighted in this review, novel nanomedicines are in many ways ideally suited for the translation of effective cancer immunotherapies. Nanomedicines can be engineered to optimize the targeted delivery of multiple signals to specific cell types using spatiotemporal accuracy not possible with other drug delivery methods. Lessons learned with first-generation nanomedicines have been used to further optimize the engineering of advanced nanocarriers for targeted cancer immunotherapies. In the short term, we predict that NPs will primarily serve as advanced drug-delivery vectors to improve targeting of specific subpopulations and synchronous delivery of co-stimulatory signals using approaches derived from existing platforms. In our view, the strongest potential for paradigm-altering impact probably lies with truly novel nanomedicines including engineered immune-cell mimetics (like *ex vivo* generated CAR-T cells). However, because these will require extensive preclinical validation before entering trials in humans, it is unlikely their clinical impact is realized for several years to come.

Acknowledgments

This work was supported by funding from National Institutes of Health/National Cancer Institute (U54CA198999 and R01 EB25651), Department of Defense (W81XWH-16-1-0530), and The University of North Carolina's Research Opportunities Initiative grant.

References

[1] R.J. Motzer, N.M. Tannir, D.F. McDermott, et al., Nivolumab plus Ipilimumab versus Sunitinib in advanced renal-cell carcinoma, N. Engl. J. Med. 378 (14) (Apr 5 2018) 1277–1290.

[2] J.D. Wolchok, V. Chiarion-Sileni, R. Gonzalez, et al., Overall survival with combined nivolumab and ipilimumab in advanced melanoma, N. Engl. J. Med. 377 (14) (Oct 5 2017) 1345–1356.

[3] M.D. Hellmann, N.A. Rizvi, J.W. Goldman, et al., Nivolumab plus ipilimumab as first-line treatment for advanced non-small-cell lung cancer (CheckMate 012): results of an open-label, phase 1, multicohort study, Lancet Oncol. 18 (1) (Jan 2017) 31–41.

[4] L. Horn, D.R. Spigel, E.E. Vokes, et al., Nivolumab versus docetaxel in previously treated patients with advanced non-small-cell lung cancer: two-year outcomes from two randomized, open-label, Phase III Trials (CheckMate 017 and CheckMate 057), J. Clin. Oncol. 35 (35) (Dec 10 2017) 3924–3933.

[5] J.R. Brahmer, C.G. Drake, I. Wollner, et al., Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates, J. Clin. Oncol. 28 (19) (Jul 1 2010) 3167–3175.

[6] J.M. Pitt, M. Vetizou, R. Daillere, et al., Resistance mechanisms to immune-checkpoint blockade in cancer: tumor-intrinsic and -extrinsic factors, Immunity. 44 (6) (Jun 21 2016) 1255–1269.

[7] R.W. Jenkins, D.A. Barbie, K.T. Flaherty, Mechanisms of resistance to immune checkpoint inhibitors, Br. J. Cancer 118 (1) (Jan 2018) 9–16.

[8] P. Sharma, S. Hu-Lieskovan, J.A. Wargo, A. Ribas, Primary, adaptive, and acquired resistance to cancer immunotherapy, Cell. 168 (4) (Feb 9 2017) 707–723.

[9] Y. Min, J.M. Caster, M.J. Eblan, A.Z. Wang, Clinical translation of nanomedicine, Chem. Rev. 115 (19) (Oct 14 2015) 11147–11190.

[10] J.M. Caster, A.N. Patel, T. Zhang, A. Wang, Investigational nanomedicines in 2016: a review of nanotherapeutics currently undergoing clinical trials, Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 9 (1) (Jan 2017).

[11] J.M. Caster, A.Z. Wang, Applying nanotherapeutics to improve chemoradiotherapy treatment for cancer, Ther. Deliv. 8 (9) (Aug 2017) 791–803.

[12] J. Fang, H. Nakamura, H. Maeda, The EPR effect: Unique features of tumor blood vessels for drug delivery, factors involved, and limitations and augmentation of the effect, Adv. Drug Deliv. Rev. 63 (3) (Mar 18 2011) 136–151.

[13] H. Maeda, Macromolecular therapeutics in cancer treatment: the EPR effect and beyond, J. Control. Release 164 (2) (Dec 10 2012) 138–144.

[14] M. Sethi, R. Sukumar, S. Karve, et al., Effect of drug release kinetics on nanoparticle therapeutic efficacy and toxicity, Nanoscale. 6 (4) (Feb 21 2014) 2321–2327.

[15] K.M. Au, Y. Min, X. Tian, et al., Improving cancer chemoradiotherapy treatment by dual controlled release of wortmannin and docetaxel in polymeric nanoparticles, ACS Nano 9 (9) (Sep 22 2015) 8976–8996.

[16] X. Tian, S.B. Warner, K.T. Wagner, et al., Preclinical evaluation of promitil, a radiation-responsive liposomal formulation of mitomycin C prodrug, in chemoradiotherapy, Int. J. Radiat. Oncol. Biol. Phys. 96 (3) (Nov 1 2016) 547–555.

[17] K.S. Huang, C.H. Yang, Y.C. Wang, W.T. Wang, Y.Y. Lu, Microfluidic synthesis of vinblastine-loaded multifunctional particles for magnetically responsive controlled drug release, Pharmaceutics 11 (5) (May 3 2019).

[18] Z. Liu, Z. Le, L. Lu, et al., Scalable fabrication of metal-phenolic nanoparticles by coordination-driven flash nanocomplexation for cancer theranostics, Nanoscale 11 (19) (May 16 2019) 9410–9421.

[19] D. Rosenblum, N. Joshi, W. Tao, J.M. Karp, D. Peer, Progress and challenges towards targeted delivery of cancer therapeutics, Nat. Commun. 9 (1) (Apr 12 2018) 1410.

[20] L.K. Bogart, G. Pourroy, C.J. Murphy, et al., Nanoparticles for imaging, sensing, and therapeutic intervention, ACS Nano 8 (4) (Apr 22 2014) 3107–3122.

[21] H. Cabral, Y. Matsumoto, K. Mizuno, et al., Accumulation of sub-100 nm polymeric micelles in poorly permeable tumours depends on size, Nat. Nanotechnol. 6 (12) (Oct 23 2011) 815–823.

[22] A.E. Hansen, A.L. Petersen, J.R. Henriksen, et al., Positron emission tomography based elucidation of the enhanced permeability and retention effect in dogs with cancer using Copper-64 liposomes, ACS Nano 9 (7) (Jul 28 2015) 6985–6995.

[23] H. Lee, A.F. Shields, B.A. Siegel, et al., (64)Cu-MM-302 positron emission tomography quantifies variability of enhanced permeability and retention of nanoparticles in relation to treatment response in patients with metastatic breast cancer, Clin. Cancer Res. 23 (15) (Aug 1 2017) 4190–4202.

[24] M.A. Miller, S. Gadde, C. Pfirschke, et al., Predicting therapeutic nanomedicine efficacy using a companion magnetic resonance imaging nanoparticle, Sci. Transl. Med. 7 (314) (Nov 18 2015) 314ra183.

[25] A.V. Makela, J.M. Gaudet, P.J. Foster, Quantifying tumor associated macrophages in breast cancer: a comparison of iron and fluorine-based MRI cell tracking, Sci. Rep. 7 (Feb 8 2017) 42109.

[26] C. Liu, T. Shaurova, S. Shoemaker, M. Petkovich, P.A. Hershberger, Y. Wu, Tumor-targeted nanoparticles deliver a vitamin D-based drug payload for the treatment of EGFR tyrosine kinase inhibitor-resistant lung cancer, Mol. Pharm. 15 (8) (Aug 6 2018) 3216–3226.

[27] M. Creixell, A.C. Bohorquez, M. Torres-Lugo, C. Rinaldi, EGFR-targeted magnetic nanoparticle heaters kill cancer cells without a perceptible temperature rise, ACS Nano 5 (9) (Sep 27 2011) 7124–7129.

[28] A.V. Nascimento, F. Gattacceca, A. Singh, et al., Biodistribution and pharmacokinetics of Mad2 siRNA-loaded EGFR-targeted chitosan nanoparticles in cisplatin sensitive and resistant lung cancer models, Nanomedicine (London) 11 (7) (Apr 2016) 767–781.

[29] A.M. Master, Gupta A. Sen, EGF receptor-targeted nanocarriers for enhanced cancer treatment, Nanomedicine (London) 7 (12) (Dec 2012) 1895–1906.

[30] O. Flores, S. Santra, C. Kaittanis, et al., PSMA-targeted theranostic nanocarrier for prostate cancer, Theranostics. 7 (9) (2017) 2477–2494.

[31] J. Hrkach, D. Von Hoff, M. Mukkaram Ali, et al., Preclinical development and clinical translation of a PSMA-targeted docetaxel nanoparticle with a differentiated pharmacological profile, Sci. Transl. Med. 4 (128) (Apr 4 2012) 128ra139.

[32] P.K.B. Nagesh, N.R. Johnson, V.K.N. Boya, et al., PSMA targeted docetaxel-loaded superparamagnetic iron oxide nanoparticles for prostate cancer, Colloids Surfaces 144 (Aug 1 2016) 8–20.

[33] V. Shirshahi, F. Shamsipour, A.H. Zarnani, J. Verdi, R. Saber, Active targeting of HER2-positive breast cancer cells by Herceptin-functionalized organically modified silica nanoparticles, Cancer nanotechnology. 4 (1-3) (2013) 27–37.

[34] M. Truffi, M. Colombo, L. Sorrentino, et al., Multivalent exposure of trastuzumab on iron oxide nanoparticles improves antitumor potential and reduces resistance in HER2-positive breast cancer cells, Sci. Rep. 8 (1) (Apr 2018) 6563.

[35] T. Kubota, S. Kuroda, N. Kanaya, et al., HER2-targeted gold nanoparticles potentially overcome resistance to trastuzumab in gastric cancer, Nanomedicine 14 (6) (Aug 2018) 1919–1929.

[36] J. Wei, J. Sun, Y. Liu, Enhanced targeting of prostate cancer-initiating cells by salinomycin-encapsulated lipid-PLGA nanoparticles linked with CD44 antibodies, Oncol. Lett. 17 (4) (Apr 2019) 4024–4033.

[37] S. Wang, M. Shao, Z. Zhong, et al., Co-delivery of gambogic acid and TRAIL plasmid by hyaluronic acid grafted PEI-PLGA nanoparticles for the treatment of triple negative breast cancer, Drug Deliv. 24 (1) (Nov 2017) 1791–1800.

[38] E. Gaio, C. Conte, D. Esposito, et al., Co-delivery of docetaxel and disulfonate tetraphenyl chlorin in one nanoparticle produces strong synergism between chemo- and photodynamic therapy in drug-sensitive and -resistant cancer cells, Mol. Pharm. 15 (10) (Oct 1 2018) 4599–4611.

[39] G.H. Kim, J.E. Won, Y. Byeon, et al., Selective delivery of PLXDC1 small interfering RNA to endothelial cells for anti-angiogenesis tumor therapy using CD44-targeted chitosan nanoparticles for epithelial ovarian cancer, Drug delivery. 25 (1) (Nov 2018) 1394–1402.

[40] Z. Yang, H. Luo, Z. Cao, et al., Dual-targeting hybrid nanoparticles for the delivery of SN38 to Her2 and CD44 overexpressed human gastric cancer, Nanoscale. 8 (22) (Jun 2 2016) 11543–11558.

[41] Y. Shamay, M. Elkabets, H. Li, et al., P-selectin is a nanotherapeutic delivery target in the tumor microenvironment, Sci. Transl. Med. 8 (345) (Jun 29 2016) 345ra387.

[42] E.I. Sega, Y. Lu, M. Ringor, C.P. Leamon, P.S. Low, Low-dose radiation potentiates the therapeutic efficacy of folate receptor-targeted hapten therapy, Int. J. Radiat. Oncol. Biol. Phys. 71 (2) (Jun 1 2008) 559–566.

[43] Y. Yang, Z. Wang, Y. Peng, J. Ding, W. Zhou, A smart pH-sensitive delivery system for enhanced anticancer efficacy via paclitaxel endosomal escape, Front. Pharmacol. 10 (2019) 10.

[44] S.M. Hoy, Patisiran: first global approval, Drugs. 78 (15) (Oct 2018) 1625–1631.

[45] T.S. Zimmermann, A.C. Lee, A. Akinc, et al., RNAi-mediated gene silencing in non-human primates, Nature. 441 (7089) (May 4 2006) 111–114.

[46] J. Heyes, L. Palmer, K. Bremner, I. MacLachlan, Cationic lipid saturation influences intracellular delivery of encapsulated nucleic acids, J. Controll. Release 107 (2) (Oct 3 2005) 276–287.

[47] J. Gilleron, W. Querbes, A. Zeigerer, et al., Image-based analysis of lipid nanoparticle-mediated siRNA delivery, intracellular trafficking and endosomal escape, Nat. Biotechnol. 31 (7) (Jul 2013) 638–646.

[48] Y. Sato, K. Hashiba, K. Sasaki, M. Maeki, M. Tokeshi, H. Harashima, Understanding structure-activity relationships of pH-sensitive cationic lipids facilitates the rational identification of promising lipid nanoparticles for delivering siRNAs in vivo, J. Control. Release 295 (Feb 10 2019) 140–152.

[49] J.P. Nam, K. Nam, S. Jung, J.W. Nah, S.W. Kim, Evaluation of dendrimer type bioreducible polymer as a siRNA delivery carrier for cancer therapy, J. Controll. Release 209 (Jul 10 2015) 179–185.

[50] A. Akinc, M. Thomas, A.M. Klibanov, R. Langer, Exploring polyethylenimine-mediated DNA transfection and the proton sponge hypothesis, J. Gene Med. 7 (5) (May 2005) 657–663.

[51] M. Wojnilowicz, A. Glab, A. Bertucci, F. Caruso, F. Cavalieri, Super-resolution Imaging of proton sponge-triggered rupture of endosomes and cytosolic release of small interfering RNA, ACS Nano 13 (1) (Jan 22 2019) 187–202.

[52] E.A. Reits, J.W. Hodge, C.A. Herberts, et al., Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy, J. Exp. Med. 203 (5) (May 15 2006) 1259–1271.

[53] K.G. Anderson, I.M. Stromnes, P.D. Greenberg, Obstacles posed by the tumor microenvironment to T cell activity: a case for synergistic therapies, Cancer Cell 31 (3) (May 15 2017) 311–325.

[54] E. Zupancic, C. Curato, M. Paisana, et al., Rational design of nanoparticles towards targeting antigen-presenting cells and improved T cell priming, J. Controll. Release 258 (Jul 28 2017) 182–195.

[55] M. Luo, H. Wang, Z. Wang, et al., A STING-activating nanovaccine for cancer immunotherapy, Nat. Nanotechnol. 12 (7) (Jul 2017) 648–654.

[56] A.W. Li, M.C. Sobral, S. Badrinath, et al., A facile approach to enhance antigen response for personalized cancer vaccination, Nat. Mater. 17 (6) (Jun 2018) 528–534.

[57] N.M. Molino, M. Neek, J.A. Tucker, E.L. Nelson, S.W. Wang, Viral-mimicking protein nanoparticle vaccine for eliciting anti-tumor responses, Biomaterials. 86 (Apr 2016) 83–91.

[58] H. Kim, L. Niu, P. Larson, et al., Polymeric nanoparticles encapsulating novel TLR7/8 agonists as immunostimulatory adjuvants for enhanced cancer immunotherapy, Biomaterials. 164 (May 2018) 38–53.

[59] G. Zhu, G.M. Lynn, O. Jacobson, et al., Albumin/vaccine nanocomplexes that assemble in vivo for combination cancer immunotherapy, Nat. Commun. 8 (1) (Dec 5 2017) 1954.
[60] R. Kuai, L.J. Ochyl, K.S. Bahjat, A. Schwendeman, J.J. Moon, Designer vaccine nanodiscs for personalized cancer immunotherapy, Nat. Mater. 16 (4) (Apr 2017) 489–496.
[61] G. Zhu, L. Mei, H.D. Vishwasrao, et al., Intertwining DNA-RNA nanocapsules loaded with tumor neoantigens as synergistic nanovaccines for cancer immunotherapy, Nat. Commun. 8 (1) (Nov 14 2017) 1482.
[62] D.Q. Liu, S. Lu, L.X. Zhang, et al., An indoleamine 2, 3-dioxygenase siRNA nanoparticle-coated and Trp2-displayed recombinant yeast vaccine inhibits melanoma tumor growth in mice, J. Controll. Release 273 (Mar 10 2018) 1–12.
[63] Y. Xia, J. Wu, W. Wei, et al., Exploiting the pliability and lateral mobility of Pickering emulsion for enhanced vaccination, Nat. Mater. 17 (2) (Feb 2018) 187–194.
[64] O.K. Appelbe, K.D. Moynihan, A. Flor, N. Rymut, D.J. Irvine, S.J. Kron, Radiation-enhanced delivery of systemically administered amphiphilic-CpG oligodeoxynucleotide, J. Controll. Release 266 (Nov 28 2017) 248–255.
[65] L.X. Zhang, X.X. Xie, D.Q. Liu, Z.P. Xu, R.T. Liu, Efficient co-delivery of neo-epitopes using dispersion-stable layered double hydroxide nanoparticles for enhanced melanoma immunotherapy, Biomaterials. 174 (Aug 2018) 54–66.
[66] A.I. Bocanegra Gondan, A. Ruiz-de-Angulo, A. Zabaleta, et al., Effective cancer immunotherapy in mice by polyIC-imiquimod complexes and engineered magnetic nanoparticles, Biomaterials. 170 (Jul 2018) 95–115.
[67] H.A. Hassan, L. Smyth, J.T. Wang, et al., Dual stimulation of antigen presenting cells using carbon nanotube-based vaccine delivery system for cancer immunotherapy, Oct Biomaterials. 104 (2016) 310–322.
[68] L. Deng, H. Liang, M. Xu, et al., STING-dependent cytosolic DNA sensing promotes radiation-induced type I interferon-dependent antitumor immunity in immunogenic tumors, Immunity. 41 (5) (Nov 20 2014) 843–852.
[69] M. Luo, Z. Liu, X. Zhang, et al., Synergistic STING activation by PC7A nanovaccine and ionizing radiation improves cancer immunotherapy, J. Controll. Release 300 (Apr 28 2019) 154–160.
[70] L. Liu, Y. Wang, L. Miao, et al., Combination immunotherapy of MUC1 mRNA nanovaccine and CTLA-4 blockade eEffectively inhibits growth of triple negative breast cancer, Mol. Ther. 26 (1) (Jan 3 2018) 45–55.
[71] L.M. Kranz, M. Diken, H. Haas, et al., Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy, Nature. 534 (7607) (Jun 16 2016) 396–401.
[72] S.M. Lim, N.L. Syn, B.C. Cho, R.A. Soo, Acquired resistance to EGFR targeted therapy in non-small cell lung cancer: mechanisms and therapeutic strategies, Cancer Treat. Rev. 65 (Apr 2018) 1–10.
[73] G. Vlacich, R.J. Coffey, Resistance to EGFR-targeted therapy: a family affair, Cancer Cell 20 (4) (Oct 18 2011) 423–425.
[74] Y. Min, K.C. Roche, S. Tian, et al., Antigen-capturing nanoparticles improve the abscopal effect and cancer immunotherapy, Nat. Nanotechnol. 12 (9) (Sep 2017) 877–882.
[75] A.V. Kroll, R.H. Fang, Y. Jiang, et al., Nanoparticulate delivery of cancer cell membrane elicits multiantigenic antitumor immunity, Adv. Mater. 29 (47) (Dec 2017).
[76] R. Yang, J. Xu, L. Xu, et al., Cancer cell membrane-coated adjuvant nanoparticles with mannose modification for effective anticancer vaccination, ACS Nano 12 (6) (June 26 2018) 5121–5129.
[77] G. Gaud, R. Lesourne, P.E. Love, Regulatory mechanisms in T cell receptor signalling, Nat. Rev. Immunol. 18 (8) (Aug 2018) 485–497.
[78] P.N. Aguiar Jr., I.L. Santoro, H. Tadokoro, et al., The role of PD-L1 expression as a predictive biomarker in advanced non-small-cell lung cancer: a network meta-analysis, Immunotherapy. 8 (4) (2016) 479–488.
[79] Y. Li, L. Liang, W. Dai, et al., Prognostic impact of programed cell death-1 (PD-1) and PD-ligand 1 (PD-L1) expression in cancer cells and tumor infiltrating lymphocytes in colorectal cancer, Mol. Cancer 15 (1) (Aug 24 2016) 55.
[80] D.T. Le, J.N. Uram, H. Wang, et al., PD-1 blockade in tumors with mismatch-repair deficiency, N. Engl. J. Med. 372 (26) (Jun 25 2015) 2509–2520.
[81] S. Jaiswal, C.H. Jamieson, W.W. Pang, et al., CD47 is upregulated on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis, Cell. 138 (2) (Jul 23 2009) 271–285.
[82] L. Luo, J. Yang, C. Zhu, et al., Sustained release of anti-PD-1 peptide for perdurable immunotherapy together with photothermal ablation against primary and distant tumors, J. Controll. Release 278 (May 28 2018) 87–99.
[83] C. Wang, W. Sun, G. Wright, A.Z. Wang, Z. Gu, Inflammation-triggered cancer immunotherapy by programmed delivery of CpG and anti-PD1 antibody, Adv. Mater. 28 (40) (Oct 2016) 8912–8920.
[84] Y. Wu, W. Gu, J. Li, C. Chen, Z.P. Xu, Silencing PD-1 and PD-L1 with nanoparticle-delivered small interfering RNA increases cytotoxicity of tumor-infiltrating lymphocytes, Nanomedicine (London) 14 (8) (Apr 2019) 955–967.
[85] S. Lian, R. Xie, Y. Ye, et al., Simultaneous blocking of CD47 and PD-L1 increases innate and adaptive cancer immune responses and cytokine release, EBioMedicine. 42 (Apr 2019) 281–295.
[86] S.Y. Li, Y. Liu, C.F. Xu, et al., Restoring anti-tumor functions of T cells via nanoparticle-mediated immune checkpoint modulation, J. Controll. Release 231 (Jun 10 2016) 17–28.
[87] Y. Wu, W. Gu, L. Li, C. Chen, Z.P. Xu, Enhancing PD-1 gene silence in T lymphocytes by comparing the delivery performance of two inorganic nanoparticle platforms, Nanomaterials 9 (2) (Jan 28 2019).
[88] X. Zhang, C. Wang, J. Wang, et al., PD-1 blockade cellular vesicles for cancer immunotherapy, Adv. Mater. 30 (22) (May 2018), e1707112.
[89] D.L. Burdette, K.M. Monroe, K. Sotelo-Troha, et al., STING is a direct innate immune sensor of cyclic di-GMP, Nature. 478 (7370) (Sep 25 2011) 515–518.
[90] J. Fu, D.B. Kanne, M. Leong, et al., STING agonist formulated cancer vaccines can cure established tumors resistant to PD-1 blockade, Sci. Transl. Med. 7 (283) (Apr 15 2015) 283ra252.
[91] D.R. Wilson, R. Sen, J.C. Sunshine, D.M. Pardoll, J.J. Green, Y.J. Kim, Biodegradable STING agonist nanoparticles for enhanced cancer immunotherapy, Nanomedicine 14 (2) (Feb 2018) 237–246.
[92] I. Melero, D. Hirschhorn-Cymerman, A. Morales-Kastresana, M.F. Sanmamed, J.D. Wolchok, Agonist antibodies to TNFR molecules that costimulate T and NK cells, Clin. Cancer Res. 19 (5) (Mar 1 2013) 1044–1053.
[93] B.D. Curti, M. Kovacsovics-Bankowski, N. Morris, et al., OX40 is a potent immune-stimulating target in late-stage cancer patients, Cancer Res. 73 (24) (Dec 15 2013) 7189–7198.
[94] M. Chen, H. Ouyang, S. Zhou, J. Li, Y. Ye, PLGA-nanoparticle mediated delivery of anti-OX40 monoclonal antibody enhances anti-tumor cytotoxic T cell responses, Cell. Immunol. 287 (2) (Feb 2014) 91–99.
[95] M. Chen, H. Ouyang, S. Zhou, J. Li, Y. Ye, Effect of PLGA nanoparticles conjugated with anti-OX40/anti-AFP mAbs on cytotoxicity of CTL cells against hepatocellular carcinoma, Xi bao yu fen zi mian yi xue za zhi = Chinese Journal Cellular Molecular Immunology 30 (4) (Apr 2014) 337–341.
[96] Y. Mi, C.C. Smith, F. Yang, et al., A dual immunotherapy nanoparticle improves T-cell activation and cancer immunotherapy, Adv. Mater. 30 (25) (Jun 2018), e1706098.
[97] A.K. Kosmides, J.W. Sidhom, A. Fraser, C.A. Bessell, J.P. Schneck, Dual targeting nanoparticle stimulates the immune system to inhibit tumor growth, ACS Nano 11 (6) (Jun 27 2017) 5417–5429.
[98] Y. Liu, X. Cao, The origin and function of tumor-associated macrophages, Cell. Mol. Immunol. 12 (1) (Jan 2015) 1–4.
[99] J. Jackute, M. Zemaitis, D. Pranys, et al., Distribution of M1 and M2 macrophages in tumor islets and stroma in relation to prognosis of non-small cell lung cancer, BMC Immunol. 19 (1) (Jan 24 2018) 3.
[100] A. Yuan, Y.J. Hsiao, H.Y. Chen, et al., Opposite effects of M1 and M2 macrophage subtypes on lung cancer progression, Sci. Rep. 5 (Sep 24 2015) 14273.
[101] C.B. Rodell, S.P. Arlauckas, M.F. Cuccarese, et al., TLR7/8-agonist-loaded nanoparticles promote the polarization of tumour-associated macrophages to enhance cancer immunotherapy, Nat. Biomed. Eng. 2 (8) (2018) 578–588.
[102] S.L. Ai, X.Y. He, B.Y. Liu, R.X. Zhuo, S.X. Cheng, Targeting delivery of oligodeoxynucleotides to macrophages by mannosylated cationic albumin for immune stimulation in cancer treatment, Mol. Pharm. 16 (6) (Jun 3 2019) 2616–2625.
[103] N. Wang, H. Liang, K. Zen, Molecular mechanisms that influence the macrophage m1-m2 polarization balance, Front. Immunol. 5 (May 2 2014) 614.
[104] K. Mao, X. Cong, L. Feng, et al., Intratumoral delivery of M-CSF by calcium crosslinked polymer micelles enhances cancer immunotherapy, Biomaterials science. 7 (7) (Jun 25 2019) 2769–2776.
[105] X. Cai, Y. Yin, N. Li, et al., Re-polarization of tumor-associated macrophages to pro-inflammatory M1 macrophages by microRNA-155, J. Mol. Cell Biol. 4 (5) (Oct 2012) 341–343.
[106] J.R. Cubillos-Ruiz, J.R. Baird, A.J. Tesone, et al., Reprogramming tumor-associated dendritic cells in vivo using miRNA mimetics triggers protective immunity against ovarian cancer, Cancer Res. 72 (7) (Apr 1 2012) 1683–1693.
[107] L. Yang, J. Sun, Q. Liu, et al., Synergetic functional nanocomposites enhance immunotherapy in solid tumors by remodeling the immunoenvironment, Adv. Sci. 6 (8) (Apr 17 2019) 1802012.
[108] S. Zanganeh, G. Hutter, R. Spitler, et al., Iron oxide nanoparticles inhibit tumour growth by inducing pro-inflammatory macrophage polarization in tumour tissues, Nat. Nanotechnol. 11 (11) (Nov 2016) 986–994.
[109] X. Tang, C. Mo, Y. Wang, D. Wei, H. Xiao, Anti-tumour strategies aiming to target tumour-associated macrophages, Immunology. 138 (2) (Feb 2013) 93–104.
[110] X. Zang, X. Zhang, H. Hu, et al., Targeted delivery of zoledronate to tumor-associated macrophages for cancer immunotherapy, Mol. Pharm. 16 (5) (May 6 2018) 2249–2258.
[111] S. Zhou, T. Zhang, B. Peng, et al., Targeted delivery of epirubicin to tumor-associated macrophages by sialic acid-cholesterol conjugate modified liposomes with improved antitumor activity, Int. J. Pharm. 523 (1) (May 15 2017) 203–216.
[112] C. Sacchetti, N. Rapini, A. Magrini, et al., In vivo targeting of intratumor regulatory T cells using PEG-modified single-walled carbon nanotubes, Bioconjug. Chem. 24 (6) (June 19 2013) 852–858.
[113] W. Ou, L. Jiang, R.K. Thapa, et al., Combination of NIR therapy and regulatory T cell modulation using layer-by-layer hybrid nanoparticles for effective cancer photoimmunotherapy, Theranostics. 8 (17) (2018) 4574–4590.
[114] R. Zhang, F. Qi, F. Zhao, et al., Cancer-associated fibroblasts enhance tumor-associated macrophages enrichment and suppress NK cells function in colorectal cancer, Cell Death Dis. 10 (4) (Mar 20 2019) 273.
[115] L. Wei, H. Ye, G. Li, et al., Cancer-associated fibroblasts promote progression and gemcitabine resistance via the SDF-1/SATB-1 pathway in pancreatic cancer, Cell Death Dis. 9 (11) (Oct 18 2018) 1065.
[116] K. Shiga, M. Hara, T. Nagasaki, T. Sato, H. Takahashi, H. Takeyama, Cancer-associated fibroblasts: their characteristics and their roles in tumor growth, Cancers. 7 (4) (Dec 11 2015) 2443–2458.
[117] A. Santi, F.G. Kugeratski, S. Zanivan, Cancer associated fibroblasts: the architects of stroma remodeling, Proteomics. 18 (5-6) (Mar 2018), e1700167.
[118] L. Miao, Q. Liu, C.M. Lin, et al., Targeting tumor-associated fibroblasts for therapeutic delivery in desmoplastic tumors, Cancer Res. 77 (3) (Feb 1 2017) 719–731.

[119] L. Miao, J. Li, Q. Liu, et al., Transient and local expression of chemokine and immune checkpoint traps to treat pancreatic cancer, ACS Nano 11 (9) (Sep 26 2017) 8690–8706.
[120] J. Zhang, L. Miao, S. Guo, et al., Synergistic anti-tumor effects of combined gemcitabine and cisplatin nanoparticles in a stroma-rich bladder carcinoma model, J. Controll. Release 182 (May 28 2014) 90–96.
[121] L. Hou, Q. Liu, L. Shen, et al., Nano-delivery of fraxinellone remodels tumor microenvironment and facilitates therapeutic vaccination in desmoplastic melanoma, Theranostics. 8 (14) (2018) 3781–3796.
[122] K. Hu, L. Miao, T.J. Goodwin, J. Li, Q. Liu, L. Huang, Quercetin remodels the tumor microenvironment to improve the permeation, retention, and antitumor effects of nanoparticles, ACS Nano 11 (5) (May 23 2017) 4916–4925.
[123] T. Ji, Y. Ding, Y. Zhao, et al., Peptide assembly integration of fibroblast-targeting and cell-penetration features for enhanced antitumor drug delivery, Adv. Mater. 27 (11) (Mar 18 2015) 1865–1873.
[124] M.J. Ernsting, B. Hoang, I. Lohse, et al., Targeting of metastasis-promoting tumor-associated fibroblasts and modulation of pancreatic tumor-associated stroma with a carboxymethylcellulose-docetaxel nanoparticle, J. Controll. Release 206 (May 28 2015) 122–130.
[125] D.F. Mardhian, G. Storm, R. Bansal, J. Prakash, Nano-targeted relaxin impairs fibrosis and tumor growth in pancreatic cancer and improves the efficacy of gemcitabine in vivo, J. Controll. Release 290 (Nov 28 2018) 1–10.
[126] S. Spranger, T.F. Gajewski, A new paradigm for tumor immune escape: beta-catenin-driven immune exclusion, J. Immunother. Cancer. 3 (2015) 43.
[127] A. Matsuda, K. Ishiguro, I.K. Yan, T. Patel, Extracellular vesicle-based therapeutic targeting of beta-catenin to modulate anticancer immune responses in hepatocellular cancer, Hepatol. Commun. 3 (4) (Apr 2019) 525–541.
[128] S. Ganesh, X. Shui, K.P. Craig, et al., RNAi-mediated beta-catenin inhibition promotes T cell infiltration and antitumor activity in combination with immune checkpoint blockade, Mol. Ther. 26 (11) (Nov 7 2018) 2567–2579.
[129] P. Duewell, A. Steger, H. Lohr, et al., RIG-I-like helicases induce immunogenic cell death of pancreatic cancer cells and sensitize tumors toward killing by CD8(+) T cells, Cell Death Differ. 21 (12) (Dec 2014) 1825–1837.
[130] M.E. Jacobson, L. Wang-Bishop, K.W. Becker, J.T. Wilson, Delivery of 5'-triphosphate RNA with endosomolytic nanoparticles potently activates RIG-I to improve cancer immunotherapy, Biomater. Sci. 7 (2) (Jan 29 2019) 547–559.
[131] Y.X. Zhang, Y.Y. Zhao, J. Shen, et al., Nanoenabled modulation of acidic tumor microenvironment reverses anergy of infiltrating T cells and potentiates anti-PD-1 therapy, Nano Lett. 19 (5) (May 8 2019) 2774–2783.
[132] M. Sadelain, I. Rivière, S. Riddell, Therapeutic T cell engineering, Nature. 545 (05/24/online 2017) 423.
[133] J. Lou, L. Zhang, G. Zheng, Advancing cancer immunotherapies with nanotechnology, Adv. Ther. 2 (4) (2019) 1800128.
[134] T.T. Smith, S.B. Stephan, H.F. Moffett, et al., In situ programming of leukaemia-specific T cells using synthetic DNA nanocarriers, Nat. Nanotechnol. 12 (04/17/online 2017) 813.
[135] C.Y. Slaney, M.H. Kershaw, P.K. Darcy, Trafficking of T cells into tumors, Cancer Res. 74 (24) (2014) 7168–7174.
[136] A.A. Wu, V. Drake, H.-S. Huang, S. Chiu, L. Zheng, Reprogramming the tumor microenvironment: tumor-induced immunosuppressive factors paralyze T cells, OncoImmunology. 4 (7) (2015/07/03 2015), e1016700.
[137] L. Tang, Y. Zheng, M.B. Melo, et al., Enhancing T cell therapy through TCR-signaling-responsive nanoparticle drug delivery, Nat. Biotechnol. 36 (07/09/online 2018) 707.
[138] F. Zhang, S.B. Stephan, C.I. Ene, T.T. Smith, E.C. Holland, M.T. Stephan, Nanoparticles that reshape the tumor milieu create a therapeutic window for effective T-cell therapy in solid malignancies, Cancer Res. 78 (13) (2018) 3718–3730.
[139] Y. Sakurai, W. Mizumura, M. Murata, et al., Efficient siRNA delivery by lipid nanoparticles modified with a nonstandard macrocyclic peptide for EpCAM-targeting, Mol. Pharm. 14 (10) (Oct 2 2017) 3290–3298.
[140] S.V. Jenkins, Z.A. Nima, K.B. Vang, et al., Triple-negative breast cancer targeting and killing by EpCAM-directed, plasmonically active nanodrug systems, NPJ Precision Oncol. 1 (1) (2017) 27.
[141] Y.V. Kucheryavykh, J. Davila, J. Ortiz-Rivera, et al., Targeted delivery of nanoparticulate cytochrome C into glioma cells through the proton-coupled folate transporter, Biomolecules 9 (4) (Apr 18 2019).
[142] Y. Zhang, M. Cheng, J. Cao, et al., Multivalent nanoparticles for personalized theranostics based on tumor receptor distribution behavior, Nanoscale. 11 (11) (Mar 14 2019) 5005–5013.
[143] K. Kim, A.D. Skora, Z. Li, et al., Eradication of metastatic mouse cancers resistant to immune checkpoint blockade by suppression of myeloid-derived cells, Proc. Natl. Acad. Sci. U. S. A. 111 (32) (Aug 12 2014) 11774–11779.
[144] Y. Qian, H. Jin, S. Qiao, et al., Targeting dendritic cells in lymph node with an antigen peptide-based nanovaccine for cancer immunotherapy, Biomaterials. 98 (Aug 2016) 171–183.
[145] W. Ou, R.K. Thapa, L. Jiang, et al., Regulatory T cell-targeted hybrid nanoparticles combined with immuno-checkpoint blockage for cancer immunotherapy, J. Controll. Release 281 (Jul 10 2018) 84–96.
[146] T.T. Smith, S.B. Stephan, H.F. Moffett, et al., In situ programming of leukaemia-specific T cells using synthetic DNA nanocarriers, Nat. Nanotechnol. 12 (8) (Aug 2017) 813–820.
[147] H.F. Moffett, M.E. Coon, S. Radtke, et al., Hit-and-run programming of therapeutic cytoreagents using mRNA nanocarriers, Nat. Commun. 8 (1) (Aug 30 2017) 389.
[148] S. Ramishetti, R. Kedmi, M. Goldsmith, et al., Systemic gene silencing in primary T lymphocytes using targeted lipid nanoparticles, ACS Nano 9 (7) (Jul 28 2015) 6706–6716.
[149] A.M. Dorrance, P. Neviani, G.J. Ferenchak, et al., Targeting leukemia stem cells in vivo with antagomiR-126 nanoparticles in acute myeloid leukemia, Leukemia. 29 (11) (Nov 2015) 2143–2153.
[150] Y. Zheng, L. Tang, L. Mabardi, S. Kumari, D.J. Irvine, Enhancing adoptive cell therapy of cancer through targeted delivery of small-molecule immunomodulators to internalizing or noninternalizing receptors, ACS Nano 11 (3) (Mar 28 2017) 3089–3100.
[151] T. Nagy-Simon, A.S. Tatar, A.M. Craciun, et al., Antibody conjugated, raman tagged hollow gold-silver nanospheres for specific targeting and multimodal dark-field/SERS/two photon-FLIM imaging of CD19(+) B lymphoblasts, ACS Appl. Mater. Interfaces 9 (25) (Jun 28 2017) 21155–21168.
[152] V. Krishnan, X. Xu, D. Kelly, et al., CD19-targeted nanodelivery of doxorubicin enhances therapeutic efficacy in B-cell acute lymphoblastic leukemia, Mol. Pharm. 12 (6) (Jun 1 2015) 2101–2111.
[153] D.E. Myers, S. Yiv, S. Qazi, et al., CD19-antigen specific nanoscale liposomal formulation of a SYK P-site inhibitor causes apoptotic destruction of human B-precursor leukemia cells, Integrative Biol. 6 (8) (Aug 2014) 766–780.
[154] H.Y. Tung, Y.C. Su, B.M. Chen, et al., Selective delivery of PEGylated compounds to tumor cells by Anti-PEG hybrid antibodies, Mol. Cancer Ther. 14 (6) (Jun 2015) 1317–1326.
[155] S. Jiang, X. Wang, Z. Zhang, et al., CD20 monoclonal antibody targeted nanoscale drug delivery system for doxorubicin chemotherapy: an in vitro study of cell lysis of CD20-positive Raji cells, Int. J. Nanomedicine 11 (2016) 5505–5518.
[156] W.K. Nevala, J.T. Butterfield, S.L. Sutor, D.J. Knauer, S.N. Markovic, Antibody-targeted paclitaxel loaded nanoparticles for the treatment of CD20(+) B-cell lymphoma, Sci. Rep. 7 (Apr 5 2017) 45682.
[157] X. Chen, Z. Zhang, S. Yang, H. Chen, D. Wang, J. Li, All-trans retinoic acid-encapsulated, CD20 antibody-conjugated poly(lactic-co-glycolic acid) nanoparticles effectively target and eliminate melanoma-initiating cells in vitro, OncoTargets Ther. 11 (2018) 6177–6187.
[158] B. Yu, Y. Mao, L.Y. Bai, et al., Targeted nanoparticle delivery overcomes off-target immunostimulatory effects of oligonucleotides and improves therapeutic efficacy in chronic lymphocytic leukemia, Blood. 121 (1) (Jan 3 2013) 136–147.
[159] M. Kanekiyo, W. Bu, M.G. Joyce, et al., Rational design of an epstein-barr virus vaccine targeting the receptor-binding site, Cell. 162 (5) (Aug 27 2015) 1090–1100.
[160] P. de la Puente, M.J. Luderer, C. Federico, et al., Enhancing proteasome-inhibitory activity and specificity of bortezomib by CD38 targeted nanoparticles in multiple myeloma, J. Controll. Release 270 (Jan 28 2018) 158–176.
[161] N. An, Y.N. Hou, Q.X. Zhang, et al., Anti-multiple myeloma activity of nanobody-based anti-CD38 chimeric antigen receptor T cells, Mol. Pharm. 15 (10) (Oct 1 2018) 4577–4588.
[162] Y.H. Huang, M.R. Vakili, O. Molavi, et al., Decoration of anti-CD38 on nanoparticles carrying a STAT3 inhibitor can improve the therapeutic efficacy against myeloma, Cancers 11 (2) (Feb 10 2019).