

Available online at www.sciencedirect.com

ScienceDirect

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

Review

Nanomaterials for modulating innate immune cells in cancer immunotherapy



Quoc-Viet Le^a, Geon Yang^a, Yina Wu^a, Ho Won Jang^b,
 Mohammadreza Shokouhimehr^{b,*}, Yu-Kyoung Oh^{a,*}

^a College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 08826, Republic of Korea

^b Department of Materials Science and Engineering, Research Institute of Advanced Materials, Seoul National University, Seoul 08826, Republic of Korea

ARTICLE INFO

Article history:

Received 18 May 2018

Revised 24 June 2018

Accepted 4 July 2018

Available online 23 August 2018

Keywords:

Cancer immunotherapy

Nanomaterials

Innate immune cells

Tumor-associated macrophages

ABSTRACT

Cancer immunotherapy has been intensively investigated in both preclinical and clinical studies. Whereas chemotherapies use cytotoxic drugs to kill tumor cells, cancer immunotherapy is based on the ability of the immune system to fight cancer. Tumors are intimately associated with the immune system: they can suppress the immune response and/or control immune cells to support tumor growth. Immunotherapy has yielded promising results in clinical practice, but some patients show limited responses. This may reflect the complexities of the relationship between a tumor and the immune system. In an effort to improve the current immunotherapies, researchers have exploited nanomaterials in creating new strategies to cure tumors via modulation of the immune system in tumor tissues. Although extensive studies have examined the use of immune checkpoint-based immunotherapy, rather less work has focused on manipulating the innate immune cells. This review examines the recent approaches and challenges in the use of nanomaterials to modulate innate immune cells.

© 2018 Shenyang Pharmaceutical University. Published by Elsevier B.V.

This is an open access article under the CC BY-NC-ND license.

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

1. Introduction

Cancer is a group of diseases characterized by out-of-control cell growth. Cancer patients have been treated with surgery, radiotherapy, and chemotherapy, but such therapies still suffer from limited efficacy, side effects, and the risk of damage

to normal tissues. Moreover, these therapies are often not effective in metastatic and/or advanced-stage tumors, resulting in curative failure.

Immune systems can function as a double-edged sword by inhibiting or promoting tumor growth (Fig. 1). In healthy people, immune systems play important roles in controlling the growth of malignant cells. In cancer patients, however,

* Corresponding authors.

E-mail addresses: mrsh2@snu.ac.kr (M. Shokouhimehr), ohyk@snu.ac.kr (Y.-K. Oh).

Peer review under responsibility of Shenyang Pharmaceutical University.

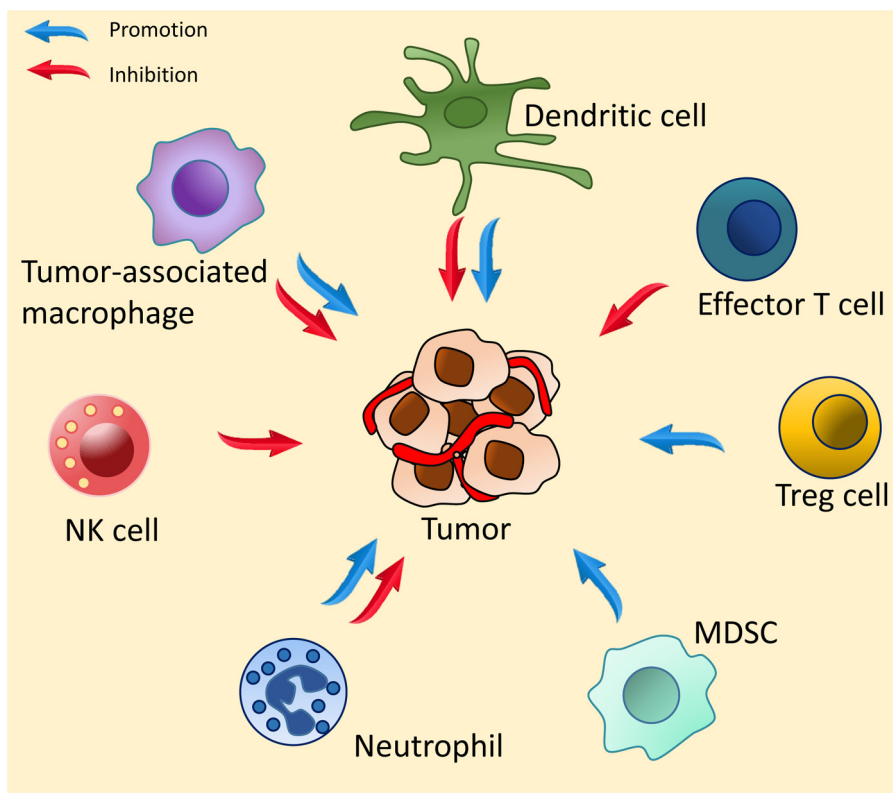


Fig. 1 – Interactions of immune cells and a tumor.

In the tumor microenvironment, immune systems can function as a double-edged sword by promoting or inhibiting tumor growth. The role of immune cells can depend on the types of such cells present at the tumor tissues.

immune systems often fail to control and may even facilitate the growth of tumor cells. The assistance of tumor-associated immune cells has even been linked to the ability of a tumor to progress to an advanced stage. For example, crosstalk between tumor and immune cells can induce angiogenesis, which supports tumor growth and metastasis by secreting various cytokines, tumor growth factors, and vascular endothelial growth factors [1–3]. However, tumors may escape immune surveillance by expressing molecules that cause immune cell anergy. Programmed death ligand 1 (PD-L1), programmed death receptor 1 (PD-1), 2,3 indoleamine oxygenase (IDO), and cytotoxic T lymphocyte antigen-4 (CTLA-4) are among the immune checkpoint molecules that are highly expressed in tumor cells or tumor-associated immune cells [2–4].

In recent years, immunotherapy has been intensively studied for its unique advantages over the current cancer therapies. Some immunotherapies can activate the immune system to efficiently scavenge tumor cells, while others reverse the exhausted state of immune cells to recover their anti-tumor effects. The activated immune cells are capable of infiltrating deeply into tumor tissues at locations that limit the entry of conventional chemotherapeutics, such as in the brain, spleen, and prostate. Chemotaxis has been exploited as a possible mechanism for the infiltration of drug-loaded neutrophils to brain tumor tissues. Chemotaxis was shown to be induced by post-surgery inflammation. Upon combination with surgery, drug-loaded neutrophils were proposed to pass

through blood-brain barrier and accumulate to the surgery sites by chemotaxis [5,6]. Immunotherapies may also offer unprecedented specificity, thereby limiting the occurrence of undesirable side effects in healthy tissues. Finally, immunotherapies may confer long-term protection from tumor recurrence [7] and overcome multi-drug resistance various tumors. The types of tumors include leukemia, breast cancer, ovarian cancer, colon carcinoma and osteosarcoma [8].

Despite the numerous merits of immunotherapy, however, the use of various materials to selectively modulate immune cells is still in its infancy. Immune systems are composed of innate and adaptive immune cells that differ in their targeting strategies, delivery strategies, and cargoes. Nanomaterials can affect the pharmacokinetic profiles of entrapped materials by protecting them from rapid degradation in the bloodstream [9,10]. Indeed, nanomaterial-mediated delivery can enhance the delivery of immunomodulators to immune cells and improve the efficacy of immunotherapy. Numerous efforts have been made to target adaptive immune cells, such as via chimeric antigen receptor-T cell therapy or through the use of antibodies against immune checkpoint inhibitors (e.g., PD-1, PD-L1, and CTLA4). The results of such studies suggest that it may be feasible to perform immunotherapy by modulating innate immune cells. Accumulating reports have focused on the pathways of innate immune cell biology and their relationship with tumors. However, even though the use of nanomaterials to modulate the activities of innate immune cells could open new avenues for cancer immunotherapy, relatively few stud-

ies have examined this topic. Here, we review the published studies that have used nanomaterials to modulate various innate immune cells, including tumor-associated macrophages (TAMs), natural killer (NK) cells, myeloid-derived suppressor cells (MDSCs), neutrophils, and dendritic cells (DCs) (Table 1).

2. Nanomaterials for modulating TAMs

Macrophages are professional phagocytes that play crucial roles in eliminating pathogens and cellular debris. They can function as antigen-presenting cells and secrete various types of soluble factors to communicate with other immune cells. TAMs are an important target immune cell type in cancer immunotherapy. For example, breast carcinoma is reportedly comprised of up to 70% TAMs [11]. In the tumor microenvironment, macrophages exert both antitumor and protumor effects. Numerous studies have reported that the functions and phenotypes of TAMs differ from those of macrophages in normal tissues [12]. At the initial stages of tumor formation, peritumoral macrophages or monocytes are recruited to tumor tissues and polarized to M1-type cells, which can exert antitumor effects. As a tumor progresses to an advanced stage, the macrophages convert from M1 type to M2 type, which can exert protumor effects by helping suppress CD8 killer T cells [12,13]. Nanomaterials have been studied for their ability to modulate the phenotypes and activities of TAMs. Iron oxide nanoparticles [14] and hyaluronic-coated manganese dioxide nanoparticles [15] are reportedly capable of reprogramming the TAM phenotype (Fig. 2A). TAMs can be activated by siRNAs encapsulated in cationic liposomes, which undergo enhanced phagocytosis [16], and gluco-mannan polysaccharide and liposomes have been used to deliver toxic agents that eliminate M2-type macrophages [17,18].

2.1. Nanomaterials for reprogramming TAMs

Superparamagnetic iron oxide nanoparticles (Fe_3O_4) have been used to deliver agents capable of modulating the TAM phenotype [14]. Ferumoxytol, which consists of carboxymethyl-dextran-coated iron oxide nanoparticles, is approved by Food and Drug Administration (USA) for intravenous use as a supplementary agent for iron deficiency in long-term kidney disease patients [19]. The intravenous administration of carboxymethyl-dextran-coated iron oxide nanoparticles was shown to promote the differentiation of TAMs to M1 type and inhibited tumor growth by 43% compared to the control group [14]. Moreover, ferumoxytol nanoparticles significantly reduced the tumor lesions in both pulmonary and liver metastasis model mice implanted with KP1 smallcell lung cancer cells. In the group treated with ferumoxytol, TAMs isolated from tumor tissues exhibited an increase in CD86 (an M1 marker) and a decrease in CD206 (an M2 marker). Ferumoxytol is believed to convert the TAM phenotype via the Fenton reaction: hydrogen peroxide secreted from pro-inflammatory M1 macrophages could react with iron to produce toxic hydroxyl radicals [20]. In another recent study reported by Silva et al. [21], the administration of dextran crosslinked-iron oxide nanoparticle in Lewis lung carcinoma tumor-bearing mice was shown to

shift the phenotypes of TAM. In the dextran crosslinked-iron oxide nanoparticle-treated group, the shift of TAM to pro-inflammatory type was notable. This study needs attention in that inorganic iron oxide nanoparticles functioned as an image contrast agent and as a TAM-reprogramming agent.

Manganese dioxide nanoparticles modified with hyaluronic acid and mannan polysaccharide have been studied for their ability to target TAMs and modulate the hypoxic tumor microenvironment [15]. Tumor hypoxia is associated with the accumulation of M2-type TAMs, which can promote tumor growth and metastasis by altering the expression levels of various genes, such as those encoding hypoxia-inducible factors (HIFs), vascular endothelial growth factor (VEGF), and matrix metalloproteinases (MMPs). The manganese dioxide nanoparticles were entrapped in hyaluronic acid and coated with mannan polysaccharide to target the mannose receptors present on TAMs. These nanoparticles reacted with hydrogen peroxide in the hypoxic tumor tissues to produce oxygen, thereby alleviating hypoxia and increasing the pH of tumor microenvironment. A later study found that the presence of a hyaluronic acid layer facilitated the differentiation of M2-type macrophages to M1-type macrophages [22]. In the 4T1 breast tumor bearing model, mannan-hyaluronic acid-manganese dioxide nanoparticles were found to reduce the expression levels of HIFs and VEGF and to shift TAMs toward M1 type, as evidenced by elevated levels of interleukin-12 and inducible nitric oxide synthase.

Recently, miR155 was found to be involved in attenuating cytokine production and polarizing M2-type macrophages toward M1 type [22]. Therefore, the specific delivery of miR155 to TAMs could be a potential strategy for overcoming immunosuppression in the tumor microenvironment. Liu et al. showed that self-assembled cationic polymeric nanoparticles formulated from poly(ethylene glycol)-b-poly(L-lysine)-b-poly(L-cysteine) hybrid polypeptides could be used to deliver miR155 to TAMs [23]. miR155 could form a complex with poly(L-lysine) via static interaction, whereas poly(L-cysteine) could crosslink via redox disulfide bonding to stabilize the micelle structure. Galactose moieties were grafted and exposed on the micelle surface to specifically target TAMs. miR155-encapsulated cationic micelles, but not free miR155, were found to elevate the expression of miR155 in mouse bone marrow-derived TAMs by about two orders of magnitude compared to the negative control group. Moreover, intratumoral injection of miR155-cationic nanocomplexes to melanoma B16F10 tumor-bearing mice was shown to upregulate markers of M1 macrophages and inhibit tumor growth more significantly than free miR155.

Interleukin-12 is a heterodimeric cytokine that is known to polarize TAMs toward antitumor M1 type macrophage [24]. Accordingly, polymeric nanoparticles entrapping interleukin-12 were investigated for their ability to shift TAMs from M2 type to M1 type [25]. Interleukin-12 was entrapped in a cationic histamine-grafted-polyethyleneglycol-b-poly(β -amino ester) polymer-forming nanostructure by the double emulsion water-in-oil method. The cytokine was found to retain its stability following entrapment in this nanostructure at pH 7.4. In a B16F10 cell-bearing mouse model of melanoma, intravenous administration of interleukin-12-loaded cationic nanoparticles was found to reduce the systemic toxicity and

Table 1 – Nanomaterials used as platforms for immunotherapy-based drug delivery with the goal of modulating innate immune cells.

Nanomaterial	Modified ligand	Cargo type	Cargo	Targeted cell	Purpose	Tumor	Injection route	Reference
Iron oxide nanoparticle				TAM	TAM reprogramming	KP1	IV	[14]
Hyaluronic acid -MnO ₂ nanoparticle	Mannan polysaccharide			TAM	TAM reprogramming	4T1	IV	[15]
Poly(L-lysine)-b-poly(L-cysteine) @ Poly(ethylene glycol)-poly(Llysine)-g-(1,2dicarboxyliccyclohexene)	Galactose	Nucleic acid	miR155	TAM	TAM reprogramming	B16F10	IT	[23]
Poly(b-amino ester)g-poly(ethylene glycol)-g-histamine		Protein	IL-12	TAM	TAM reprogramming	B16F10	IV	[25]
Hyaluronic acid-protamine @ cationic liposome		Nucleic acid	CD47 siRNA	TAM	TAM activation	B16F10	IV	[16]
Carboxylated polystyrene	Calreticulin, HER2 antibody	Protein		TAM	TAM activation	E0771/E2	IV	[29]
Gluko-mannan polysaccharide	Glucomannan	Chemical	Alendronate	TAM	TAM depletion	S180	IT	[17]
Liposome		Chemical	Clodronate	TAM	TAM depletion	KPC	IP	[18]
Cationic liposome		Nucleic acid	Plasmid encoding TUSC2	NK cell	NK cell activation	CMT167	IV	[43]
Fe ₃ O ₄ @ SiO ₂ nanoparticle				NK cell	NK cell migration	RPMI8226	IV	[44]
Liposome		Chemical	Lauroyl gemcitabine	MDSC	MDSC depletion	EG07-OVA B16F10	SC	[47]
Mesoporous Silica @ liposome		Chemical	All trans retinoic acid	MDSC	MDSC differentiation	B16F10	IV	[49]
Liposome	T1 aptamer	Chemical	Doxorubicin	MDSC	MDSC depletion	4T1	IV	[48]
Denatured albumin + TA99 Ab		Chemical	Pyropheophorbide-a	Neutrophil	Neutrophil mediated delivery	B16	IV	[54]
Gold nanoparticle	CD11b antibody			Neutrophil	Neutrophil mediated delivery	3LL	IV	[56]
Cationic liposome		Chemical	Paclitaxel	Neutrophil	Neutrophil mediated delivery	G422	IV	[57]
HDL mimicking nanodisc		Nucleic acid, peptide	CpG ODN, antigen peptide	DC	DC activation	B16F10	SC	[65]
Poly(ethylene glycol)-b-poly-2-(hexamethylene-imino) ethyl methacrylate		Peptide	Antigen peptide	DC	DC activation	B16F10 MC38 TC-1	SC	[69]
Poly(lactic-co-glycolic) acid		Chemical	Indocyanine green, imiquimod	DC	DC activation	CT26	IV	[67]

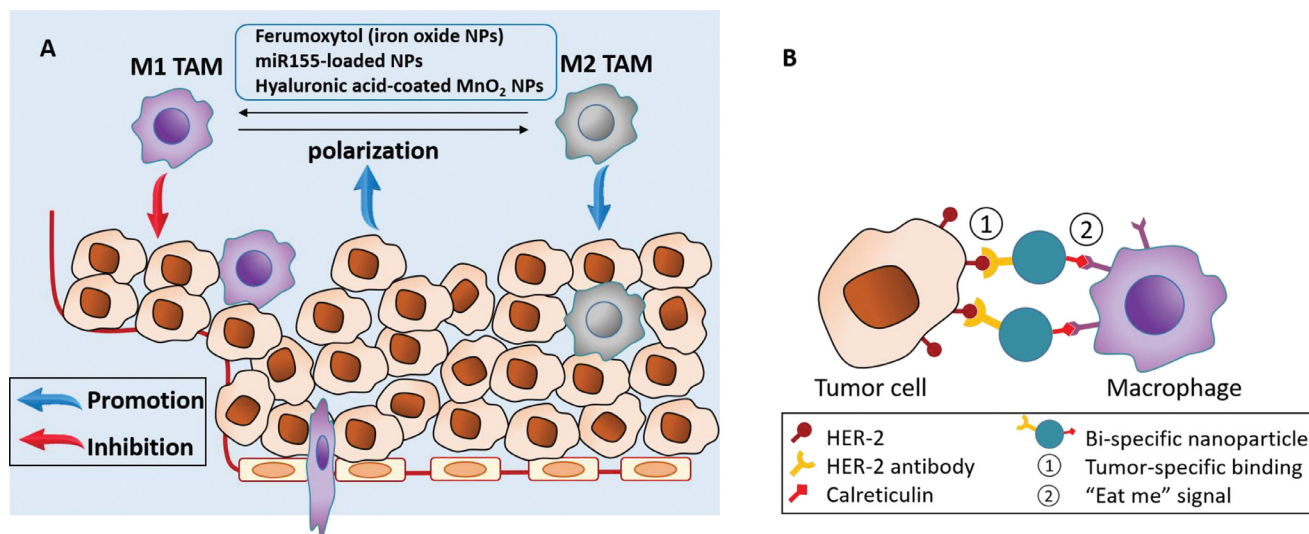


Fig. 2 – Strategies for targeting TAMs in cancer immunotherapy.

(A) Delivery of TAM-reprogramming agents that can convert M2-type TAMs to M1 type were found to reverse the pro-tumorigenic effect of TAMs [14,15,23]. **(B)** Dual-conjugated nanoparticles were decorated with HER-2 antibody and calreticulin. The binding of nanoparticles to HER-2-expressing cancer cells induced macrophages to phagocytize the cancer cells via calreticulin recognition [29].

exert TAM dependent antitumor effects. Tumor tissue sections displayed an increase of induced nitric oxide synthase (a biomarker of M1-type TAMs) and a decrease of arginase-1 (a biomarker of M2-type macrophages). This study is particularly interesting because the conversion of M2-type TAMs to M1-type TAMs was directly visualized in tumor tissue sections.

TAM reprogramming strategy has an advantage over TAM depletion in that it could preserve the high density of macrophage populations in tumor tissues. Pro-inflammatory M1-type macrophages in the tumor tissues can synergize with other immune cells for exerting antitumor activity. It has been reported that M1-type TAM directly inhibited tumor growth as well as boosted the antitumor activity of adaptive immune cells [26]. In the patients treated with rituximab combination therapy, the high density of TAM has been reported to correlated with the better therapeutic outcome [12]. This study suggests that the preservation of properly tamed TAM would be beneficial in anticancer therapy. However, the conversion into pro-inflammatory M1-type macrophages observed after systemic administration of iron oxide nanoparticle [14] or interleukin-12-loaded polymeric nanoparticles [27] may unbalance immune systems and cause systemic inflammation [28].

Inorganic materials like iron oxide nanoparticles, manganese dioxide nanoparticles exerted direct or indirect effect on the phenotype shifting of TAM by various mechanisms. Unlike inorganic materials, cationic polymeric nanoparticles per se did not possess the capability to reprogram TAM [23,25]. Rather, the cationic polymeric nanoparticles mainly played a role as a carrier of therapeutic cargoes such as siRNA or cytokine. In this field, more studies need to be done for design of multifunctional nanomaterials which may carry therapeutic cargoes and reprogram TAM.

2.2. Nanomaterials for activating TAMs

Nanomaterials have been studied for their ability to activate the anergic function of TAMs by boosting their ability to phagocytize cancer cells. As TAM-activating strategies, researchers have investigated indirect activation via the siRNA-mediated silencing of CD47 [16] or the enhanced exposure of calreticulin on cancer cells [29].

In the first study, cationic liposomes were complexed with CD47-specific siRNAs for the indirect activation of TAMs [16]. CD47 is considered to be a self-marker that acts as a "don't eat me" signal; its binding to signal regulatory protein alpha receptor on macrophages has been shown to inhibit the cancer cell phagocytosis of macrophages [30]. CD47-specific siRNAs and hyaluronic acid were complexed with cationic protamine, and this core complex was encapsulated in a pegylated liposome composed of the cationic lipid, DOTAP (N-[1-(2,3dioleoyloxy)propyl]-N,N,N-trimethylammonium methyl-sulfate), DSPE-PEG2000 (1,2distearoyl-sn-glycero-3-phosphoethanolamine-N-(methoxy (polyethyleneglycol)-2000), and cholesterol. Intravenous injection of CD47 siRNA-loaded cationic liposomes significantly inhibited B16F10 melanoma tumor growth and reduced metastatic lung nodules compared to those observed in the carrier-alone and untreated groups. In contrast, administration of CD47 siRNA-loaded cationic liposomes did not exhibit any ability to inhibit tumors in a macrophage depletion model. Although the authors did not directly measure the levels of CD47 in tumor tissues, their findings suggest that macrophages are a critical part of this anti-CD47 siRNA-based immunotherapy.

In the second study, dual protein-modified nanoparticles were used to promote the antitumor effect of TAMs by including a pro-phagocytic protein [29]. Carboxylated polystyrene

nanoparticles were co-conjugated with an antibody against human epidermal growth factor receptor 2 (HER-2) and calreticulin. HER-2 is known to be highly expressed on 20% of human breast cancers [31]. Calreticulin was used to increase phagocytosis of the nanoparticles. These dual-conjugated nanoparticles not only specifically bound to HER-2-overexpressing cancer cells, they also enhanced the exposure of calreticulin on the cancer cell surface and thereby produced the “eat me” signal to induce phagocytosis of macrophages (Fig. 2B). In a breast tumor-bearing model, both intratumoral and intravenous administration of dual-modified nanoparticles could facilitate the tumor recognition of macrophages. Tumor tissue sections showed increased numbers of tumor-infiltrating macrophages and CD8+T cells. This study is notable in that the dual-modified nanoparticles delayed the growth of HER2-expressing tumors but not HER2-negative tumors. In this study, the authors aimed to expect the enhanced immunotherapy effect by co-conjugation of HER-2 antibody and calreticulin. The presence of HER-2 antibody on the nanoparticles may contribute to the recognition of tumor cells. Tumor cells bound with the nanoparticles are then positive with calreticulin, an “eat-me” signal, which can contribute to the recruiting of immune cells to attack tumor cells. The ability of dual protein-modified nanoparticles to exert antitumor effects even at a distance supports the notion that immunotherapy may be a powerful tool for treating recurrent tumors after surgery or chemotherapy.

The activation of TAM has an advantage of promoting the engulfment of cancer cells and enhancing the antigen presentation processes of macrophages [29]. The enhancement of calreticulin-mediated “eat me” signal with the suppression of “don’t eat me” signal by CD47 blockade would be an interesting strategy to sensitize the phagocytic activity of macrophages. However, the activation of TAM may not stand alone as monotherapy. The administration of SIRP- α , a natural ligand of CD47, as an CD47 blocker, was shown to be insufficient to induce the phagocytosis of macrophages. Rather, CD47 blocker was effective as an adjuvant to reduce the threshold of phagocytosis [12]. The rationale of combining macrophage activation with other immune therapies needs to be studied in the future.

2.3. Nanomaterials for depleting TAMs

Given the protumor effects of M2-type TAMs on tumor angiogenesis, metastasis, and immune invasion, researchers have sought to deliver toxic agents that can eliminate these macrophages [17,18]. For example, a gluco-mannan polysaccharide from *Bletilla striata*, which has a high affinity for a mannose receptor abundantly expressed in macrophages, was used to develop a macrophage-targeting carrier. This backbone was conjugated with alendronate (4-amino-1-hydroxybutylidene-1,1-bisphosphonate), a bisphosphonate compound that causes apoptosis of macrophages, to form nanoparticles [17]. Following intratumoral injection of alendronate-loaded polysaccharide nanoparticles into sarcoma S180 tumor-bearing mice, the authors observed a high-level distribution of the nanoparticles to cells expressing the macrophage marker, F4/80, an 84.5% reduction of TAMs in the tumor tissue, and a significant inhibition of

tumor growth. By comparison, macrophages were reduced by only 17.0% in the corresponding mice treated with free alendronate. The depletion of macrophages in alendronate-polysaccharide nanoparticle-treated mice was correlated with decreases in blood vessel formation and the levels of VEGF and MMP-9.

As another TAM-depleting strategy, the bisphosphonate compound, clodronate, was formulated in liposomes [18]. Clodronate-encapsulated liposomes were intravenously injected into KPC pancreatic tumor-bearing mice, and the effects of macrophage depletion on primary tumor growth and metastasis were examined. The tumor incidence did not significantly differ between the clodronate-loaded liposome- and control liposome-treated groups. However, the incidence of lung metastasis was dramatically reduced in the clodronate-loaded liposome-treated group relative to the control group (less than 10% versus approximately 50%, respectively). The successful depletion of TAMs was supported by the observed decreases in the level of circulating VEGF and tumor microvessel density.

Sialoadhesin, also named as Siglec-1, is a cell adhesion protein dominantly expressed on the surfaces of TAM. Sialic acid, a derivative from N-acetylneuraminic, has a strong binding affinity to Siglec-1 [32]. Decoration of drug-loaded liposomes with sialic acid has been shown to eliminate TAM specifically [33–35]. In these studies, hydrophobic moieties such as octadecylamine [33,35] and cholesterol [34] were tethered to sialic acid. Resulting amphiphilic derivatives of sialic acid were incorporated into liposome structure for TAM binding. The encapsulation of anticancer drugs in sialic acid-decorated liposomes showed greater antitumor effect compared with plain liposomes. Interestingly, the use of sialic acid-decorated liposomes containing anticancer drugs showed specific depletion of TAM at tumor environment and “shedding” of tumor tissues [34]. These studies provided evidences that the depletion of TAM could be achieved using sialic acid-decorated nanocarriers.

TAM depletion strategy would be suitable for combination therapy with drugs such as alendronate or clodronate since systemic administration of clodronate-loaded liposomes was reported to deplete macrophages. However, TAM depletion has limitations to increase the possibility of opportunistic infections to immune suppressed cancer patients. Especially, non-specific systemic deletion of macrophages can increase the susceptibility of cancer patients to infectious disease. In alveolar macrophage-deleted animals were reported to be more susceptible to viral infections [36,37]. Given the possible risk of infection, TAM depletion strategy should be cautiously studied. Specific deletion of TAM using nano delivery systems needs to be studied further to minimize the risk of systemic infection.

Taken together, the findings from the existing studies indicate that macrophages act as a double edged sword in the tumor microenvironment, where their phenotypic conversion can enable them to promote or inhibit tumors of various stages. Various TAM manipulation-based strategies have been used, including the reprogramming, activation, and elimination of these macrophages. No study to date has directly compared the therapeutic effects of these three concepts. In clinical practice, the decision to employ activation or elimination

of TAMs may depend on the tumor stage, tumor histology and patient's condition.

3. Nanomaterials for modulating NK cells

NK cells, which are defined as innate effector lymphocytes, were first described in 1975 [38]. They represent an important type of cytotoxic lymphocyte in the innate immune system. NK cells can kill cancer cells without activating complements or antibodies, and thus confer a much faster immune response than the adaptive immune system [39]. At an early stage of cancer, tumor cells are recognized and eliminated by NK cells that are recruited by damage signals secreted from tumor-adjacent cells [40]. Cytokines such as interleukin-12, -15, and -18 can activate NK cells, which function as cytotoxic effectors [41]. NK cells can release proteins such as perforin and proteases to peripheral cancer cells, and thereby increase the porosity of target cell membranes [42]. Compared to TAMs, far fewer studies have sought to modulate NK cells. However, some nanomaterials have been studied for their abilities to activate NK cells [43] or modulate their movement [38,44].

Cationic liposomes loaded with plasmid DNA encoding the TUCS2 gene were studied for their ability to activate NK cells [43]. TUSC2 expression is decreased in most lung cancer patients, and this may be related to their low survival rate [45]. Cationic liposomes composed of DOTAP and cholesterol were complexed to plasmid DNA encoding the TUCS2 gene. Upon intravenous injection of the lipoplexes into Kras-mutant syngeneic model mice, the population of NK cells was increased in the tumor microenvironment. This appeared to reflect the TUCS2 upregulation-triggered stimulation of proinflammatory cytokines and the interleukin-15 cytokine pathway. Since these cationic liposomes lacked any specific targeting ligand, it is unclear which specific cell type(s) took up the lipoplexes. However, this study importantly showed that the cationic liposome-mediated delivery of a gene could activate NK cells.

Magnetic nanoparticles were studied for their ability to modulate the movement of NK cells to tumor tissues [44]. Superparamagnetic iron oxide (Fe_3O_4) nanoparticles were decorated with a silica layer and modified with PET-silane and fluorescent dye. NK cells were isolated and loaded with magnetic nanoparticles under a magnetic field. Human B cell lymphoma-bearing mice were intravenously injected with the magnetic nanoparticle-loaded NK cells and exposed to an external magnetic field (340 G/mm). During the magnetic field exposure, a strong fluorescent dye signal was detected in the tumor tissues, apparently reflecting the migration of the magnetic nanoparticle-loaded NK cells. However, the intensity of the fluorescent signal in the tumor tissues rapidly decreased after the magnetic field was discontinued, and there was only limited retention of the magnetically guided NK cells in the tumor tissues. Despite this relatively short retention of NK cells at the tumor tissues, the externally controlled movement of immune cells appears to warrant further research, as does the design of new nanomaterials with decreased toxicity and the ability to maintain the natural functions of nanomaterial-loaded immune cells.

4. Nanomaterials for modulating MDSCs

MDSCs are immature myeloid cells that are unable to differentiate to mature forms, such as dendritic cells, granulocytes, or macrophages. In the tumor microenvironment, the secretions of interleukin- 1β , interleukin-6, prostaglandin E2, VEGF, and interferon- γ inhibit the normal maturation of these myeloid progenitor cells. Furthermore, the accommodation of MDSCs at tumor tissues may suppress T cell proliferation and NK cell activation while promoting the differentiation of regulatory T cells [46]. The nanomaterial-mediated selective modulation of MDSCs at tumor tissues could enable the recovery of immune suppression and open a new avenue for immunotherapy [47–49]. Similar to the nanomaterial-mediated modulation of NK cells, relatively few studies have used nanomaterials to modulate MDSCs. To date, nanomaterials have been studied for the purposes of depleting [47,48] or modulating the differentiation [46] of MDSCs.

Gemcitabine-encapsulated lipid nanocapsules were exploited to deplete MDSCs [47]. Gemcitabine is nucleotide analog that is used to treat various solid tumor types, such as ovarian cancer, non-small cell lung cancer, and pancreatic adenocarcinoma. In addition to its anticancer effects, gemcitabine was reported to selectively kill MDSCs when given at a low dose [50,51]. In one study, lauroyl-modified gemcitabine was inserted into pegylated lipid nanocapsules by the phase-inversion method [44]. Following subcutaneous injection of fluorescence dye-tagged gemcitabine nanocapsules into EG07-OVA lymphoma tumor-bearing mice, the nanocapsules were found to accumulate at the tumor and spleen, both of which showed reduced populations of MDSCs. The combination of low-dose gemcitabine nanocapsules with adoptive T cell therapy enhanced the antitumor effect and survival rate compared to that seen with adoptive T cell therapy alone. These results suggested that MDSC depletion could provide a favorable microenvironment for cytotoxic T cell proliferation.

Another strategy for depleting MDSCs involved the use of MDSC-targeting aptamer-modified liposomes [48]. An aptamer is a single-strand oligonucleotide that can fold into a 3D structure to perform antibody-like specific binding to a target. In this study, a 74-mer aptamer (T1) was surface conjugated to pegylated liposomes encapsulating doxorubicin (Fig. 3A) and screened for binding to various breast tumor cell lines and MDSCs in the tumor microenvironment. The aptamer-modified liposomes were shown to kill the tumor cells and reverse immune suppression via MDSC depletion. In a highly metastatic 4T1 breast cancer orthotropic mouse model, intravenous injection of T1 aptamer-conjugated liposomes eliminated MDSCs in the blood, spleen, and tumor tissues. This effect further activated the antitumor immune response, as evidenced by an elevation of tumor-infiltrating cytotoxic CD8 + T cells.

MDSC can also be manipulated to differentiate toward cell types with antitumor activities. In a recent study, all-trans retinoic acid was co-delivered with interleukin-2 and doxorubicin by mesoporous silica nanoparticles, in an effort to enable combined chemotherapy and immunotherapy [49]. All-trans retinoic acid reportedly shifts the differentiation of MDSCs toward antitumor immune cells (e.g., mature DCs, macrophages,

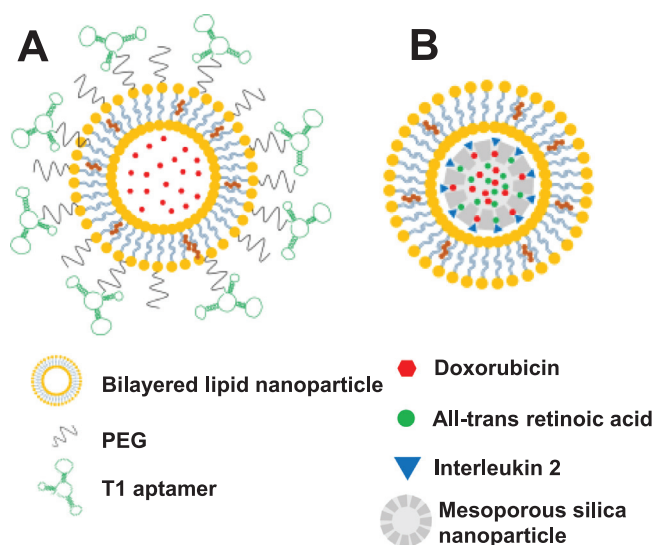


Fig. 3 – Nanoformulations for modulation of MDSC. (A) Doxorubicin-loaded liposomes were decorated with T1 aptamers, which show selective binding affinities for MDSCs and cancer cells Liu, [48]. (B) All-trans retinoic acid was entrapped in the pores of liposome coated-mesoporous silica nanoparticles together with the therapeutic agents, doxorubicin and interleukin-2. All-trans retinoic acid could suppress the differentiation of MDSCs in tumor tissues, leading to tumor inhibition Kong, [49].

and granulocytes) to facilitate the tumor-specific immune response [52]. Multidrug-loaded mesoporous silica nanoparticles were coated with lipids to improve their aqueous stability and biocompatibility (Fig. 3B). In a B16F10 melanoma tumor model, intravenous administration of these nanoparticles increased the maturation of tumor-resident DCs with a corresponding decrease in the density of MDSCs.

In the context of MDSC modulation-based approaches, specific MDSC-targeting approaches should be studied further. Although Liu and colleagues [48] very recently used MDSC aptamer modified liposomes, their T1 aptamer could bind to both tumor cells and MDSCs. In the differentiation studies [49], the lipid nanomaterials used to modulate MDSCs also carried cytotoxic anticancer drugs. The delivery of cytotoxic anticancer drug-loaded nanoparticles might kill and/or enhance the differentiation of MDSCs, confusing the issue. Future studies that focus on modulating the differentiation of MDSCs should use MDSC-specific nanomaterials that can exert the desired functions without killing MDSCs.

5. Nanomaterials for modulating neutrophils

Neutrophils (also known as neutrocytes) are the most abundant leukocytes differentiated from stem cells in the bone marrow. They are indispensable for the defense against intruding infections and for innate immune responses [53]. Neutrophils can rapidly transmigrate into injured or infected tissues in response to chemotaxis. The assembly of nanocarriers with circulating neutrophils may create a new opportunity for

the directed delivery of therapeutic drugs. Thus, neutrophil-mediated drug delivery has been studied for its potential in cancer immunotherapy.

In one study, photosensitizer-loaded albumin nanoparticles and a tumor antigen-specific antibody were used as a co-treatment to enhance the recruitment of neutrophils to tumor tissues [54]. This study utilized the previously reported binding of albumin nanoparticles to neutrophils via Fc γ RIII receptors [55]. Upon injection, the photosensitizer (pyropheophorbide-a-loaded albumin nanoparticles) were expected to bind to neutrophils in the bloodstream. To guide the neutrophils to tumor tissues, the researchers co-injected the TA99 antibody, which is a melanoma gp75 antigen-specific monoclonal antibody that can recruit neutrophils to melanoma tumor sites via antibody-dependent cell-mediated cytotoxicity. Following irradiation with a laser at 660 nm for photodynamic therapy, the tumor growth was more strongly suppressed in the nanoparticle and antibody co-treated group compared to the other groups.

In another study by the same research group, CD11b antibody-decorated gold nanoparticles were used to enhance the infiltration of neutrophils into tumor tissues [56] (Fig. 4). The CD11b antibody was used as a biomarker for activated neutrophils. To enhance the infiltration of neutrophils to tumor tissues, mice were preinjected with pyropheophorbide and illuminated at 660 nm. The researchers hypothesized that photosensitization-induced inflammation at tumor tissues might guide the gold nanoparticle-bound neutrophils to infiltrate the tumor tissues. Indeed, the CD11b-antibody-decorated gold nanoparticle-treated group showed reduced tumor growth and prolonged survival compared to the pegylated gold nanoparticle-treated group.

Another inflammation-mediated neutrophil recruitment strategy was investigated in a postoperative glioma tumor model [57]. In this study, anticancer drug-carrying liposomes were harbored inside the neutrophils rather than as an externally bound form. For *ex vivo* loading, neutrophils were isolated and incubated with paclitaxel-containing liposomes. The inflammation at operation sites was utilized to trigger the infiltration of neutrophils through the blood-brain barrier. In the study, operative-site inflammation was shown to increase the release of proinflammatory cytokines, such as interleukin-10 and CXCL1. Upon intravenous infusion, the liposome-loaded neutrophils were found to accumulate in the glioma tumor tissues, as evidenced by fluorescence from the dye-tagged liposomes. The group treated with liposome-bearing neutrophils showed higher survival compared to those treated with free Taxol or liposomes alone. This study suggested that it may be feasible to deliver nanomaterial-loaded neutrophils to brain tumor tissues, where the blood-brain barrier typically limits the access of conventional nanomaterials. However, it might be difficult to optimize the dose of anticancer drugs in neutrophils, and early drug release could damage the cells and decrease their ability to home to tumor tissues.

Song and colleagues decorated pixantrone-loaded liposomes with poly(sialic acid)-octadecylamine for neutrophil-mediated delivery to lung cancer [58]. Poly(sialic acid) was used for binding of the liposomes to L-selectin which is highly expressed on the surfaces of peripheral blood neutrophils [59]. Poly(sialic acid)-modified liposomes pro-

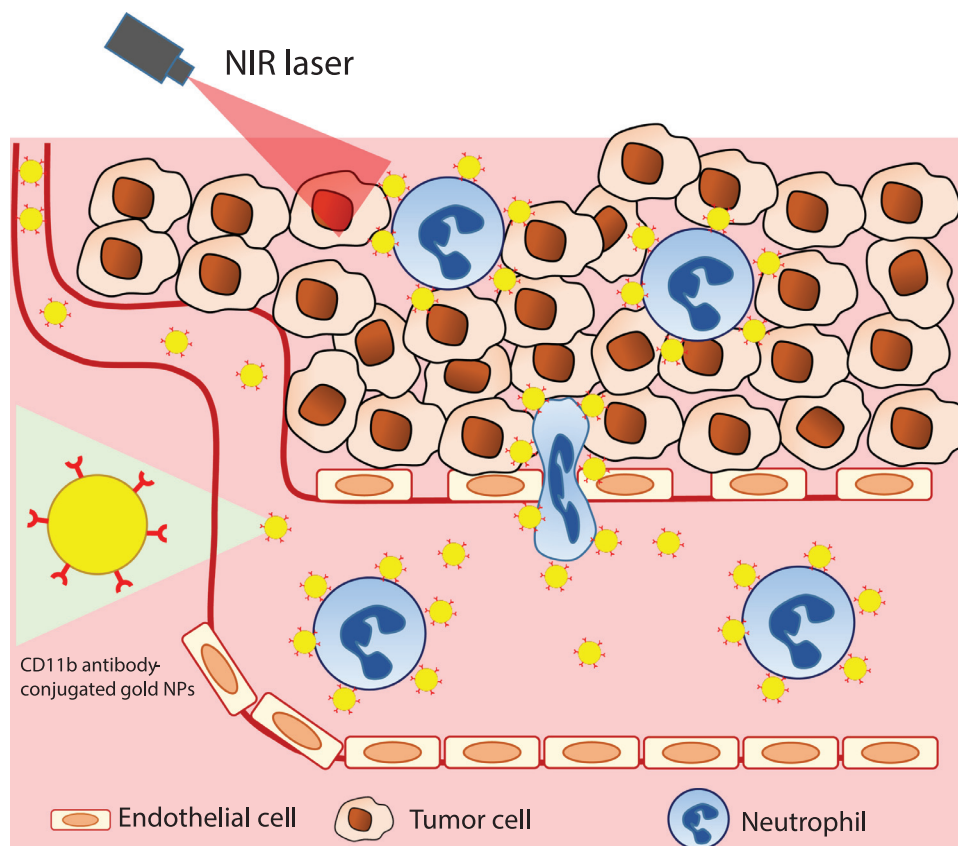


Fig. 4 – Neutrophil-mediated delivery of nanocarriers.

CD11b antibody-modified gold nanoparticles were bound to neutrophils. The gold nanoparticle-loaded neutrophils were recruited to stimulus-induced inflammatory tumor sites, facilitating the deep penetration of gold nanoparticles into the tumor tissues for photothermal therapy [56].

vided greater uptake to neutrophils compared to plain liposomes and polyethyleneglycol-modified liposomes. In A549 tumor-bearing animal model, poly(sialic acid)-modified liposome-treated group showed the highest anticancer effect among the groups tested. This study shows the potential of sialic acid modification for targeting neutrophils by nanocarriers.

6. Nanomaterial for modulating DCs

In the immune system, DCs act as interfacing cells that connect the innate and adaptive immune responses [60,61]. In the innate immune response, DCs express various types of pattern recognition receptors, such as toll-like receptors (TLRs), stimulator of interferon genes (STING), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), retinoic acid inducible gene I (RIG-I)-like receptors (RLRs), and C-type lectins. These molecules are responsible for activating DCs upon pathogen exposure or cellular damage [62]. Activated DCs secrete cytokines that recruit and activate NK cells to eliminate pathogens and other dangerous factors, such as cancer cells [63]. In addition, DCs are potent professional antigen-presenting cells that act as key immune orchestra-

tors via their ability to capture and process antigens for T cell activation. The activation of pattern-recognition receptors via the recognition of danger associated molecule patterns from dying tumor cells results in the maturation of DCs, leading to the spontaneous priming of T cell responses [60]. In the tumor microenvironment, however, the immunosuppression-induced self-tolerance of cancer cells will limit DC-mediated immune responses [64]. Numerous immunomodulators, such as TLR agonists and STING agonists, have been developed to boost the activities of DCs during cancer treatment. Unfortunately, these immunomodulators have issues with instability and side effects. To overcome the current challenges, researchers have sought to use nanomaterials to deliver these immunomodulators. To maximize the effect of immunomodulators, nanomaterials have been designed to co-deliver immunomodulators with cancer vaccines [65,66] or combine them with photothermal therapy [67] (Fig. 5).

Short synthetic single-chain oligonucleotides with unmethylated cytosines and guanines, called CpG ODN, mimic the properties of pathogenic bacterial DNA. CpG ODN was reported to specifically bind TLR-9 receptor, leading to activation of DCs. Several clinical trials have used CpG ODN, such as in combination with chemical anticancer drugs against

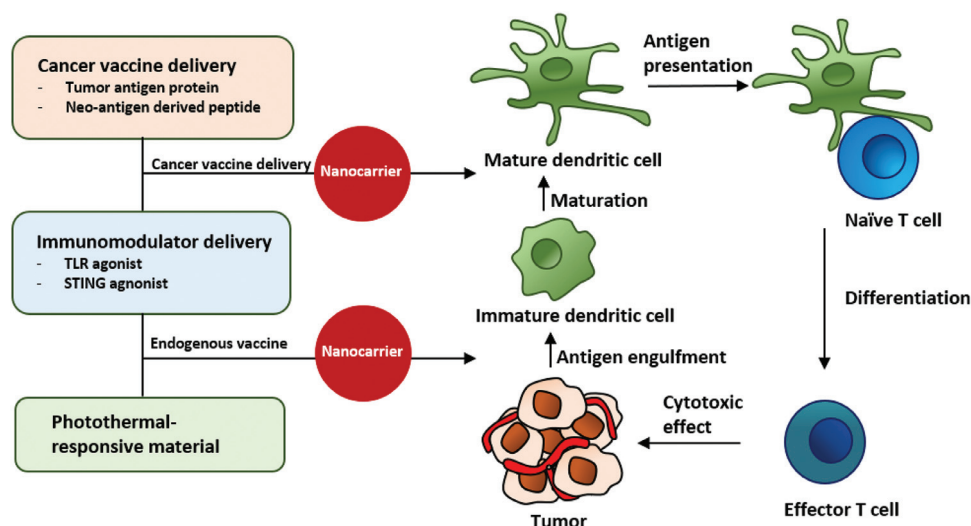


Fig. 5 – Immunomodulator-loaded nanomaterials for the modulation of DCs. The combination of immunomodulators with a cancer vaccine (vaccine-adjuvant delivery) or photothermal therapy (endogenous vaccine) could evoke an effective DC-primed immune response.

non-small cell lung cancer (NCT00070629) or melanoma (NCT00070642), and with cancer vaccines against colorectal cancer (NCT00669292) and skin cancer (NCT01149343). Due to the outstanding efficacy of CpG ODN as an adjuvant in cancer treatment, several studies have integrated them into nanocarriers in an effort to enhance the efficacy of a co-delivered cancer vaccine. In a recent study, CpG ODN was modified with cholesterol to form a nanostructure with a high-density lipoprotein (HDL)-derived peptide [65]. This complexation formed a stable HDL nanodisc in the presence of lipids, possibly due to a lipid-stabilizing effect of the HDL peptide. Neoantigen-derived peptides selected from melanoma and colorectal cancer were then conjugated into HDL nanodiscs as a vaccine-adjuvant system. These nanodiscs conferred more efficient delivery of CpG ODN to DCs at draining lymph nodes, compared to free CpG ODN. Moreover, the nanodiscs enhanced the cellular uptake of cultured DCs, promoting a stronger and more durable antigen-presentation ability. In line with the DC activation observed *in vitro*, subcutaneous injection of HDL nanodiscs elicited 47-fold and 31-fold greater cytotoxic T lymphocyte responses compared to free components and CpG ODN, respectively, in melanoma tumor bearing mice. Vaccination with HDL nanodiscs was found to protect mice from tumor challenge and reduce metastatic lesions in the lung.

STING, which is a transmembrane protein located in the endoplasmic reticulum, is activated when cells are infected with intracellular pathogens, leading to the production of type I interferon. Activation of STING reportedly promotes the maturation and antigen-presentation capability of DCs [68]. A STING-agonizing polymer was recently developed to enhance the efficacy of a delivered cancer antigen vaccine [69]. In this study, a diblock copolymer (called PC7A, composed of PEG and poly 2-(hexamethyleneimino) ethyl methacrylate derivatives containing cyclic tertiary amine side chains) was for-

mulated with a peptide antigen as a nanovaccine. The tertiary amine structure of PC7A rendered it pH-sensitive, allowing the antigens to be released and escape from the endosome to interact with STING in the cytoplasm. The encapsulation of a model antigen with PC7A produced 50 nm-sized nanoparticles. After subcutaneous injection, these nanoparticles were found to distribute to draining lymph nodes. PC7A had a high binding affinity for STING, with a K_d value of 1.3 μM . *In vitro* examination using bone marrow-derived DCs showed that these nanoparticles had an adjuvant effect on DC maturation and enhanced the expression levels of maturation markers, including CD80 and CD86. In mouse models of melanoma, colorectal cancer, and lymphoma, vaccination with PC7A nanoparticles yielded greater tumor inhibition than vaccination with the free antigen. In the lymphoma model (TC-1 tumor-bearing mice), vaccination with human papilloma virus-derived antigen peptide loaded in PC7A nanoparticles was found to extend survival in 100% of the treated mice.

Recently, photothermal therapy has attracted a great deal of attention in the context of treating cancer [70,71]. In photothermal therapy, light energy is converted to heat through light responsive materials. Heat generation reportedly induces cell damage and the release of damage associated molecules, such as adenosine triphosphate (ATP), calreticulin, high mobility group box 1 (HMGB1), and heat-shock proteins. These molecules are known as danger signals that recruit and activate DCs [72,73]. Meanwhile, the tumor shrinkage that follows photothermal therapy is a rich source of tumor antigens that are, in turn, engulfed by DCs for specific immune priming. Given this, the combination of a vaccine adjuvant (e.g., a TLR agonist) with photothermal therapy has been investigated as a heat-induced endogenous vaccine therapy for the treatment of cancer [67,74].

Imiquimod is a TLR7/8 agonist that was approved by the FDA for treating basal skin carcinoma; it is sold as a topical cream under the name Aldara® [75]. In a study seeking to exploit this agonist in an endogenous vaccine strategy, imiquimod was co-encapsulated with the photothermal-responsive agent, indocyanine green, in the hydrophobic compartment of biodegradable polymeric poly(lactic-co-glycolic) acid nanoparticles. Intravenous administration of indocyanine green/imiquimod-loaded polymeric nanoparticles showed accumulation at tumor tissues. Thereafter, irradiation of the tumor site with near infrared light was found to ablate the primary tumors of CT26 colon tumor-bearing mice. Moreover, this elimination of the primary tumor enhanced the maturation and antigen presentation of DCs. When this indocyanine/imiquimod-loaded polymeric nanoparticle-based photothermal therapy was combined with the CTLA-4 immune checkpoint antibody, impressive anticancer effects were observed. These included the dramatic suppression of the distant tumor model and reduction of metastatic nodules in the lung.

7. Challenges and future perspectives

Recent pioneering studies have opened a new era in the nanomaterial-based modulation of innate immune cells in tumor tissues. Nanomaterials can overcome the poor solubility, low selectivity, and high toxicity of chemotherapeutics [76]. Numerous nanomaterials, such as lipid-based nanoparticles, polymeric nanoparticles, and inorganic nanoparticles, have been designed as carriers for small molecules, proteins, and nucleic acids [77–79]. However, numerous issues must be resolved before such applications can be translated to the clinic. Among these issues are safety concerns. Although most studies provided *in vitro* cell viability data to support the safety of nanomaterials, more in-depth toxicity studies must be performed in both the short and long terms. In particular, the modulation of innate immune cells can also evoke immune toxicity due to the enhanced interaction of cytotoxic drug-loaded nanomaterials with immune cells. Thus, the possibility of immune toxicity should be carefully examined.

Future studies should also address the direct targeting of nanomaterials to specific immune cells. Most of the existing studies have used the inhibition of tumor growth as a major end point of immune cell-targeted nanomaterials. The collection of data supporting the targeting of specific immune cells and illuminating the intracellular functions of endocytosed nanomaterials may support and inform the development of immune cell-targeting nanomaterials. There is a general lack of known ligand molecules that can be used to differentiate between immune cells of normal and tumor tissues. For example, mannose receptors and galactose receptors can be present on both normal macrophages and TAMs. The use of ligands that recognize receptors universal to both normal and tumor-associated immune cells may not maximize the targeting of nanomaterials to tumor tissue-specific immune cells. Further studies are needed to identify more specific ligand molecules or biomarkers for MDSCs and other innate immune cells.

Another issue is the need to identify a suitable time point for evaluating the efficacy of immunotherapy in an *in vivo* model. Unlike conventional chemotherapy or surgery, immunotherapy may require more time to yield clinical effects due to the delayed activity of the immune system [80]. A benefit of immunotherapy may be observed only after several months from the start of treatment, making it difficult for researchers to draw early conclusions regarding the effectiveness of an immunotherapy.

The selection of tumor models should also be considered carefully. Each tumor type may have a different characteristic immune cell profile [61]. Moreover, the tumor stage can affect the cell populations in tumor tissues, such as seen in patient tumor tissue-derived tumor models. These potential differences could affect the outcomes of the various nanomaterial-based immune cell-modulating approaches.

Many of the existing studies have demonstrated the interaction of nanomaterials and the target immune cells solely at the level of cell-based *in vitro* experiments. Although such work is an essential step in testing the hypotheses, it does not comprise proof-of-concept. Researchers should seek to design *in vitro* systems that mimic the complexity of the crosstalk that occurs between tumor cells and host immune cells *in vivo*. Moreover, most of the existing *in vivo* studies have been performed in mice, whose immune systems are quite different from those of human [81–84]. Another goal of the field should be to develop new animal models that more closely mimic the human immune system.

One approach to mimic the human immune system is to co-transplant human immune cells with tumor xenograft [85]. However, humanized mice cannot support the development of human innate immune cells. For proper function of transplanted human innate immune cells, Rongvaux and colleagues developed two strains of mice, MITRG and MISTRG [86]. These mice were generated to have Rag2^{-/-} Il2rg^{-/-} 129xBalb/c (N2) genetic background. In MISTRG mice, four genes encoding human M-CSF, IL-3, GM-CSF and thrombopoietin were knocked-in to replace the mouse respective loci for secretion of human cytokines. Moreover, MISTRG mice were also knocked-in with BAC transgene encoding human SIRP α . Expression of human SIRP α on mouse phagocytes played a role to tolerate the signal from CD47 of grafted-human cells. However, these animal models are focused on hum macrophage-mimicking models for TAM-based immunotherapy. Humanized mouse model for neutrophils, NK cell should be investigated more in future studies.

Immunotherapy takes advantage of immune system activities. However, given that immune cells and tumor cells engage in complex relationships and interactions, monotherapy may not be sufficient to activate the immune system. The combination of nanocarrier-delivered immunotherapy with another modality has shown great promise and warranted additional development. For example, neutrophil-targeting nanoparticles with a photosensitizer were combined with photodynamic therapy [54]. In the study, the irradiation of laser onto the tumor tissues induced the generation of reactive oxygen species at the tumor tissues. The combination of neutrophil delivery and photodynamic therapy enhanced the anticancer effect of immunotherapy with prolonged survival of mice. Moreover, the combination of laser with neutrophil-

targeting gold nanoparticle was used to trigger the inflammation at the tumor site and guide the nanoparticle-bound neutrophils to the tumor tissues [56].

Clinical data have shown that the combined application of CTLA-4 and PD-1 antibodies, which affect two different immune checkpoints at different stages of the immune response, could improve the overall survival of melanoma patients [87]. Many studies have utilized combined strategies involving immune checkpoint inhibitors (e.g., PD-L1, PD-1, or CTLA-4 antibodies) and a therapeutic drug-loaded nanocarrier [67,88,89]. These co-treatments have been found to synergize the outcome effect of immunotherapy on primary tumors, distant tumors, and models of metastasis. This suggests that nanomaterial-based innate immune cell modulation might be combined with immune checkpoint antibody therapy for enhanced clinical outcomes.

8. Conclusion

Immunotherapy has been extensively studied and has shown promise in both preclinical and clinical trials. Researchers have examined the use of nanomaterials to modulate TAMs, NK cells, neutrophils, MDSCs and DCs. Although progress has been made in efforts to activate, delete, differentiate, and/or increase the infiltration of such cells into tumor tissues, this field is still in its infancy. Future studies will require the selective design of nanomaterials that can recognize target innate immune cells, and the use of carefully designed experiments and evaluation strategies. Given the importance of innate immune cells in tumor tissues, nanomaterials for specific innate immune cell modulation may have strong potential for redirecting the current path of immunotherapy.

Conflicts of interest

The authors declare that there is no conflicts of interest.

Acknowledgments

This study was funded by research grants from the Ministry of Science and Future Planning, Republic of Korea (NRF-2018R1A2A1A05019203; NRF-2018R1A5A2024425), and the Korean Health Technology R&D Project (No. HI15C2842), Ministry of Health & Welfare, Republic of Korea.

REFERENCES

- [1] De Palma M, Biziato D, Petrova TV. Microenvironmental regulation of tumour angiogenesis. *Nat Rev Cancer* 2017;17(8):457–74.
- [2] Chen DS, Mellman I. Oncology meets immunology: The cancer-immunity cycle. *Immunity* 2013;39(1):1–10.
- [3] Janssen LME, Ramsay EE, Logsdon CD, Overwijk WW. The immune system in cancer metastasis: friend or foe? *J Immunother Cancer* 2017;5:79.
- [4] Palucka AK, Coussens LM. The basis of oncoimmunology. *Cell* 2016;164(6):1233–47.
- [5] Bernardes-Silva M, Anthony DC, Issekutz AC, Perry VH. Recruitment of neutrophils across the blood-brain barrier: the role of E- and P-selectins. *J Cereb Blood Flow Metab* 2001;21(9):1115–24.
- [6] Joice SL, Mydeen F, Couraud PO, et al. Modulation of blood-brain barrier permeability by neutrophils: in vitro and in vivo studies. *Brain Res* 2009;1298:13–23.
- [7] Kottke T, Evgin L, Shim KG, et al. Subversion of NK cell and TNF-alpha immune surveillance drives tumor recurrence. *Cancer Immunol Res* 2017;5(11):1029–45.
- [8] Curiel TJ. Immunotherapy: A useful strategy to help combat multidrug resistance. *Drug Resist Updat* 2012;15(1-2):106–13.
- [9] Jiang W, Von Roemeling CA, Chen Y, et al. Designing nanomedicine for immuno-oncology. *Nat Biomed Eng* 2017;1:1–11.
- [10] Choi YH, Han HK. Nanomedicines: current status and future perspectives in aspect of drug delivery and pharmacokinetics. *J Pharm Investig* 2018;48(1):43–60.
- [11] Webb SD, Owen MR, Byrne HM, Murdoch C, Lewis CE. Macrophage-based anti-cancer therapy: modelling different modes of tumour targeting. *Bull Math Biol* 2007;69(5):1747–76.
- [12] Mantovani A, Marchesi F, Malesci A, Laghi L, Allavena P. Tumour-associated macrophages as treatment targets in oncology. *Nat Rev Clin Oncol* 2017;14(7):399–416.
- [13] Noy R, Pollard JW. Tumor-associated macrophages: From mechanisms to therapy. *Immunity* 2014;41(1):49–61.
- [14] Zanganeh S, Hutter G, Spittler R, et al. Iron oxide nanoparticles inhibit tumour growth by inducing pro-inflammatory macrophage polarization in tumour tissues. *Nat Nanotechnol* 2016;11(11):986–94.
- [15] Song M, Liu T, Shi C, et al. Bioconjugated manganese dioxide nanoparticles enhance chemotherapy response by priming tumor-Associated macrophages toward M1-like phenotype and attenuating tumor hypoxia. *ACS Nano* 2016;10(3):633–47.
- [16] Wang Y, Xu Z, Guo S, et al. Intravenous delivery of siRNA targeting CD47 effectively inhibits melanoma tumor growth and lung metastasis. *Mol Ther* 2013;21(10):1919–29.
- [17] Zhan X, Jia L, Niu Y, et al. Targeted depletion of tumour-associated macrophages by an alendronate-glucomannan conjugate for cancer immunotherapy. *Biomaterials* 2014;35(38):10046–57.
- [18] Griesmann H, Drexel C, Milosevic N, et al. Pharmacological macrophage inhibition decreases metastasis formation in a genetic model of pancreatic cancer. *Gut* 2017;66(7):1278–85.
- [19] Lu M, Cohen MH, Rieves D, Pazdur R. FDA report: Ferumoxytol for intravenous iron therapy in adult patients with chronic kidney disease. *Am J Hematol* 2010;85(5):315–319.
- [20] Sindrilaru A, Peters T, Wieschalka S, et al. An unrestrained proinflammatory M1 macrophage population induced by iron impairs wound healing in humans and mice. *J Clin Invest* 2011;121(3):985–97.
- [21] da Silva MC, Breckwoldt MO, Vinchi F, et al. Iron induces anti-tumor activity in tumor-associated macrophages. *Front Immunol* 2017;8(3):1479.
- [22] Rayahin JE, Buhrman JS, Zhang Y, et al. High and low molecular weight hyaluronic acid differentially influence macrophage activation. *ACS Biomater Sci Eng* 2015;1(7):481–93.
- [23] Liu L, Yi H, He H, Pan H, Cai L, Ma Y. Tumor associated macrophage-targeted microRNA delivery with dual-responsive polypeptide nanovectors for anti-cancer therapy. *Biomaterials* 2017;134:166–79.
- [24] Watkins SK, Egilmez NK, Suttles J, Stout RD. IL-12 rapidly alters the functional profile of tumor-associated and tumor-infiltrating macrophages in vitro and in vivo. *J Immunol* 2007;178(3):1357–62.

- [25] Wang Y, Lin YX, Qiao SL, et al. Polymeric nanoparticles enable reversing macrophage in tumor microenvironment for immunotherapy. *Biomaterials* 2017;112:153–63.
- [26] Williams CB, Yeh ES, Soloff AC. Tumor-associated macrophages: unwitting accomplices in breast cancer malignancy. *NPJ Breast Cancer* 2016;2:15025.
- [27] Liu YY, Qiao LN, Zhang SP, et al. Dual pH-responsive multifunctional nanoparticles for targeted treatment of breast cancer by combining immunotherapy and chemotherapy. *Acta Biomater* 2017;66:310–24.
- [28] Oishi Y, Manabe I. Macrophages in age-related chronic inflammatory diseases. *Npj Aging Mech Dis* 2016;2:16018.
- [29] Yuan H, Jiang W, Von Roemeling CA, Qie Y, Liu X, Chen Y, et al. Multivalent bi-specific nanobiocjugate engager for targeted cancer immunotherapy. *Nat Nanotechnol* 2017;12(8):763–9.
- [30] Weiskopf K. Cancer immunotherapy targeting the CD47/SIRP α axis. *Eur J Cancer* 2017;76:100–9.
- [31] Owens MA, Horten BC, Da Silva MM. HER2 amplification ratios by fluorescence in situ hybridization and correlation with immunohistochemistry in a cohort of 6556 breast cancer tissues. *Clin Breast Cancer* 2004;5(1):63–9.
- [32] Nath D, Hartnell A, Happerfield L, et al. Macrophage-tumour cell interactions: Identification of MUC1 on breast cancer cells as a potential counter-receptor for the macrophage-restricted receptor, sialoadhesin. *Immunology* 1999;98(2):213–19.
- [33] She Z, Zhang T, Wang X, et al. The anticancer efficacy of pixantrone-loaded liposomes decorated with sialic acid-octadecylamine conjugate. *Biomaterials* 2014;35(19):5216–25.
- [34] Zhou S, Zhang T, Peng B, et al. Targeted delivery of epirubicin to tumor-associated macrophages by sialic acid-cholesterol conjugate modified liposomes with improved antitumor activity. *Int J Pharm* 2017;523(1):203–16.
- [35] Sun J, Song Y, Lu M, et al. Evaluation of the antitumor effect of dexamethasone palmitate and doxorubicin co-loaded liposomes modified with a sialic acid-octadecylamine conjugate. *Eur J Pharm Sci* 2016;93:177–83.
- [36] Kim HM, Kang YM, Ku KB, et al. The severe pathogenicity of alveolar macrophage-depleted ferrets infected with 2009 pandemic H1N1 influenza virus. *Virology* 2013;444(1–2):394–403.
- [37] Schneider C, Nobs SP, Heer AK, et al. Alveolar macrophages are essential for protection from respiratory failure and associated morbidity following influenza virus infection. *PLoS Pathog* 2014;10:e1004053.
- [38] Cheng M, Zhang J, Jiang W, Chen Y, Tian Z. Natural killer cell lines in tumor immunotherapy. *Front Med* 2012;6(1):56–66.
- [39] Guillerey C, Huntington ND, Smyth MJ. Targeting natural killer cells in cancer immunotherapy. *Nat Immunol* 2016;17(9):1025–36.
- [40] Law AMK, Lim E, Ormandy CJ, Gallego-Ortega D. The innate and adaptive infiltrating immune systems as targets for breast cancer immunotherapy. *Endocr Relat Cancer* 2017;24(4):R123–44.
- [41] Fang F, Xiao W, Tian Z. NK cell-based immunotherapy for cancer. *Semin Immunol* 2017;31:37–54.
- [42] Iannello A, Debbeche O, Samarani S, Ahmad A. Antiviral NK cell responses in HIV infection: I. NK cell receptor genes as determinants of HIV resistance and progression to AIDS. *J Leukoc Biol* 2008;84(1):1–26.
- [43] Meraz IM, Majidi M, Cao X, et al. TUSC2 immunogene therapy synergizes with anti-PD-1 through enhanced proliferation and infiltration of natural killer cells in syngeneic Kras-mutant mouse lung cancer models. *Cancer Immunol Res* 2018;6:163–77.
- [44] Jang ES, Shin JH, Ren G, et al. The manipulation of natural killer cells to target tumor sites using magnetic nanoparticles. *Biomaterials* 2012;33(22):5584–92.
- [45] Prudkin L, Behrens C, Liu DD, et al. Loss and reduction of Fus1 protein expression is a frequent phenomenon in the pathogenesis of lung cancer. *Clin Cancer Res* 2008;14(1):41–7.
- [46] Wesolowski R, Markowitz J, Carson WE. Myeloid derived suppressor cells - a new therapeutic target in the treatment of cancer. *J Immunother Cancer* 2013;1(1):10.
- [47] Sasso MS, Lollo G, Pitorre M, et al. Low dose gemcitabine-loaded lipid nanocapsules target monocytic myeloid-derived suppressor cells and potentiate cancer immunotherapy. *Biomaterials* 2016;96:47–62.
- [48] Liu H, Mai J, Shen J, et al. A novel DNA aptamer for dual targeting of polymorphonuclear myeloid-derived suppressor cells and tumor cells. *Theranostics* 2018;8(1):31–44.
- [49] Kong M, Tang J, Qiao Q, et al. Biodegradable hollow mesoporous silica nanoparticles for regulating tumor microenvironment and enhancing antitumor efficiency. *Theranostics* 2017;7(13):3276–92.
- [50] Suzuki E, Kapoor V, Jassar AS, Kaiser LR, Albelda SM. Gemcitabine selectively eliminates splenic Gr-1+/CD11b+ myeloid suppressor cells in tumor-bearing animals and enhances antitumor immune activity. *Clin Cancer Res* 2005;11:6713–21.
- [51] Le HK, Graham L, Cha E, Morales JK, Manjili MH, Bear HD. Gemcitabine directly inhibits myeloid derived suppressor cells in BALB/c mice bearing 4T1 mammary carcinoma and augments expansion of T cells from tumor-bearing mice. *Int Immunopharmacol* 2009;9(7):900–9.
- [52] Mirza N, Fishman M, Fricke I, et al. All-trans-retinoic acid improves differentiation of myeloid cells and immune response in cancer patients. *Cancer Res* 2006;66(18):9299–307.
- [53] Borregaard N. Neutrophils, from marrow to microbes. *Immunity* 2010;33(5):657–70.
- [54] Chu D, Zhao Q, Yu J, Zhang F, Zhang H, Wang Z. Nanoparticle targeting of neutrophils for improved cancer immunotherapy. *Adv Healthc Mater* 2016;5(9):1088–93.
- [55] Wang Z, Li J, Cho J, Malik AB. Prevention of vascular inflammation by nanoparticle targeting of adherent neutrophils. *Nat Nanotechnol* 2014;9(3):204–10.
- [56] Chu D, Dong X, Zhao Q, Gu J, Wang Z. Photosensitization priming of tumor microenvironments improves delivery of nanotherapeutics via neutrophil infiltration. *Adv Mater* 2017;29(27):1–7.
- [57] Xue J, Zhao Z, Zhang L, et al. Neutrophil-mediated anticancer drug delivery for suppression of postoperative malignant glioma recurrence. *Nat Nanotechnol* 2017;12(3):692–700.
- [58] Luo X, Hu L, Zheng H, et al. Neutrophil-mediated delivery of pixantrone-loaded liposomes decorated with poly (sialic acid)-octadecylamine conjugate for lung cancer treatment. *Drug Deliv* 2018;25(1):1200–12.
- [59] Coussens LM, Werb Z. Inflammation and cancer. *Nature* 2002;420(6917):860–7.
- [60] Berraondo P, Minute L, Ajona D, Corrales L, Melero I, Pio R. Innate immune mediators in cancer: between defense and resistance. *Immunol Rev* 2016;274(1):290–306.
- [61] Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. *Nat Immunol* 2013;14:1014.
- [62] Pearce EJ, Everts B. Dendritic cell metabolism. *Nat Rev Immunol* 2015;15(1):18–29.
- [63] Persson CM, Chambers BJ. Plasmacytoid dendritic cell-induced migration and activation of NK cells in vivo. *Eur J Immunol* 2010;40(8):2155–64.
- [64] Li R, Fang F, Jiang M, et al. STAT3 and NF- κ B are simultaneously suppressed in dendritic cells in lung cancer. *Sci Rep* 2017;7:45395.

- [65] Kuai R, Ochyl LJ, Bahjat KS, Schwendeman A, Moon JJ. Designer vaccine nanodiscs for personalized cancer immunotherapy. *Nat Mater* 2017;16(4):489–96.
- [66] Wilson DR, Sen R, Sunshine JC, Pardoll DM, Green JJ, Kim YJ. Biodegradable STING agonist nanoparticles for enhanced cancer immunotherapy. *Nanomedicine: NBM* 2018;14(2):237–46.
- [67] Chen Q, Xu L, Liang C, Wang C, Peng R, Liu Z. Photothermal therapy with immune-adjuvant nanoparticles together with checkpoint blockade for effective cancer immunotherapy. *Nat Commun* 2016;7:13193.
- [68] Corrales L, McWhirter SM, Dubensky TW, Gajewski TF. The host STING pathway at the interface of cancer and immunity. *J Clin Invest* 2016;126(7):2404–11.
- [69] Luo M, Wang H, Wang Z, et al. A STING-activating nanovaccine for cancer immunotherapy. *Nat Nanotechnol* 2017;12(7):648–54.
- [70] Shim G, Ko S, Kim D, et al. Light-switchable systems for remotely controlled drug delivery. *J Control Release* 2017;267:67–79.
- [71] Guo X, You J. Near infrared light-controlled therapeutic molecules release of nanocarriers in cancer therapy. *J Pharm Investig* 2017;47(4):297–316.
- [72] Zhou F, Nordquist RE, Chen WR. Photonics immunotherapy-A novel strategy for cancer treatment. *J Innov Opt Health Sci* 2016;9(1):1–11.
- [73] Ogawa M, Tomita Y, Nakamura Y, et al. Immunogenic cancer cell death selectively induced by near infrared photoimmunotherapy initiates host tumor immunity. *Oncotarget* 2017;8(6):10425–36.
- [74] Li L, Yang S, Song L, Zeng Y, Gong C. An endogenous vaccine based on fluorophores and multivalent immunoadjuvants regulates tumor micro-environment for synergistic photothermal and immunotherapy. *Theranostics* 2018;8(3):860–73.
- [75] Smits ELJM, Ponsaerts P, Berneman ZN, Van Tendeloo VFI. The use of TLR7 and TLR8 ligands for the enhancement of cancer immunotherapy. *Oncologist* 2008;13(8):859–75.
- [76] Choi JY, Thapa RK, Yong CS, Kim JO. Nanoparticle-based combination drug delivery systems for synergistic cancer treatment. *J Pharm Investig* 2016;46(4):325–39.
- [77] Gupta B, Yong CS, Kim JO. Solid matrix-based lipid nanoplatforms as carriers for combinational therapeutics in cancer. *J Pharm Investig* 2017;47(6):461–73.
- [78] Ma X, Williams RO. Polymeric nanomedicines for poorly soluble drugs in oral delivery systems: an update. *J Pharm Investig* 2018;48(1):61–75.
- [79] Fogli S, Montis C, Paccosi S, et al. Inorganic nanoparticles as potential regulators of immune response in dendritic cells. *Nanomedicine* 2017;12(14):1647–60.
- [80] Hoos A, Britten CM. The immuno-oncology framework enabling a new era of cancer therapy a new era of cancer therapy. *Oncoimmunology* 2012;1(3):334–9.
- [81] Mestas J, Hughes CCW. Of mice and not men: Differences between mouse and human immunology. *J Immunol* 2004;172(5):2731–8.
- [82] Mak IWY, Evaniew N, Ghert M. Lost in translation: Animal models and clinical trials in cancer treatment. *Am J Transl Res* 2014;6(2):114–18.
- [83] Zschaler J, Schlorke D, Arnhold J. Differences in innate immune response between man and mouse. *Crit Rev Immunol* 2014;34(5):433–54.
- [84] Tao L, Reese TA. Making mouse models that reflect human immune responses. *Trends Immunol* 2017;38(3):181–93.
- [85] Ngiow SF, Loi S, Thomas D, Smyth MJ. Mouse models of tumor immunotherapy. *Adv Immunol* 2016;130:1–24.
- [86] Rongvaux A, Willinger T, Martinek J, et al. Development and function of human innate immune cells in a humanized mouse model. *Nat Biotechnol* 2014;32(4):364–72.
- [87] Wolchok JD, Chiarion-Sileni V, Gonzalez R, et al. Overall survival with combined nivolumab and ipilimumab in advanced melanoma. *N Engl J Med* 2017;377(14):1345–56.
- [88] He C, Duan X, Guo N, et al. Core-shell nanoscale coordination polymers combine chemotherapy and photodynamic therapy to potentiate checkpoint blockade cancer immunotherapy. *Nat Commun* 2016;7:12499.
- [89] Wang C, Sun W, Wright G, Wang AZ, Gu Z. Inflammation-triggered cancer immunotherapy by programmed delivery of CpG and anti-PD1. *Adv Mater* 2016;28(40):8912–20.