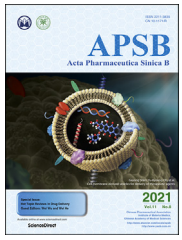
Acta Pharmaceutica Sinica B 2021;11(8):2172–2196

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



REVIEW

Magnetic systems for cancer immunotherapy

Nicole B. Day, William C. Wixson, C. Wyatt Shields IV*

Department of Chemical & Biological Engineering, University of Colorado, Boulder, CO 80303, USA

Received 30 November 2020; received in revised form 5 February 2021; accepted 25 February 2021

KEY WORDS

Drug delivery;
Immunotherapy;
Magnetic hyperthermia;
Magnetic nanoparticles;
Microrobotics;
Biomaterials

Abstract Immunotherapy is a rapidly developing area of cancer treatment due to its higher specificity and potential for greater efficacy than traditional therapies. Immune cell modulation through the administration of drugs, proteins, and cells can enhance antitumoral responses through pathways that may be otherwise inhibited in the presence of immunosuppressive tumors. Magnetic systems offer several advantages for improving the performance of immunotherapies, including increased spatiotemporal control over transport, release, and dosing of immunomodulatory drugs within the body, resulting in reduced off-target effects and improved efficacy. Compared to alternative methods for stimulating drug release such as light and pH, magnetic systems enable several distinct methods for programming immune responses. First, we discuss how magnetic hyperthermia can stimulate immune cells and trigger thermoresponsive drug release. Second, we summarize how magnetically targeted delivery of drug carriers can increase the accumulation of drugs in target sites. Third, we review how biomaterials can undergo magnetically driven structural changes to enable remote release of encapsulated drugs. Fourth, we describe the use of magnetic particles for targeted interactions with cellular receptors for promoting antitumor activity. Finally, we discuss translational considerations of these systems, such as toxicity, clinical compatibility, and future opportunities for improving cancer treatment.

Abbreviations: BW, body weight; CpG, cytosine-phosphate-guanine; DAMP, damage associated molecular pattern; EPR, enhanced permeability and retention; FFR, field free region; HSP, heat shock protein; HS-TEX, heat-stressed tumor cell exosomes; ICD, immunogenic cell death; IVIS, *in vivo* imaging system; MICA, MHC class I-related chain A; MPI, magnetic particle imaging; ODNs, oligodeoxynucleotides; PARP, poly(adenosine diphosphate-ribose) polymerase; PDMS, polydimethylsiloxane; PEG, polyethylene glycol; PLGA, poly(lactic-*co*-glycolic acid); PNIPAM, poly(*N*-isopropylacrylamide); PVA, poly(vinyl alcohol); SDF, stromal cell derived-factor; SID, small implantable device; SLP, specific loss power.

*Corresponding author. Tel.: +1 303 735 4763.
E-mail address: Charles.Shields@colorado.edu (C. Wyatt Shields).

Peer review under responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2021.03.023

1. Introduction

Immunotherapy is a promising strategy for guiding immune activation against tumor growth and metastasis by mobilizing native immune cells of the body. The two primary methods of regulating tumor activity with immunotherapy are educating or enhancing immune cell activation, such as with cancer vaccines[1], or correcting dysfunctional immune pathways within the tumor microenvironment, such as through checkpoint inhibition[2]. Research into immunotherapy has grown in recent years due to its advantages over traditional treatment options, such as higher efficiency and specificity and reduced systemic toxicity compared to chemotherapy or radiotherapy[1–4]; however, approved drugs for immunotherapy are often used as second-line treatment options or in combination with more established treatments such as chemotherapy or radiotherapy. While checkpoint inhibitors are currently being investigated as first-line treatment options, high cost[5–7] and variable patient responses[8] are significant limiting factors for the advancement of these immunotherapies[9]. Additionally, challenges remain in the delivery of immunomodulatory drugs due to the difficulty of penetrating solid tumors and the immunosuppressive tumor microenvironment, which can result in insufficient immune activation and difficulty regulating dosages to maintain potency without off-target effects[10,11]. Even clinically successful immunotherapies such as immune checkpoint inhibitors only benefit a subset of patients[12,13]. Therefore, there is an emerging interest among engineers and immunologists alike to spatially and temporally control immunotherapies.

Researchers from materials science and nanotechnology have sought to fulfill the exigent need for improved delivery of, and control over, immunotherapies[14,15]. Remotely controlled systems have been explored at length, employing stimuli such as magnetics, light, pH, heat, or acoustics to cause chemical or physical changes that activate a therapeutic mechanism[16–19]. Magnetic systems offer several advantages over other methods of remotely controlled immunotherapies, including biocompatibility of some magnetic materials like iron oxide and titanium[20], large field penetration depths, multifunctionality of magnetic materials, and high specificity for interaction with magnetically susceptible species over native tissue, all of which enable a wide variety of mechanisms to regulate immune interactions with high spatiotemporal control[19]. While magnetic systems also have use in applications beyond immunotherapy, such as for delivering other cancer agents like chemotherapies or manipulating cellular targets for regenerative medicine[21], the specificity of cellular activation timelines, spatial dependence, and precise patient dosing necessary for efficacious immunotherapy makes the use of magnetics particularly promising[15]. In this review, we discuss different methods of immunotherapeutic drug delivery and immunomodulation *via* magnetic materials, their uses in cancer treatment, and systems that have promise for future adaptation to immunotherapies (Fig. 1). Additionally, we discuss the recent advances in coupled diagnostics and therapy (termed theranostics) *via* magnetic materials as well as the challenges and potential of magnetic immunomodulatory systems for use in clinical settings.

2. Magnetic systems

Magnetic particles are generally composed of single or multiple crystals of an inorganic magnetic material[22]. The first clinical use of magnetic particles was as a negative contrast agent for magnetic resonance imaging (MRI) in 1996[23], and since then, the US Food and Drugs Administration (FDA) has approved certain maghemite (γ-Fe_2O_3) and magnetite (Fe_3O_4) superparamagnetic iron oxide nanoparticles (SPIONs) for diagnostic and therapeutic use[22,24]. However, despite the benefits of superparamagnetism and the biocompatibility of iron oxide nanoparticles, which generally accumulate in the liver or spleen after intravenous injection and break down into iron suitable for storage, only a handful of SPION products are used clinically[25,26]. Other types of magnetic materials of biological relevance include paramagnetic and ferromagnetic materials (Table 1), although they currently lack instances of clinical translation to the best of our knowledge. Magnetization in response to an applied field is also shown in Fig. 2.

Nickel and cobalt-based particles have also been studied as magnetic materials, although are used less frequently in an independent manner than iron and iron oxide due to their toxicity and inferior magnetic susceptibility[28,29]. Additionally, manganese oxides have been used increasingly as theranostic platforms in the last two decades, although not as commonly as iron oxides due to their later introduction to biomedicine and the need to improve therapeutic efficiency[30]. Regardless of material, magnetic particles are highly versatile; in addition to their fundamental magnetism, alternative properties can be imparted to magnetic particles through surface modification, incorporation into other particle systems[31], and variation of size[32] and shape[33,34]. The native interaction of unstimulated magnetic materials and immune cells has also been explored[35], but we will focus on the immunomodulatory activity of such particles under the influence of a

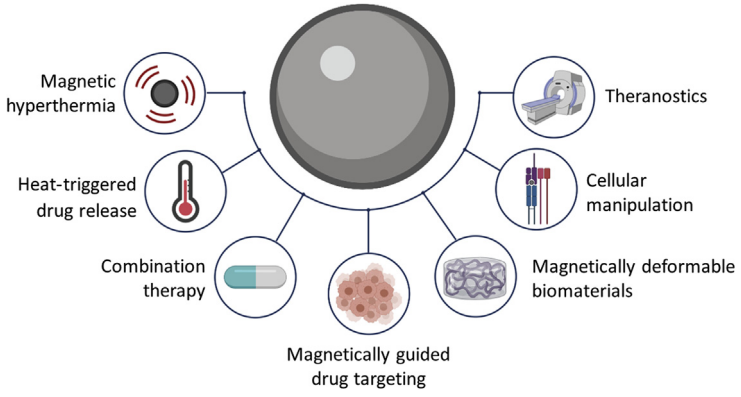
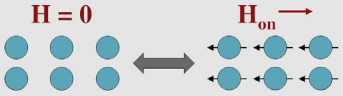
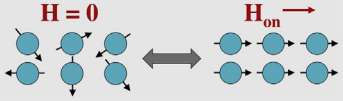
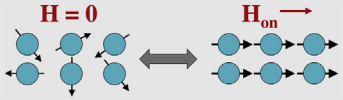
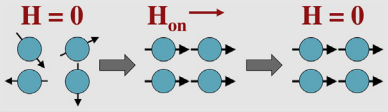


Figure 1 Different approaches that exploit magnetic materials for immunotherapy. Created with BioRender.

Table 1 Properties of magnetic materials. Adapted with permission from Ref. 27. Copyright © 2017 Taylor & Francis.

Magnetic material	Description	Schematic illustration
Diamagnetic	Forms a weak response against an applied magnetic field.	
Paramagnetic	Aligns with an applied magnetic field. Magnetic moment is not retained when the field is removed.	
Superparamagnetic	Develops a mean magnetic moment only in an applied magnetic field. Nonmagnetic when the applied magnetic field is removed.	
Ferromagnetic	Highly susceptible to an applied magnetic field. Retains a dipole, producing a magnetic field after the applied field is removed.	

magnetic field. Furthermore, despite the importance of particle properties in the performance of magnetic systems, we will not discuss their synthesis, characterization, or optimization in this review, as they are well described elsewhere[30,36–41].

2.1. *Magnetic hyperthermia*

Magnetic hyperthermia (MH, or magnetic fluid hyperthermia) is a phenomenon in which magnetic energy is transformed into thermal energy through the rapid oscillations of magnetic nanoparticles due to an alternating magnetic field (AMF)[42]. Localized magnetic induction heating of cancerous tissues has garnered significant attention as a therapeutic tool since its initial proposal in 1957[43]. Modern manifestations leverage single-domain magnetic nanoparticles under an AMF to heat tissues in a highly specific manner[44,45]. Nanometer-scale thermal measurements reveal that heat generated from nanoparticles in AMFs dissipates on subcellular scales, which makes it an attractive system for drug targeting to microscopic zones[46]. The most common material to induce MH is surface-coated SPIONs because of their biocompatibility, colloidal stability, and magnetic behavior[47,48]. Optimal particles are under 200 nm to cross the endothelial barrier in capillaries and reach tumor sites[24,49,50]. SPIONs of 10–30 nm with a high heating power per unit mass are among the preferred size range for MH and have the additional benefit of escaping phagocytes for a longer circulating half-life than particles greater than 30 nm[50]. However, despite the breadth of academic work on MH in biological systems, its use in clinical settings still lags, with regulatory approval primarily limited to specific cancer centers in Europe[25,51].

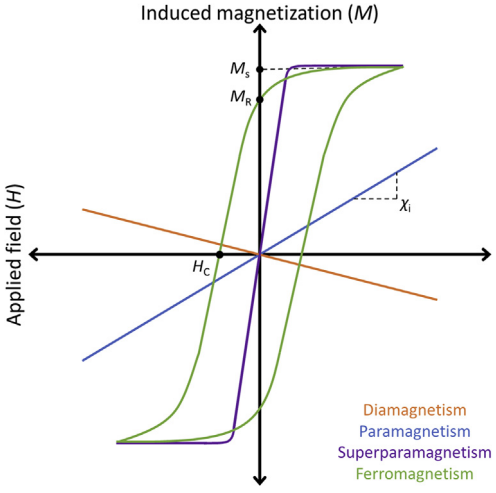


Figure 2 Generalized magnetization curves of diamagnetic, paramagnetic, superparamagnetic, and ferromagnetic materials. M_S, saturation magnetization; M_R, remanent magnetization; H_C, coercive field threshold; χ_i, magnetic susceptibility.

Heating by MH is attributed to the heat losses inherent in the generation of eddy currents, hysteresis losses, and relaxation losses[42,44]. Relaxation heating is described by two mechanisms, Brownian and Néel relaxation. Brownian relaxation results from the magnetic effects on the random (Brownian) rotation of particles in suspension[52], while Néel relaxation originates from particles with a single paramagnetic domain (superparamagnetism). Relaxation of this single magnetic domain generates loss of magnetic potential to heat[53]. An AMF induces a cycle of magnetization and relaxation through the varied amplitude, creating a heat source described by the specific loss power (SLP, the amount of released heat) as Eq. (1):

$$\mathrm{SLP} = \alpha \times \frac{4\mu_0 M_s H_{\max}}{\rho} \times f \tag{1}$$

where μ_0 is fluid viscosity, M_s is the saturation magnetization, H_{max} is the magnetic field amplitude, ρ is the mass density of nanoparticles, f is the magnetic field frequency, and α is a dimensionless parameter dependent on nanoparticle degree of alignment, anisotropy, and interparticle interactions[54]. This method of heating has been shown to raise local temperatures to 40–43 °C, which is sufficiently elevated above typical body temperatures to impact immune cell activity and stimulate thermosensitive materials for therapeutic applications[17]. While higher temperatures can be reached with magnetic stimulation of SPIONs, they can result in damage to healthy cells and tissue ablation[25,55]. This section will cover the application of MH to cancer treatment and motivate its emerging use in immunotherapy.

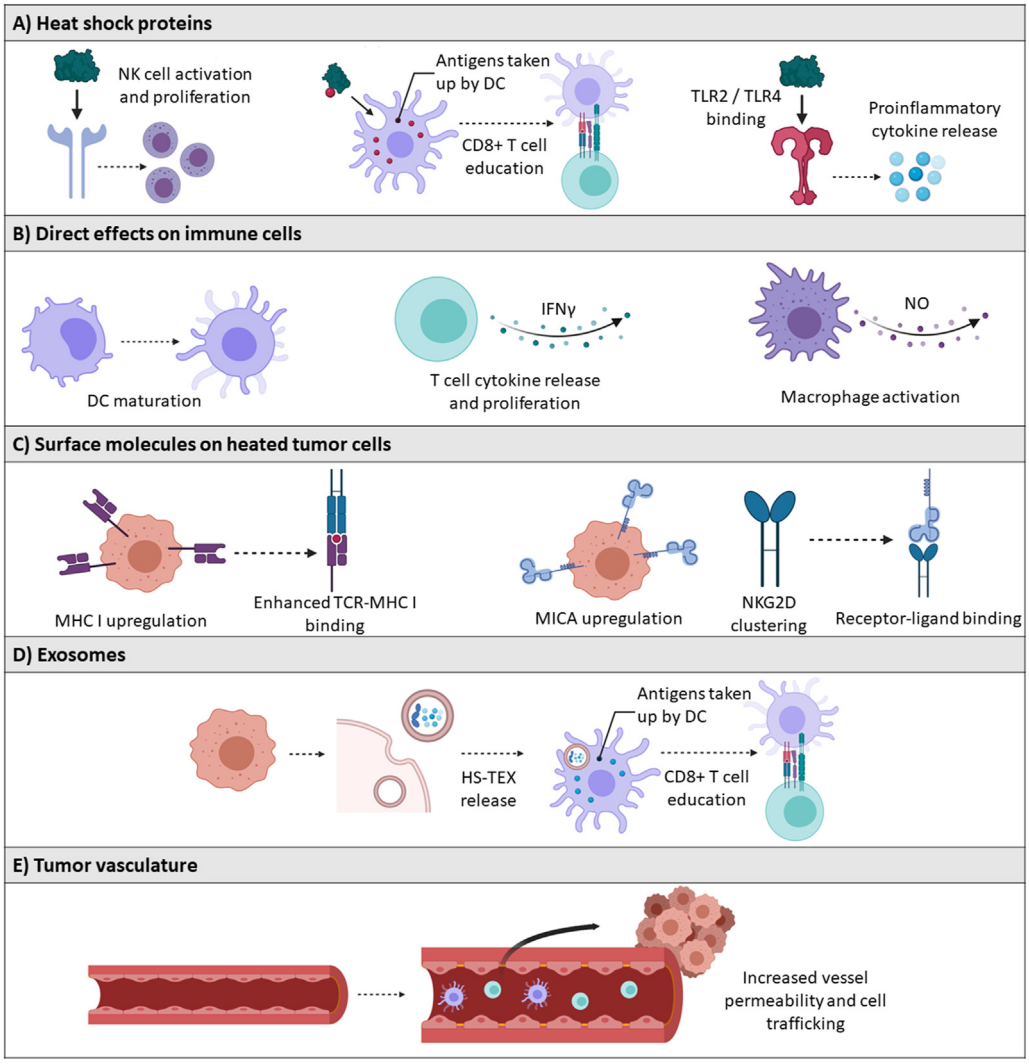


Figure 3 Mechanisms of immune modulation by magnetic hyperthermia. (A) Cellular release of heat shock proteins (green) due to heat stress results in natural killer cell activation and proliferation, cytotoxic $CD8^+$ T cell education, and proinflammatory cytokine response. (B) Dendritic cells, T cells, and macrophages have enhanced anticancer activity in response to local heating. (C) MCH I and MICA are upregulated on tumor cells. (D) Increased exosome release enhances cytotoxic T cell education by dendritic cells. (E) Tumor vasculature responds to temperature increase by allowing increased immune cell permeation. Created with BioRender. Adapted with permission from Ref. 56. Copyright © 2014 Taylor & Francis.

2.1.1. Local magnetic hyperthermia as immunotherapy

There are multiple mechanisms of action for immunotherapy within tumors using local MH, some of which have been previously reviewed in detail[55–59]. An advantage to further developing these methods is the ability to achieve potent immunomodulation without the addition of drugs, which often elicit adverse reactions[25]; however, hyperthermia is still most commonly applied as an adjunct to chemotherapy or radiotherapy rather than as a primary treatment[60,61]. This may be due in part to the lack of understanding of optimal treatment parameters for clinical use across tumor types, which makes repeatable therapy difficult[58,62].

Historically, methods to induce local heating of tumor cells included submerging the tumor site in a hot water bath or inserting a metallic probe to deliver microwaves across the tumor[56,63]. Similar to tumor excision, the goal of hyperthermia is to eliminate tumor cells and a margin of healthy cells around the tumor site, but with heat-induced cell death instead of physical extraction[64]. This method results in cell debris that is cleared by macrophages and other phagocytes and replaced by healthy tissue. In contrast to an insertable probe or hot water bath, which are imprecise with respect to both location and temperature[56], magnetic nanoparticles have the potential to penetrate solid tumors noninvasively and produce precise and uniform heating over a wider temperature range. Precise heating with magnetic nanoparticles can induce immunogenic cell death (ICD) or elicit other sustained antitumoral immune responses through a variety of mechanisms, illustrated in Fig. 3. ICD is a distinct apoptotic pathway caused by cellular stresses such as heat, which result in the release of damage-associated molecular patterns (DAMPs)[65]. DAMPs are immunostimulatory and act as danger signals, enabling processing of tumor-associated antigens released during cell death. This promotes long-term immunological memory against tumors[66] and

has even been examined as an alternative cancer vaccine[14]. Photothermal therapy (PTT), radiotherapy, and certain chemotherapies are common inducers of ICD for treatment of various types of cancer[14,65]. Additionally, thermotherapy has been shown to elicit the endoplasmic reticulum stress response and generate reactive oxygen species much like ICD; however, ICD has only been observed at temperatures above the traditional range of MH (>43 °C)[67]. A high SLP and heat potential of SPIONs are necessary for causing cell death, and it is known that higher temperatures lead to greater cytotoxicity[24,68]. However, there is evidence that even higher temperatures (*e.g.*, above 45 °C) can result in necrotic cell death that does not correlate with prolonged immunity[55,56,69,70], indicating that MH-mediated ICD and thermal ablation must be studied further before use as a therapeutic method.

The heat shock response (Fig. 3A) is the best understood mechanism by which MH induces anti-tumor immunity. Stress caused by increased intracellular temperature upregulates transcription of highly conserved heat shock proteins (HSPs), which protect cells from heat-induced apoptosis[71]. When tumors are heated with MH, some HSPs act in a protumoral manner by preventing thermal ablation of the cancer cells, while others act to protect nearby cells through forming an immune response against the heated cancer cells. Of the latter type, HSP70, a 70 kDa protein, has been shown to cause a potent proinflammatory response *via* several pathways[56,57,72]. In addition to their role in cryoprotection, HSPs are molecular chaperones. HSP70 can bind to and transport tumor-associated proteins to antigen presenting cells (APCs)[71,73], which subsequently present their tumor antigens on MHC I for education of cytotoxic $CD8^+$ T cells to mount an antigen-specific attack against tumors[71]. HSP70 also binds to toll-like receptor (TLR)2 and TLR4 on APCs, activating the NF-κB signaling pathway, resulting in increased secretion of proinflammatory cytokines like tumor necrosis factor alpha (TNFα)[74,75]. In addition to activation from exposure to proinflammatory cytokines released due to HSP70, natural killer cells (NKs) recognize HSP70, resulting in enhanced proliferation and cytolytic activity[72,76]. Similarly, Tanaka et al.[77] demonstrated activation of dendritic cells (DCs) by HSP70 released from magnetite cationic liposomes *via* MH, resulting in regression of melanomas in C57BL/6 mice. However, the HSP subgroup produced in response to MH varies between tumor types and heating conditions, with the resultant immune response being a sum of the protumoral and antitumoral HSP behaviors[56]. Moreover, due to the cryoprotective function of HSPs, use of MH must be carefully timed to prevent thermotolerance from HSP overexpression resulting from overly frequent treatments[57,78,79]. As such, the complete action of HSP-induced anti-tumor immunogenicity has not yet been fully elucidated[57].

Other effects of hyperthermia acting directly on immune cells have been studied (Fig. 3B). An early example of this came from Duff and Durum, who showed that T lymphocyte proliferation in response to interleukin (IL)-1 and IL-2 was enhanced with 39 °C heat treatment, a temperature similar to fever conditions[80]. Successive fever-range (<41 °C) heat treatment studies have shown similar results in other immune cells[81]; 39.5 °C heating of cytotoxic $CD8^+$ T cells increased interferon (IFN)γ production and tumor cytotoxicity[82]; DCs matured in response to 39.5−41 °C heating independently of the heat shock response[83]; and macrophages upregulated inducible nitric oxide (NO) synthase (iNOS, a proinflammatory enzyme) after 39.5 °C heat treatment[84]. However, Kalber et al.[85] demonstrated that mesenchymal stem cells loaded with SPIONs, injected into tumors, and heated +4.3 °C did not have an effect on tumor size or growth characteristics, indicating that conditions for different tumor types require accurate tuning of MH conditions for effective immune responses. Regardless, the activation of immune cells by MH could be a promising adjuvant technique for immunotherapies such as cancer vaccination due to the ability for MH to mimic local fever conditions. Time, duration, and temperature of MH must be considered carefully due to the specificity of immune cell activation timelines and responses[81].

Several other immune responses to local heating have been identified with a direct effect on tumor cells (Fig. 3C). Ito et al.[86] demonstrated that MHC I is upregulated on tumor cells in response to 43 °C MH, resulting in enhanced recognition by $CD8^+$ T cells and subsequent cytotoxic action. MHC I is amply expressed on nascent tumors, but it is often depleted as tumors progress, making their recovery an effective strategy for immunotherapies[87]. Heating can also elicit antitumor responses from NKs, as shown by Ostberg et al.[88]; distinctive clustering of the NKG2D receptor occurred on NKs in response to a local temperature of 39.5 °C, identical to their response to the proinflammatory cytokine IL-2. The NKG2D receptor recognizes the MHC class I-related chain A (MICA) ligand, which was also upregulated on the surface of tumor cells in the same heated environment. This NK activity induced by MH is a promising method of highly specific therapy.

Tumor cells have been shown to release higher levels of exosomes when under stress, such as from hypoxia, inflammation, and heat (Fig. 3D)[89–92]. Exosomes are nanovesicles that contain different compositions of RNA and proteins, which can act as either immunosuppressive signaling factors for tumor growth or as antigens for programming antitumoral immune responses. In MH, enhanced proinflammatory signaling from macrophages and T cells as well as from elevated temperatures can cause this cellular stress response and increase paracrine signaling from tumor exosomes[89,90,93]. The natural tumor microenvironment is hypoxic, which generates exosomes that promote tumor progression, angiogenesis, and metastasis[93]. However, Dai et al.[90] showed that heat-stressed tumor cells released exosomes (HS-TEX) containing carcinoembryonic antigen, HSP70, and MCH I, which instead result in activation of DCs and immunogenicity. The release of HSP70 in HS-TEX has been shown by other groups as well[94,95]. Some HS-TEX have also been shown to contain cytokines responsible for chemoattraction and activation of DCs and T cells[89]. Additionally, protumoral regulatory T cells differentiated into potent antitumor T helper 17 (T_h17) cells *via* enhanced DC secretion of IL-6 caused by HS-TEX[95]. As such, heat-stressed tumor exosomes have been proposed as components for cancer nanovaccines[89,90,95,96]. However, as with many of these therapeutic methods, MH conditions must be carefully tuned to facilitate the release of HS-TEX with enhanced tumor immunogenicity while avoiding the generation of immunosuppressive exosomes[56].

The final mechanism of MH as a standalone immunotherapy relies on physical changes caused by local temperature increases that enhance immune cell recruitment and increase drug penetration into tumors (Fig. 3E). Hypoxia and immunosuppression of the tumor microenvironment are caused in part by reduced blood vessel density and blood flow to the tumor[60]. However, increased permeability of the tumor vasculature, arteriole diameter, and oxygenation have all been demonstrated to occur in response to 40−43 °C temperatures, which allow for improved transport of DCs and T cells into tumor sites from the draining lymph

nodes[97–99]. Furthermore, MH within tumors has been shown to enhance the recruitment of immune cells by expressing vascular adhesion molecules such as ICAM-1 at higher levels due to increased secretion of IL-6[100]. Iron oxide nanocubes have also been shown to destroy the structure of the extracellular matrix within tumors *via* MH, resulting in improved drug and nanoparticle penetration[101].

Due to the interconnectedness of the immune system, these mechanisms have substantial overlap and can work synergistically to form a potent and sustained therapeutic response. Local MH can generate these effects as a standalone therapy or work in conjunction with other immunotherapies, as discussed in Section 2.1.3. Careful tuning of, or improvements upon, the conditions used in many of these studies (*e.g.*, nanoparticle size, field frequency, field strength, and treatment duration) may improve immune responses. However, some of the constraints of MH therapy are due to intrinsic limitations of an AMF as the stimulus. In particular, whole body AMF exposure can result in adverse heating effects due to the accumulation of SPIONs in off-target locations, while regional AMF exposure can be ineffective for deep tissue penetration[102]. Therefore, emphasis must be placed on improving the transport of SPIONs to target tissues or the precision of magnetic field stimulation to increase the specificity of MH to support clinical translation.

2.1.2. *Heat-triggered drug release*

To improve safety and efficacy of immunotherapies, controlled drug delivery has emerged as an important area of study. Two fundamental goals of this field are the protection of therapeutic cargo and the use of noninvasive stimuli to control the biodistribution of drugs[103]. Thermosensitive nanocarriers are therefore promising systems for spatiotemporal control of drug release. Kumar and Mohammad propose two distinct mechanisms for drug release mediated by MH: (i) heat-triggered crevice formation within a larger polymeric matrix, releasing drugs through expanded pores, and (ii) engineering thermosensitive linkers between the drug and the magnetic nanoparticle[104]. Yatvin et al. demonstrated the first iteration of the former mechanism by developing a hyperthermia-triggered drug release system employing liposomes with a phase transition temperature of 42 °C to release protein synthesis inhibitors in *Escherichia coli*[105]. This early example provided a basis for later research to study different materials, more advanced heating mechanisms, and a variety of drugs in application to cancer therapy.

Many drug delivery systems with heat-triggered release have focused on chemotherapeutic drugs, consistent with the ubiquity of chemotherapy in clinical settings. Doxorubicin (DOX) is a common chemotherapeutic drug used for studying new release systems due to its well-characterized cancericidal effects[106]. Early iterations of heat-triggered release were achieved by means other than MH. One such system incorporated ammonium bicarbonate and DOX into a liposome, causing temperature-controlled cavitation through the decomposition of ammonium bicarbonate to CO_2 and other products at hyperthermic temperatures[107]. Another system employed more complex chemistries and a model fluorophore instead of therapeutic drug, using MH and a reversible click and Diels–Alder reaction to controllably link and unlink the fluorophore from poly (ethylene oxide) and maleimide-modified iron oxide nanoparticles, respectively. As shown in Fig. 4, the use of thermosensitive bonds enabled control over fluorophore release and system tunability to allow conjugation of a variety of therapeutic agents[108].

This study and another using citric acid-modified magnetic nanoparticles incorporated into DOX-containing liposomes showed feasibility of using external magnetic fields to spatiotemporally control drug release, building off of the earlier work that used non-MH induction sources[109]. Additional drug carriers have since been developed to improve uptake by tumor cells[110], release other chemotherapeutic drugs[111,112], or use differing heat-triggered release mechanisms[113].

Only in recent years have researchers examined MH-mediated drug release in the context of immunotherapy. Checkpoint inhibitors such as anti-programmed cell death (PD)1, anti-PD ligand (PD-L)1, and anti-cytotoxic T-lymphocyte-associated protein (CTLA)4 are promising agents to treat metastatic cancers, prompting research into combining these drugs with MH[62]. Even though checkpoint inhibitors are generally less toxic than most chemotherapies, they can produce proinflammatory cytokine storms that lead to dose-limiting toxic effects. These constraints have necessitated further research into techniques that remotely control the release of immunomodulatory drugs[114]. The broad range of drugs used in MH suggests the possibility of expanding this technique beyond chemotherapy, such as to checkpoint inhibitors, cytokines, antigens, and other vaccine components[115].

Future work in heat-triggered drug release systems must optimize MH platforms to improve spatial resolution of drug delivery and to further prevent toxicity or overactivation of immune responses. Encapsulation of SPIONs in hydrogels, such as in microparticle gels made of the highly thermoresponsive polymer *N*-isopropylacrylamide, is a popular method of controlling drug release. However, SPIONs have been shown to experience physical hindrance of Brownian relaxation when encapsulated within hydrogels of mesh sizes on the scale of hydrodynamic particle size; inhibited relaxation of magnetic nanoparticles was shown to prevent efficient local heating and may affect drug release in temperature-dependent systems, necessitating careful tuning of optimal properties for polymeric platforms[52]. Additionally, guiding the drug delivery platform to the tumor site is important to prevent off-target immune responses or cell ablation, which is discussed in Section 2.2. Moreover, most research has examined the effects of immediate cell death caused by release of drugs using MH, not the long-term effects of immune modulation in conjunction with MH[116]. Further optimization of clinical concentrations, particle coatings, and iron oxide structure is needed to verify toxicity levels, especially over multiple rounds of treatment[117,118]. Given the positive results seen in controlled release of checkpoint inhibitors using MH, greater work in remote release systems of immunotherapies such as anti-PD1 and anti-CTLA4 is warranted. However, a barrier to application of some immunotherapies to MH-triggered release systems is heat sensitivity; temperature-sensitive payloads such as cytokines may denature in response to the temperatures used for drug release[119].

2.1.3. *Combination therapy*

We define combination therapy as strategies that use MH in conjunction with more conventional therapies, including chemotherapy or other immunotherapies, to achieve synergistic effects with respect to both the primary tumor and metastases. Frequently, combination therapy aims to induce the abscopal effect, a phenomenon that refers to an adaptive immune response that can attack distant metastatic colonies. While there is insufficient data to show that the abscopal effect can occur by MH alone, recent evidence suggests that it can be induced by combining MH with immunotherapy[120]. Early evidence of synergy between MH and

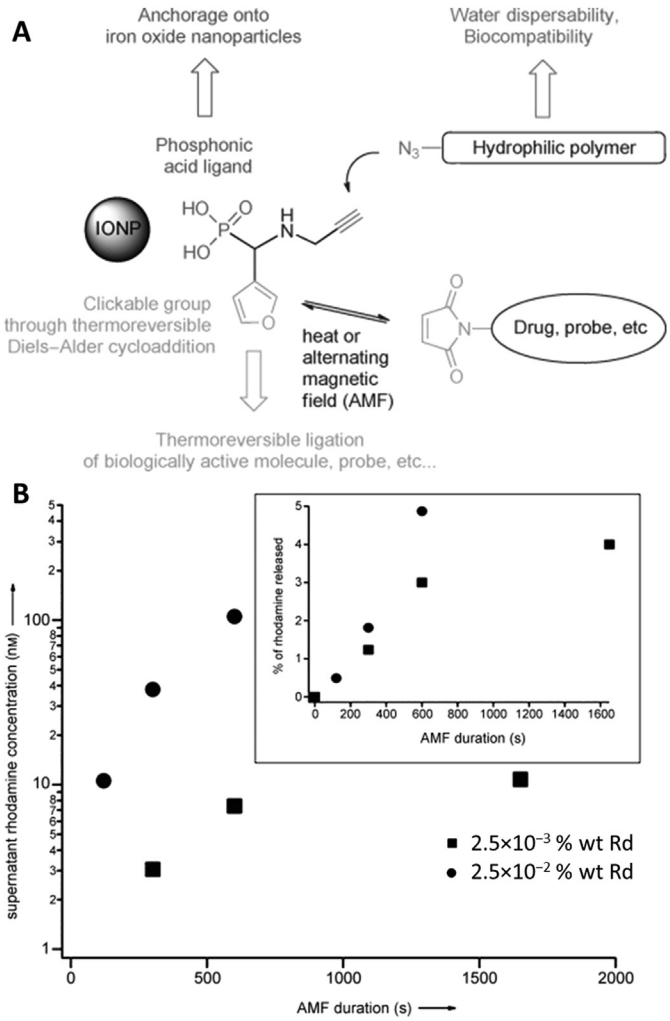


Figure 4 Orthogonal click reaction for release of a model fluorophore, rhodamine (Rd), using magnetic hyperthermia. (A) Scheme for attachment of iron oxide nanoparticles (IONPs) to a polymeric carrier and a drug. (B) Rd release profiles at two different concentrations over 30 min. Reproduced with permission from Ref. 108. Copyright © 2013 John Wiley and Sons.

immune stimulation was provided by a series of papers that showed greater tumor cell clearance when MH was combined with TNFα gene therapy[121], IFNs[122], granulocyte-macrophage colony-stimulating factor (GM-CSF)[86], and DCs[123] than any of the treatments independently. As the field of immunotherapy has advanced, so too has the understanding of combination therapy.

Hyperthermia used in combination with chemotherapy has been known to generate synergistic effects on drug potency, even before sophisticated *in vivo* thermal control techniques such as MH and PTT were established[124]. With the advent of MH, this combination could be achieved while avoiding whole body or even regional hyperthermia. Using SPIONs functionalized with cisplatin (a chemotherapeutic drug) in an AMF, Babincová et al.[125] quantitatively demonstrated synergism through reduced rat sarcoma cell viability *in vitro*. They further proposed using a gradient magnetic field for guiding the drug to the target site, underlining the multifunctional nature of magnetic systems. One proposed mechanism for the observed synergism is hyperthermia-induced upregulation of HSPs, inciting an immune response, as discussed previously[126]. Further demonstrating the enhanced therapeutic efficacy of combining chemotherapy and MH, magnetic mesoporous silica nanoparticles were shown to enable the coregulation of temperature and pH for drug release. This allowed the prevention of undesirable toxic effects due to early drug release at physiological pH outside of cells, while gaining the benefits of the combination therapy after release inside of cells[128].

FDA approval of anti-CTLA4 and anti-PD1 checkpoint inhibitors caused a renewed interest in combination therapy over the last decade[116]. Researchers continue to find synergistic effects when localized heating—whether by MH, PTT, or other methods—is combined with checkpoint inhibitors. Similarly to MH, PTT can impart local heating through laser stimulation of iron oxide nanoparticles[129]. Wang et al.[130] used PTT instead of MH and proposed a mechanism by which thermal ablation of tumor cells caused antigen release for uptake by APCs, triggering a potent immune response. When a checkpoint inhibitor was co-administered, the immunosuppressive activity of regulatory T cells was inhibited in secondary tumors, allowing T_h cells to respond to distal metastases. Clarity on the exact synergistic mechanisms still lag, indicating a need for additional work to fully optimize this approach[131].

Recent work has demonstrated a similar synergy by using MH, which has the advantage of deeper penetration over PTT. Polyethylene glycol (PEG)-coated (or PEGylated) iron nanoparticles administered in combination with anti-CTLA4 drugs demonstrated

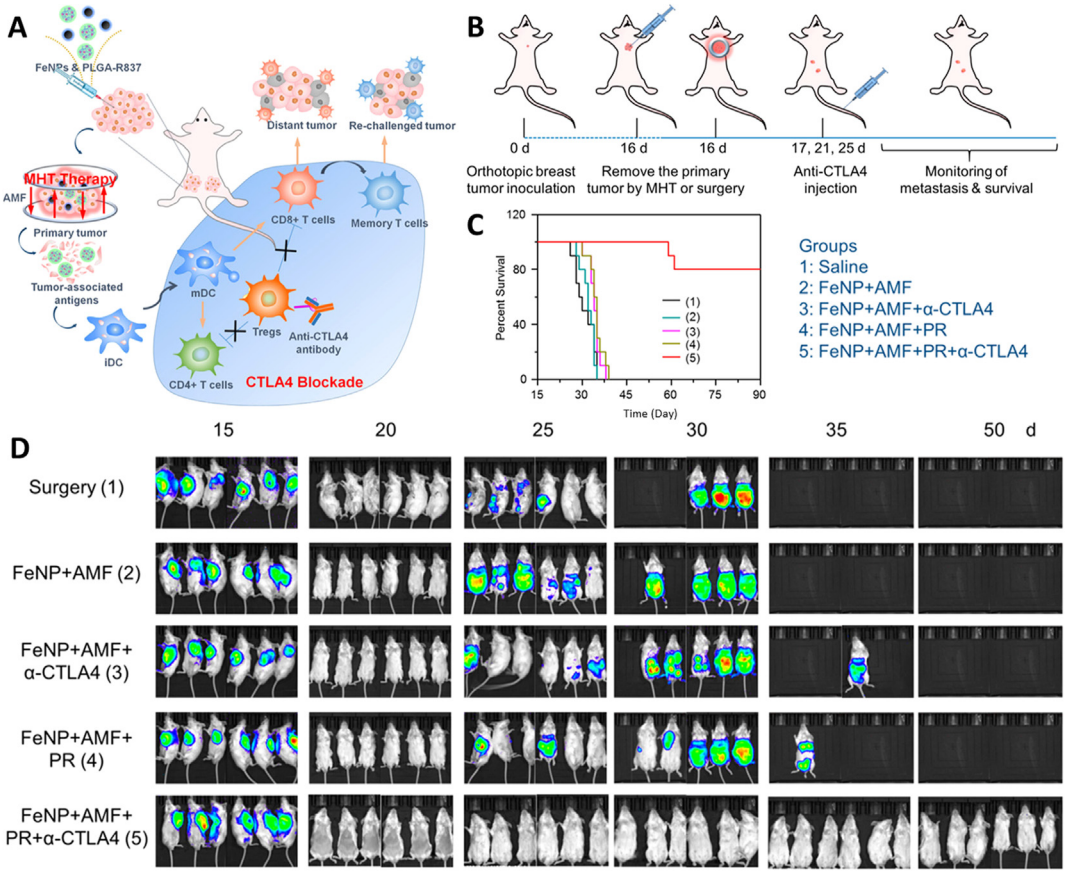


Figure 5 MH plus CTLA4 blockade on an orthotopic 4T1 breast tumor model. (A) Mechanism of immunotherapy through combination of MH from iron oxide nanoparticles (FeNPs), poly (lactic-*co*-glycolic acid) (PLGA) nanoparticles encapsulating imiquimod (R837) (PR), and anti-CTLA4 antibodies (α-CTLA4). T cells are educated for metastatic tumor cell clearance. (B) Schematic timeline of MH and CTLA4 blockade treatments to inhibit tumor metastases. The primary tumor was surgically removed 16 days after inoculation for monitoring of metastatic tumors. (C) Survival of mice with various treatments over 90 days. Combined MH, PR, and α-CTLA4 therapy resulted in 80% survival ($n = 10$ per group). (D) *In vivo* bioluminescence images tracking firefly luciferase-expressing 4T1 breast cancer cells after surgery alone, MH treatment, MH and α-CTLA4 treatment, MH and PR treatment, or MH, α-CTLA4, and PR treatment. Reproduced with permission from Ref. 127. Copyright © 2019 American Chemical Society.

superior shrinkage of secondary tumors compared to MH or a CTLA4 blockade alone, as shown in Fig. 5. Further, the combination showed greater long-term immune memory than through anti-CTLA4 alone[127]. To improve the relatively low energy efficiency of MH *versus* photon-based systems such as PTT, another study used nanoparticles with magnetically hard cobalt iron oxide cores and magnetically soft manganese iron oxide shells with greater MH performance due to the interface between magnetically hard and soft materials. These particles, combined with an AMF and anti-PD-L1 treatment, initiated a cascade of immune responses involving DCs, $CD8^+$ T cells, and proinflammatory cytokines that promoted the abscopal effect and secondary tumor elimination[132]. However, use of a cobalt core may reduce translational potential due to toxicity issues.

Magnetic combination therapies are rapidly evolving, with great promise for improving patient outcomes. Proposed systems for combinatorial treatment continue to incorporate innovative ideas from nanotechnology with proven immunomodulatory drugs into a single system[133–135]. Further elucidation of the mechanisms underlying the synergism of local heating and therapeutic drugs will enable rational design of combination therapy systems that may make their way toward clinical use.

2.2. *Magnetically guided drug targeting*

Drug carriers containing immunomodulatory drugs can take many forms[136,137]. Some carriers rely on high dosage administered systemically to passively reach the desired site, however, this often results in off-target and adverse effects[138,139]. In the context of cancer therapy, inclusion of active targeting functionalities can result in increased drug accumulation within tumors, tumor cells, or immune cells and allows for reduced dosages due to increased specificity[2]. While some drug delivery systems take advantage of the accumulation of nanoparticles that can occur in certain tumor types with leaky vasculatures and impaired lymphatic drainage, known as the enhanced permeability and retention (EPR) effect, this process is passive[140]. A recent meta-analysis showed that the fraction of non-magnetically guided particles that reach the tumor was 0.7% of the injected dose[141], demonstrating a critical need for improved targeting systems. A common mechanism of active tumor targeting is ligand-receptor binding, such as hyaluronic acid to CD44, which is highly expressed on some cancer cells, while the other primary method is using an external field as a driver[137,142].

Table 2 summarizes some of the key characteristics of targeted drug delivery systems. Magnetically guided drug targeting involves carriers that contain a magnetic moiety and encapsulate or display the therapeutic drug. These parameters can have an impact on the rates of (i) delivery to the target site, (ii) drug release, and (iii) clearance or degradation. Certain immunotherapies have drawbacks that limit their therapeutic potential, including rapid degradation or clearance, poor cellular uptake[143], lack of cellular or tumor specificity[144], and inability to penetrate tumor tissue[145,146]; however, improved targeting techniques may increase drug stability or reduce clearance by enabling specific delivery and uptake. Additionally, conjugation of drugs to magnetic nanoparticles has been shown to improve properties like drug circulation time and solubility[147]. Magnetically guided drug targeting has been used in chemotherapy and photodynamic therapy. Due to the importance of spatial control, targeted delivery, and drug stabilization, magnetically guided systems are actively being studied for immunotherapy as well[110,148,149].

The most common magnetic moieties in targeted drug delivery systems are core−shell particles and SPIONs that are either coated with drugs or dispersed within a polymer matrix. Magnetic core−shell particles are most often composed of magnetic cores with a shell constituent that contains drugs[151]. As such, they have the advantage of easily controlling drug release *via* the alteration of shell properties to enable diffusion, erosion, or swelling[150]. SPIONs are also highly versatile, and they have structural properties that can enable efficient drug targeting and the ability to conjugate a variety of therapeutic moieties from glycopeptides for vaccines to small molecules[140,152]. While these drug carriers most often take the form of spherical particles, active or self-propelling microscale robotic particles, or so-called "microbots", are an alternative that generally have more conformational variety to enable distinct patterns of movement. Microbots fabricated from or containing magnetic materials enable highly specific targeting and can be controlled either in bulk or individually[153]. While microbots that involve magnetic actuation for cargo release will be discussed in Section 2.3.2, microbots that can undergo magnetic locomotion often have enhanced speed, specific navigational capabilities, and high levels of targeting precision due to their structural and material properties[154]. As the field of microscale robotics is rapidly growing, there are many promising locomotive systems that have not yet been applied to drug delivery[155−159] or have only tested release of cargo for other applications such as chemotherapy[160−162] or tissue regeneration[163].

To direct the targeting of active drug carriers, applied magnetic fields are used both external and internal to the body. External magnetic fields are the primary choice of stimulus due to their ease of use, low invasiveness, and control over the applied field conditions. A high-gradient field, which is static, but rapidly decreases in strength with distance from the source, can be used to overcome the hydrodynamic force of blood flow acting on drug carriers and is thus highly dependent on particle properties such as size and magnetic susceptibility[164]. Other important considerations for influencing the drug carrier trajectory include particle magnetization as well as drag and buoyancy forces[165]. While tumors deep within the body can be difficult to reach with external magnets, optimization of magnetic field conditions have been studied to improve targeting. This includes the use of oppositely polarized magnets to increase field penetration depth or a combination of external magnets that are placed to target remote locations in the body[166−168]. However, there are still limitations to using external magnetic fields for targeting. As a gradient field is required to direct particles, external fields have the potential to cause stronger attraction to the surface of the body than the target location. Some have proposed overcoming this issue by using implanted magnets[169,170]. Permanent magnetic implants often take the form of ferromagnetic stents, wires, or spherical seeds and can improve upon the targeting of drugs at sites deeper in the body by external fields. However, the downside is the increased invasiveness required for implantation[171−173]. Optimal implants have a long-range, low gradient field that can form a local, high gradient field when magnetized. Therefore, the magnetic particles used for targeting should be designed to only aggregate at the site of interest in response to a high gradient field[172]. Both internal and external magnetic stimuli have been shown to significantly increase accumulation of magnetic drug carriers in tumors, tumor cells, and immune cells, resulting in amplified antitumoral effects.

Table 2 Fundamental characteristics of a targeted drug delivery system. Adapted with permission from Ref. 150. Copyright © 2019 Dove Medical Press Limited.

Features	Effect on drug delivery system
Efficiency of carrier delivery to the targeted sites	• An adequate amount of a drug should be delivered to the desired sites • Free drug should reach a particular concentration in the targeted area
Rate of carrier elimination from the targeted sites	• Drug should remain in the desired area for a sufficient time • Elimination process of carrier drug conjugate should not be faster than the delivery process
Duration between drug release and drug removal in the targeted sites	• Drug should retain a specific concentration in the targeted tissues for sufficient time to act on it
Rate of drug release at non-targeted sites	• Releasing the drug at non-targeting tissue will reduce the amount of drug delivered to the intended areas • Systemic toxicity will increase

2.2.1. *Magnetically guided accumulation of drugs in tumors*

Drugs are incorporated into magnetic carriers with SPIONs primarily by encapsulation, surface coating, and conjugation. Chiang et al.[174] demonstrated encapsulation by fabricating multifunctional fucoidan-dextran particles containing SPIONs and conjugating the checkpoint inhibitor anti-PD-L1 and T cell agonists anti-CD3 with anti-CD28. While combining checkpoint inhibitors with other immune modulators can have significant therapeutic effects, the risk for toxicity often increases[175]; however, this magnetically targeted drug carrier resulted in improved median survival of tumor-burdened mice compared to soluble anti-PD-L1 and reduced adverse events due to a significant increase in tumor accumulation compared to the non-targeted formulation (*i.e.*, ≈16% of the injected dose per gram reached

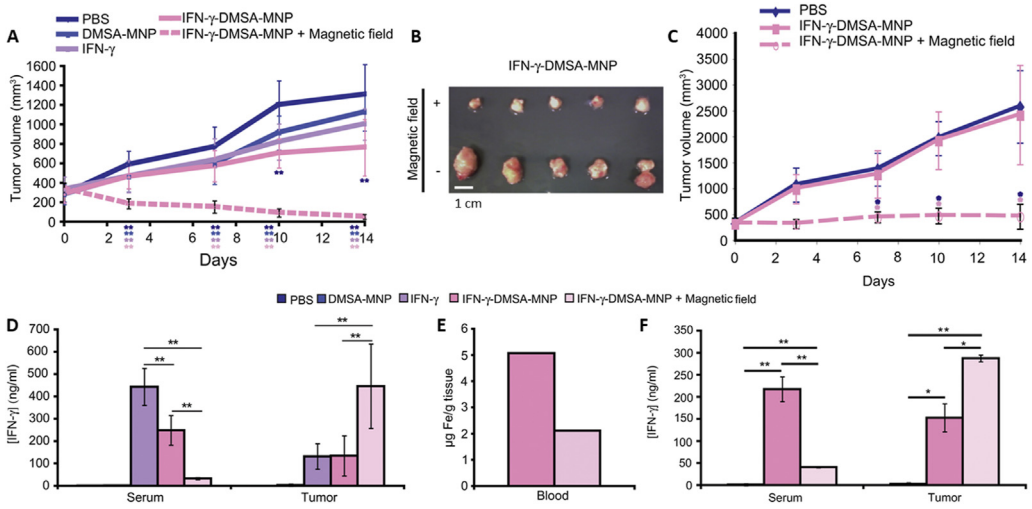


Figure 6 Targeted accumulation of IFNγ (IFNγ-dimercaptosuccinic acid-coated magnetic nanoparticles, DMSA-MNP, with a magnetic field) in murine (A, B) pancreatic (Pan 02) and (C) 3-methylcholatrene (3-MCA)-induced tumors reduces mean tumor volume compared to mice treated with PBS, DMSA-MNPs, soluble IFNγ, or IFNγ-DMSA-MNP without a magnetic field. Data show mean tumor volume ± SD ($n = 30$; $*P < 0.05$; $**P < 0.001$). (D) IFNγ levels in serum and Pan02 tumors. Data show mean ± SD ($n = 10$; $**P < 0.001$). (E) Decreased concentration of nanoparticles in blood samples with magnetic targeting. (F) IFNγ levels in serum and 3-MCA tumors. Data show mean ± SD ($n = 10$; $*P < 0.05$; $**P < 0.001$). Reproduced with permission from Ref. 176. Copyright © 2011 Elsevier.

the tumor)[174]. Instead of encapsulation, another system used SPIONs coated in dimercaptosuccinic acid to direct the surface adsorption of IFNγ. When exposed to an external magnetic field, SPIONs accumulated in the tumors of C57BL/6 mice and locally released the proinflammatory cytokine, resulting in reduced tumor size and increased macrophage and T cell infiltration, as shown in Fig. 6[176]. Grifantini et al.[177] further demonstrated the versatility of SPIONs with two SPION platforms for accumulation within a tumor. The monoclonal antibody mAb 198.3 (a drug that binds to FAT1, a cadherin that is broadly expressed in colorectal cancers) was embedded in erythro-magneto-hemagglutinin virosomes (erythrocytes entrapping SPIONs) or bound to SPIONs directly. Each of these platforms were directed to the tumor using an external magnet and caused reduced tumor mass, while not exhibiting any immunological or cytotoxic reactions.

PTT and photodynamic therapy are often used in conjunction with other therapeutic platforms that contain SPIONs. Recently, Zhang et al.[178] described such a system using SPIONs for targeting of a tumor and use in PTT. While the SPIONs also functioned as MRI guides, theranostics will be discussed in Section 3. PEG polyphenols with SPION cores were loaded with an immunostimulant, R837 hydrochloride, and tumors were targeted *via* an external magnetic field, resulting in a median iron accumulation at least 3x greater than the untargeted equivalent. Photothermal ablation caused release of tumor-associated antigens, initiating a potent immune response enhanced by the release of R837 within the tumor. A core−shell helical microbot with surface-bound SPIONs was also designed for multiple uses of SPION functionality. Inspired by flagellar propulsion, the microbots efficiently locomoted to tumors under a rotating magnetic field for laser irradiation, causing both cargo release and PTT treatment[162]. While DOX was the drug tested in this system, the combination of PTT and immunomodulatory drug release to tumors has been shown to enhance therapeutic potency in other systems and could therefore be applied to this drug carrier for efficacious use[130].

Another innovative system for the delivery of DOX used magnetics for both directing and releasing drugs *via* a two-component millirobot. The drug carrier, containing a permanent magnet, was directed through the body using an external magnetic field to a site near the tumor; due to its relatively large size compared to many traditional microbots, the device was constrained to large body canals such as the urethra, spine, and pancreatic duct. After reaching the target site, the second millirobot component—a piston also embedded with a permanent magnet—was directed to the same location by the same means. Once a critical distance between the carrier and piston were reached, attraction between the two components dominated the external field forces and the piston compressed a drug-loaded hydrogel within the carrier to initiate drug release. While DOX and an external field was used for validation of the system, the authors propose use of this millirobot for targeted delivery of a variety of therapeutic agents and clinical flexibility through the use of a coil system, an MRI scanner, or a permanent magnet for navigation[179]. Another microbot locomotive system was presented by Steager et al.[180]; U-shaped microbots composed of SPIONs embedded within SU-8 photoresist exhibited a stick-slip motion in response to an external rocking field. These microbots demonstrated manipulation and transportation of cells and capture and transportation of chemically loaded poly (lactic-*co*-glycolic acid) (PLGA) microbeads, which could be used for containment and delivery of immunomodulatory drugs. A similar system of swimming helical microbots also demonstrated transport of microbeads[181]. While no drug release was demonstrated for either of these systems, their application to immunotherapy would enable efficient targeting of tumor sites with sustained delivery from drug-loaded particles through the well-characterized diffusive or degradation-mediated release of cargo from PLGA[182,183].

Locomotive microbots are a promising method for targeted drug delivery when operated by magnetic stimuli; however, much of the characterization of microbots focuses on speed and

precision of their motion, leaving the question of their potential improvement over traditional drug delivery systems unanswered. Similarly, particle-based targeting drug delivery systems often use treatment efficacy (*e.g.*, shrinking tumor volume) as an indication of targeting success. Quantitative evaluations of accumulation and delivery efficiency compared to non-targeted delivery systems must be studied more thoroughly in future work.

2.2.2. *Magnetically enhanced cellular uptake*

While improved tumor accumulation is the most common goal of magnetic drug targeting systems for cancer therapy, external fields and implanted magnets can be used to improve immunotherapy by other methods. Several groups have demonstrated innovative uses of magnetic targeting moieties to increase the uptake of drugs or particles by immune cells, either in combination with passive ligand-receptor interactions or to impart magnetic targeting abilities to the cells. Single-cell targeting requires more precision and magnetic control than tumor targeting, for which microbots are well-suited; however, few of such systems have been developed for the purpose of releasing drugs for immunotherapy. One example by Mhanna et al.[184] demonstrated micrometer precision of liposome-functionalized corkscrew microswimmers for delivery of calcein—a model water-soluble drug—to single cells *via* an external rotating field. However, more work should be performed in this area to fully utilize the benefits of microbots for precise single-cell drug targeting (*e.g.*, the targeted delivery of RNA and CRISPR-Cas9 components to diseased cells).

Another system using magnetic particles without microscale robotic locomotion used 3-aminopropyltriethoxysilane-modified SPIONs to target delivery of synthetic unmethylated cytosine-phosphate-guanine (CpG) oligodeoxynucleotides (ODNs), a TLR9 agonist[185]. CpG ODN internalization by inflammatory cells results in a significant increase in the production of chemokines and cytokines and the upregulation of costimulatory molecules on T cells[186,187]. However, CpG ODN treatment can elicit nonspecific inflammation, and it has a propensity for rapid enzymatic degradation, which may contribute to its weak clinical response[144,185]. While intratumoral injection of CpG ODNs addresses these issues to some extent, their retention within tumors remains low[188]. Therefore, a combination of magnetic targeting and receptor-ligand binding was developed to improve cellular uptake of CpG ODNs into DCs located within solid tumors *via* TLR9, which resulted in decreased tumor volume and weight in multiple tumor models as compared to free CpG ODNs, shown in Fig. 7[185]. Another group also used CpG ODNs as an immunomodulator, creating PEG-modified amine magnetic mesoporous silica nanoparticles with bound CpG ODNs for enhanced uptake by macrophages *in vivo* compared to free CpG ODNs[144].

Targeting with SPIONs has also been employed to increase RNA transfection in DCs for cancer vaccines. Grippin et al.[1,189] showed that SPIONs contained within RNA-loaded liposomes enabled a threefold higher transfection efficiency than electroporation when an external magnetic field was applied *in vitro* prior to vaccination. Efficiency increased with SPION concentration within the liposome, and this method also caused DC activation. Since a significant barrier to clinical translation of cancer vaccines is the lack of ability to predict patient responses to treatment, the authors also used MRI to track DC migration and found that increased trafficking to lymph nodes was a strong predictor of a potent antitumor response from the cancer vaccine. While combination diagnostic and therapeutic platforms are discussed in Section 3, SPIONs used in systems such as these are often successfully employed for tracking in addition to targeting.

2.3. *Magnetically deformable biomaterials*

In addition to guiding the transport of drug carriers, magnetic particles can be incorporated into biomaterials to facilitate drug release by magnetically induced structural changes. Polymer deformation and microbot actuation activated by magnetic stimuli are commonly used to enable temporal control over the release of encapsulated drugs in well-defined locations. However, the majority of these systems have been applied to release chemotherapeutic drugs. Despite being perhaps the least explored application of magnetic systems within immunotherapy, magnetically deformable biomaterials are a promising platform for immunotherapy due to their ability to minimize off-target effects and align the kinetics of drug release with the timescales of cellular activation.

2.3.1. *Magnetically responsive drug carriers*

The primary forms of magnetically deformable drug carriers are core−shell particles and ferrogels. The former has been described for targeted delivery. The latter, ferrogels, are magnetically functionalized polymers that have also been used extensively as delivery vehicles for a range of therapeutic cargo. Ferrogels are produced through dispersion of magnetic particles as a powder or fluid within a polymer matrix, most often formed into spherical particles for *in vivo* testing after validation in bulk form[190]. Methods for synthesizing ferrogel drug carriers and bulk ferrogels have been described elsewhere[191−193]. While ferrogels may also enable magnetic targeting, their primary use is for temporal control over drug release due to their ability to deform in response to a magnetic stimulus. Many of these magnetically responsive systems were developed over a decade ago; however, there have been surprisingly few attempts to apply these systems to immunotherapies, despite the critical need for improved control over the release of immunomodulatory drugs. Therefore, we will describe core−shell particle and ferrogel carriers as platforms that may be adapted to deliver immunomodulatory drugs in future work.

Gelatin-SPION sponge-like ferrogels are early examples of systems which could enable magnetic control over drug release. However, while stimuli-responsive polymers are often designed to activate drug release, Hu et al.[194] demonstrated the retardation of release kinetics from their ferrosponges when a magnetic field was turned on. Interparticle attraction due to dipole formation in response to a magnetic field caused contraction of the polymer network and shrinkage of the diffusive pathways for their model drug, cobalamin. A similar study created poly (vinyl alcohol) (PVA) ferrogels by mixing various sizes of iron oxide particles into the polymer network, with the aim of pausing drug release with magnetic stimulation and resuming release when the field was removed. When a magnetic field was turned on, diffusion of the model drug—also cobalamin—through the pores was halted and replaced by drug accumulation at the interface. After removal of the field, the PVA ferrogel pores reopened and there was a burst release of the drug due to the prior accumulation. Strength of drug burst could be tuned through magnetic particle size as well as field conditions and duration, demonstrating promise for more applications in immunotherapy[195].

A potentially more translational ferrogel was developed by Zhao et al.[196], which used the same mechanism of magnetic particle attraction within an elastic matrix, but to initiate cargo

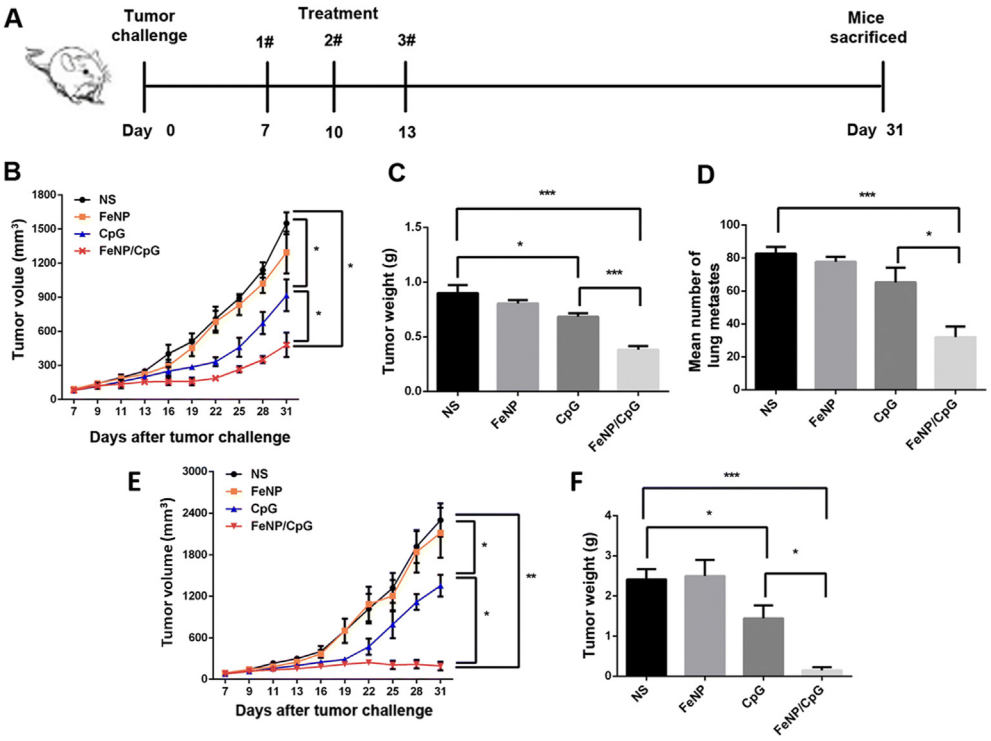


Figure 7 Magnetic targeting of CpG ODNs (CpG for convenience) to DCs increases therapeutic efficacy. (A) Schedule for treatment with iron nanoparticle (FeNP)/CpG particles in C26 colon cancer and 4T1 breast cancer-inoculated mice through intratumoral injection. (B) 4T1 tumor growth over 31 days in response to normal saline (NS), FeNP, free CpG, and targeted FeNP/CpG treatment. (C) Average 4T1 tumor weight after treatment. (D) Average number of tumorous nodules in the lung as an indication of metastasis. (E) C26 tumor growth over 31 days. (F) Average C26 tumor weight after treatment. All data mean ± SEM ($n = 10$), $^{*}P < 0.05$, $^{*}P < 0.01$, and $^{***}P < 0.001$. Reproduced with permission from Ref. 185. Copyright © 2018 Springer Nature.

release instead of interrupting it (Fig. 8A–G). The group demonstrated release of mitoxantrone (a chemotherapeutic drug), plasmid DNA, stromal cell derived-factor (SDF)-1α (a chemokine), and fibroblasts from an alginate-SPION ferrogel. The ferrogel was synthesized with millimeter to centimeter macropores to enable repeatable contraction and relaxation in response to a cyclic magnetic field, allowing cargo to escape in a pulsatile manner due to local convection within the pores. This was an early example of remote, controlled release of immunomodulatory agents *via* magnetic deformation: SDF-1α as an immunomodulator[197] and plasmid DNA as a cancer vaccine component[198]. It may be possible for immune cells, such as DCs—which are similar in size to fibroblasts—to also be released from this system[199]. Similar systems have since been developed, such as a scaled down macroporous ferrogel for implantation in mice using biphasic SPION incorporation[200], ferrogel microbeads with macropores for release of the protein ovalbumin, fluorescein isothiocyanate (FITC)-labeled dextran, and mitoxantrone (Fig. 8H–K)[201], and a ferrogel for sequential release of two different payloads[202].

Another common mechanism of on-demand drug release from ferrogels is by increased polymer permeability for quicker drug diffusion through physical disruption of the gel matrix by magnetic particles. As has been shown in many systems, the magnetic field conditions have a significant impact on the magnetic particle response[202]. As such, a high-frequency AMF was used to stimulate SPIONs bound to a chitosan matrix, resulting in bursts of variable magnitude of the model drug cobalamin[203]. Another group used ferromagnetic gold-coated cobalt nanoparticles embedded within poly (sodium styrene sulfonate)/poly (allylamine hydrochloride) microcapsules, which rotated in response to an AMF to cause distortion of the polymer chains and allow higher permeability of model macromolecules[204].

Structural disruption is also a cause of drug release in magnetic core−shell particles. For example, Liu et al.[205] prepared nanospheres with SPION cores and a shell layer of thermosensitive Pluronic F127. The SPIONs heated when exposed to a high-frequency AMF, causing the polymer shell to experience a rapid volume decrease and expel encapsulated DOX. While this system may have potential to encase and deliver immunomodulatory drugs, the resting temperature of 15 °C necessary to stabilize this polymer is significantly below body temperature, and the release temperature of 35 °C would cause a state of permanent particle collapse *in vivo*; therefore, further optimization using a different polymer type with higher contraction temperature must be used for translation of this system, which could release drugs in response to MH or local increases in temperature within the tumor microenvironment. This has since been demonstrated with a magnetic particle coated with thermosensitive poly (*N-iso*-propylacrylamide) (PNIPAM) for triggered drug release by MH[206].

Hu et al.[207] also presented a core−shell particle with an atypical structure of a silica core with a single-crystalline iron oxide shell. This configuration of magnetic moiety and polymer created an impenetrable casing to prevent premature drug release from the core. Short (60 s) exposure to a high-frequency AMF

induced vibration of the iron oxide shell and created nanoscale cracks, allowing triggered release of fluorophore as a model drug that was reversible by removing the AMF. Longer (5 min) exposure created more significant cracking for faster drug release, but this cracking was irreversible. This method in particular may be advantageous for immunotherapies due to the lack of heat generation that could denature certain payloads. While none of these systems for remotely controlled drug release have yet been applied to immunotherapies, a recent example using near-infrared PTT as the stimulus released immunomodulators *via* phase change of a polymer[208]. Therefore, there is an opportunity to combine well-characterized magnetic core−shell particles with immunomodulatory drugs.

2.3.2. *Magnetic actuation*

New types of microactuators are being rapidly developed for drug delivery. Distinct from propulsive microbots described in Section 2.2, actuation in this section refers to a motion or shape-change caused by an external magnetic field or MRI scan. There are numerous examples of fundamental work being done to improve magnetically responsive soft robots and microactuators, which may lead to increased research in the area of drug release in the coming years[209–211]. Additionally, actuation depends on mechanical changes in the system rather than temperature fluctuations to modulate polymer phases for drug release—a potential limitation for the application of thermally unstable drugs—and therefore, actuating materials have substantial versatility in the drug payload they deliver. However, despite significant interest in this area[212–214], there are few examples of magnetically responsive actuators that can release drugs on demand without the use of MH or ferrogel permeability.

Actuators can be used in high concentrations as a "swarm"[153], generally of nano- or microdevices, or used singularly as relatively large (millimeter-scale) devices. One such device was fabricated by Lee et al.[215]; the model chemotherapeutic drug 5-fluorouracil (5-FU) was released in multiple doses over several weeks using a magnetically actuated small implantable device (SID), shown in Fig. 9. The SID held the drug in solution within a barrel component, containing inlet ports to a plunger and outlet ports to the body. Magnetic attraction between the plunger and barrel prevented drug release in a resting state, but could repeatedly be opened or closed, pulling drug solution out of the barrel and expelling it from the SID, to release multiple doses of drug in a highly controlled manner with the application and removal of an

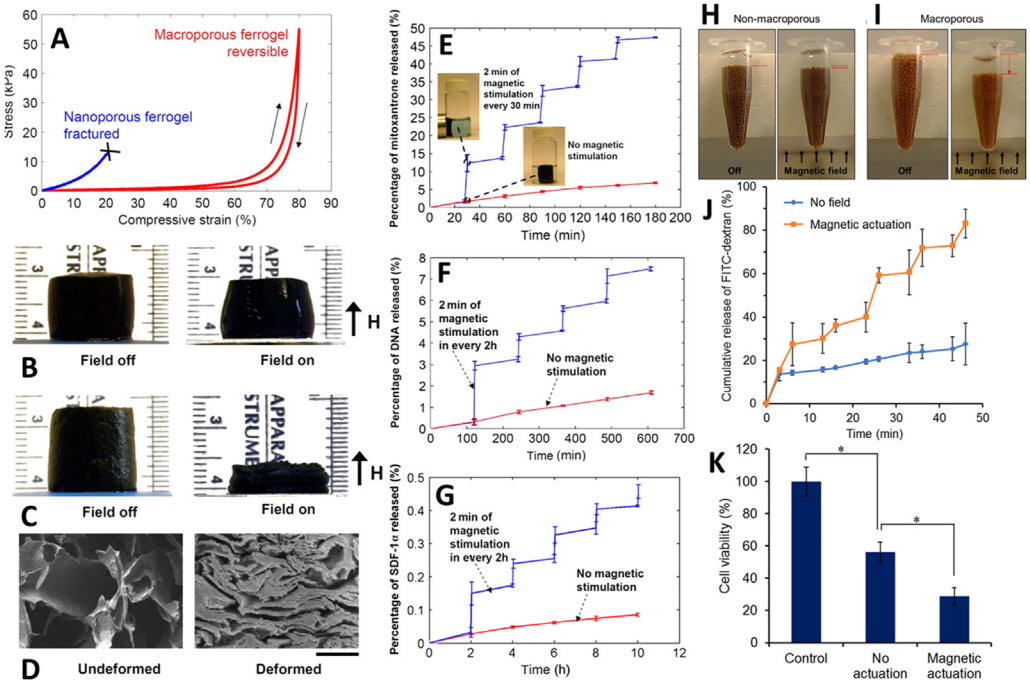


Figure 8 Macroporous alginate-SPION ferrogels enable controlled release of payloads in response to a magnetic field. (A) Nano- and macroporous ferrogel compression data. (B) A nanoporous ferrogel cylinder compresses ~5% in response to a vertical ~38 A/m^2 magnetic field gradient. (C) A macroporous ferrogel cylinder compresses ~70% in response to the same magnetic field. (D) SEM images of a lyophilized macroporous gel in an undeformed and a deformed state (scale bar = 500 μm). (E) Cumulative mitoxantrone release from the macroporous ferrogel, subject to no magnetic stimulation (control) or to 2 min of magnetic stimulation every 30 min (experimental). (F) Cumulative plasmid DNA release from the macroporous ferrogel, subject to no magnetic stimulation (control) or to 2 min of magnetic stimulation every 2 h (experimental). (G) Cumulative SDF-1α release from macroporous ferrogel, subject to the same conditions described in F. Data show mean ± SD (n = 3−5). (A)–(G) Reproduced with permission from Ref. 196. Copyright © 2011 National Academy of Science of the United State of America. (H) Non-macroporous microbeads show negligible volume change when exposed to a 500 mT magnetic field. (I) Macroporous microbeads undergo significant collective volume change due to pore collapse when exposed to the same magnetic field. (J) Cumulative dextran release from the macroporous ferrogel microbeads, subject to no magnetic stimulation (control) or to 1 min of magnetic stimulation every 10 min (experimental). (K) Viability of 4T1 tumor cells after 15 min of incubation with empty microparticles (control) or particles containing mitoxantrone without magnetic stimulation (no actuation) or with magnetic stimulation (magnetic actuation). Data show mean ± SD, *P < 0.05. (H)–(K) Reproduced with permission from Ref. 201. Copyright © 2017 American Chemical Society.

external magnet. Dosage could be altered through the number of outlet ports, number of sequential actuations, and concentration of drug in solution[215]. Pirmoradi et al.[216] created another relatively large actuating device containing drug in solution in a cylindrical microreservoir (diameter = 6 mm, depth = 550 μm), sealed on top with a SPION-polydimethylsiloxane (PDMS) ferrogel membrane over a single microaperture. Magnetic actuation of the SPIONs within the ferrogel initiated membrane deformation, creating a force of up to 1.3 mN within the reservoir under a 200 mT magnetic field, and pushing drug solution out of the aperture. This method resulted in a 10-fold increase in model drug methylene blue release compared to a resting state.

There is significant opportunity for more work on magnetically actuating materials to release immunomodulatory drugs, which has enormous potential for safer, more efficacious administration of immunotherapies. A more fundamental understanding of the interactions between actuators and the immune system must be examined as well, as very little work has yet been done in this area. Further, the long-term fate and biocompatibility of non-biodegradable actuators must be examined before clinical translation. Yasa et al.[217] found that structural locomotion parameters of magnetic helical microswimmers affected their immunogenicity; therefore, further studies should be conducted to understand the properties of other magnetic actuating and locomoting drug carriers that may elicit undesirable immune responses. Typically, biomaterials are designed to avoid recognition by immune cells, which may require tuning of structural and behavioral properties, in addition to material or surface coating. However, exploiting a proinflammatory response to actuating materials may also be a potential route of immunomodulation for cancer therapy as a type of material-based adjuvant.

2.4. *Cellular activation and manipulation*

Hyperthermia and drug targeting are the most commonly used strategies for magnetic immunotherapy, but there are other unique and innovative methods of modulating cancer immunity with magnetic fields that do not involve heat induction, magnetic targeting, or triggered drug release. Activation and manipulation of immune cells through binding or internalization of functionalized magnetic particles enables a distinct way to combat the immunosuppressive tumor microenvironment.

2.4.1. *Surface binding of magnetic particles*

Antitumoral immune responses can be induced through many different signaling pathways. For example, T cells recognize antigens presented by MHCs on the surfaces of APCs *via* binding to their T cell receptors (TCRs). Surface co-receptors CD4 or CD8 must also experience antigen-specific binding of an MHC domain, and nonspecific CD28 receptors must be bound by APC B7 costimulatory molecules to cause intracellular signaling for differentiation into either $CD4^+$ helper T cells or $CD8^+$ cytotoxic T cells and subsequent

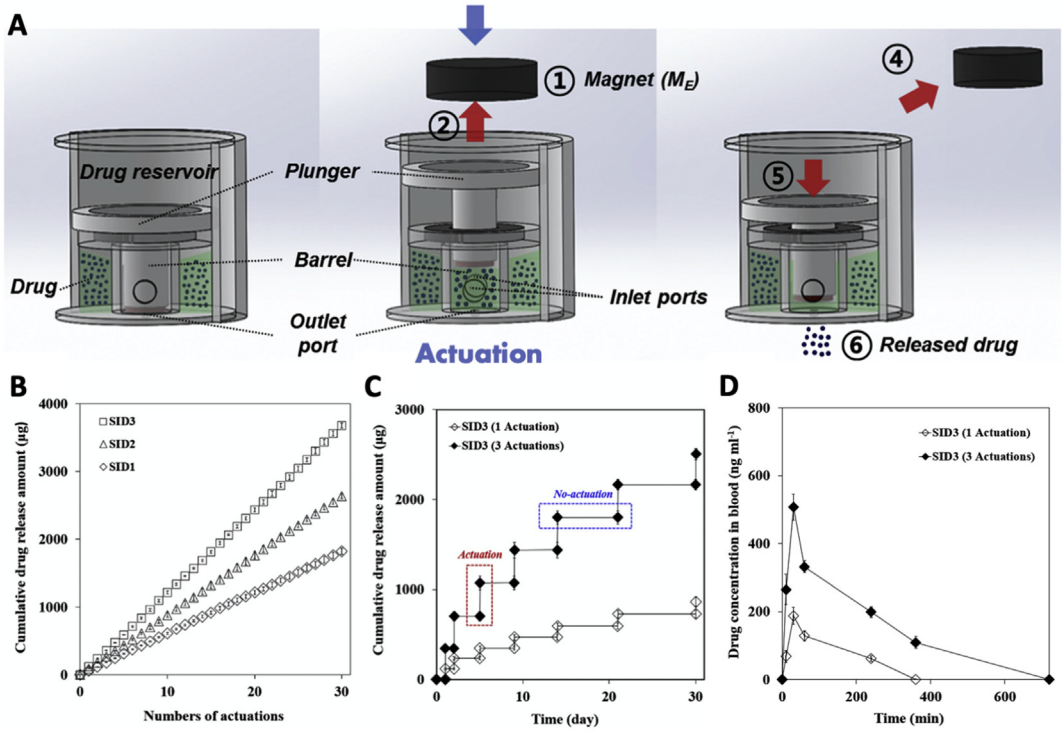


Figure 9 Small implantable device (SID) with magnetic actuation for controlled drug release. SID with an 18 mm diameter and a 13 mm height, with a total volume of ~3.3 mL. (A) Schematic illustration of SID operation. External magnet is applied, ② moving the plunger upward, and ③ sucking the drug solution into the barrel. ④ Magnet is removed, ⑤ releasing the plunger, and ⑥ pushing the drug solution out of the outlet port. The port and barrel are oppositely polarized for tight attachment and prevention of drug release without an external magnet. (B) Cumulative release of model drug, 5-fluorouracil (5-FU) in PBS *in vitro* over 30 actuations of the SID with 1, 2, or 3 outlet ports (port diameter = 700 μm). (C) Long-term cumulative release of 5-FU from SID3, with either 1 or 3 actuations at each time point. (D) 5-FU concentration in blood after SID3 implantation in a subcutaneous pocket in the dorsal area of rats with several short-term actuations. Error bars = mean ± SD. Reproduced with permission from Ref. 215. Copyright © 2018 Elsevier.

clonal expansion. In addition to their important role in combating tumor growth, activated $CD8^+$ T cells release cytokines for both autocrine and paracrine signaling to amplify the developing immune response[218]. While the immune system is extremely complex, there are also many potential routes for artificial modulation within these complex pathways; as such, functionalized magnetic particles have been used to imitate ligand binding to receptors for initiation of cellular signaling for increased stimulation of immune cells to better infiltrate and destroy immunosuppressive tumors.

Complex magnetic manipulations are often required for control of single-cell interactions such as artificial ligand binding[219]. However, Lee et al.[219] demonstrated the use of Janus particles for T cell stimulation with single-cell precision that required only handheld magnets. A thin film of nickel coating one hemisphere of the particles enabled decoupled rotation and locomotion, while anti-CD3 antibody ligands bound to the other hemisphere imparted specific TCR complex binding and subsequent activation *in vitro*. Therefore, the Janus particles could turn on cytotoxic $CD8^+$ T cells with high precision for enhanced tumor cell recognition and attack[220]. Furthermore, the ability to selectively target single cells with simplified magnetic stimuli may expedite clinical translation due to the lack of complex magnetic systems.

Two other studies examined immune cell activation for antitumor action through induced mast cell receptor and TCR clustering, which are in the same family of multi-chain immune recognition receptors[221]. Mast cell receptor clustering is caused naturally by IgE antibody−receptor complexes on the cell surface interacting with multivalent antigens, causing oligomerization and subsequent release of vesicle granules and histamines for the initiation of an antitumor proinflammatory signaling cascade[221,222]. Mannix et al.[223] exploited this mechanism by first coating SPIONs with dinitrophenyl-lysine antigen ligands for binding to IgE-receptor complexes in pretreated mast cells. Superparamagnetism of the bound nanoparticles resulted in magnetic attraction *via* dipole formation when exposed to a magnetic field, causing particle aggregation and clustering of their attached receptors for induction of an proinflammatory response. While mast cell degranulation in response to allergen antigens is a significant contributor to anaphylaxis[224], and other cell types may be better suited targets, this mechanism of induced receptor clustering is adaptable to many other cell types and receptor pathways[223]. Perica et al.[225] illustrated this through the use of functionalized SPIONs for TCR clustering. Chimeric MHC-Ig dimers (for antigen presentation signaling) and anti-CD28 antibodies (for costimulatory molecule signaling), both of which are necessary to mount a T cell response, were bound to the SPIONs. Particle aggregation *via* magnetic field exposure and subsequent TCR clustering caused increased T cell expansion both *in vitro* and *in vivo* and resulted in reduced tumor growth *in vivo*[226]. More work can be done to cause manipulations of cellular processes *via* magnetic particle binding to receptors, as this mechanism has also been demonstrated in a wide range of biomedical applications such as tissue engineering and regenerative medicine[21].

2.4.2. *Internalization of magnetic particles*

The interaction between unstimulated SPIONs and macrophages has been studied, indicating that SPION internalization may have a proinflammatory/anticancer effect; however, this response relies on nanoparticle material properties[160,227,228]. Despite cellular effects, the internalization of SPIONs can enable locomotion of immune cells using a magnetic field and is of rising interest for immunotherapy[229,230]. To this aim, Wu et al.[231] developed polydopamine-coated SPIONs for internalization by NKs. It has been demonstrated that improved NK infiltration into tumors results in improved outcomes; however, NKs cannot passively infiltrate the tumor microenvironment through the EPR effect. Therefore, NKs were guided to tumors *via* targeting of the internalized magnetic particles for improved tumor infiltration to inhibit tumor growth without affecting NK viability. Another example of internalized magnetic particles are those aimed to target and retain T lymphocytes in secondary lymphoid organs for improving adoptive T cell therapies, which often have a low proportion of administered cells reach the site of interest. Since T cells are not as phagocytotic as NKs, 3-aminopropyl-triethoxysilane-coated SPIONs often associated with T cell membranes instead of undergoing internalization. However, *in vivo* targeting to, and retention within, murine lymph nodes was successful for both internalized and surface-bound SPIONs using an external magnet[232]. This method could be expanded to deliver T cells to tumors as well. While this method of immune cell targeting may be applicable to a broad range of cell types, eventual lysosomal degradation of SPIONs in macrophages has been demonstrated and may be a limiting factor for other types of cells[233].

Enhancing immune cell trafficking to target locations within the body can improve cellular activation or education. Furthermore, some groups have integrated a therapeutic agent to the system, using immune cells as drug carriers in addition to enhancing their cellular transport. Numerous examples of this strategy exist across immune cell types, such as internalization of magnetic nanoparticles loaded with antigen peptide by DCs for increased migration to the lymph nodes as a cancer vaccine[234] and magnetically labeled macrophages carrying an oncolytic virus for infiltrating tumors to reduce tumor growth and metastasis[235]. Low toxicity and few reported effects on natural cell functioning make internalization of magnetic particles for cellular trafficking an exciting area of continued study[236].

3. Theranostics

Theranostics describes technologies armed with both diagnostic and therapeutic capabilities. Magnetic particles are increasingly used for theranostics of cancer, as treatment often depends heavily on disease progression as well as tumor location, size, metastasis, and overall immune status[237]. While other reviews characterize theranostics with more detail[25,237−243], the field cannot be overlooked in a discussion of magnetic innovations for drug delivery and cancer treatment.

Magnetic particle imaging (MPI) and MRI are the primary methods used for imaging magnetic nanoparticles, and both have been used for tracking the biodistribution of immunomodulatory drugs. For instance, injected magnetic particles or drug carriers loaded with magnetic particles can be taken up by phagocytes and monitored as they traffic to tumors, lymph nodes[189], or other inflammatory sites[244,245]. These innovations are crucial for developing a deeper understanding of immunotherapies and increasing their clinical use; however, they are not the focus of theranostics. Most often, cancer theranostics takes the form of monitoring tumor prognosis during the course of targeted drug or MH treatment.

3.1. *Magnetic resonance imaging*

MRI is a noninvasive anatomical imaging technique that has been used clinically to study tissue for many years. In response to a

uniform magnetic field, MRI detects relaxation of endogenous cell nuclei magnetic moments[237]. This method is commonly enhanced in clinical settings through intravenous injection of paramagnetic contrast agents, the most common of which are gadolinium-based[246]. Protons from water molecules near the studied tissue undergo relaxation in response to magnetic field stimulation of the contrast agent, resulting in a stronger signal for detection[237,247]. MRI agents move passively in the blood and are retained within tumors longer than healthy tissue due to the characteristic hypervascularity and perfusion of solid tumors. Therefore, for cancer diagnosis, substantial knowledge can be gleaned from scanning or actively monitoring the accumulation and clearance of contrast agents within tumors. While several SPION agents such as ferumoxytol, ferumoxide, and ferucarbotran have been approved for clinical trials, none are used clinically as MRI contrast agents[247]; however, imparting more control over contrast agents, such as through active targeting, has been a recent area of interest[247]. For this, SPIONs have been studied heavily, with the additional advantage of the ability to perform therapy in combination with MRI. Furthermore, patients with chronic kidney disease cannot safely excrete gadolinium, demonstrating the need for a larger pool of MRI contrast agents[248].

Several of the therapeutic mechanisms described so far are compatible with MRI, the most common of which is MH. Since MRI and MH are well-characterized, many recent studies have focused on the design of particle systems that are optimally functional for each, such as dual-ligand and magnetic tumor-targeting SPIONs, which were shown to increase tumor penetration and accumulation[249], tetramethylammonium hydroxide-coated nickel ferrite particles with highly efficient heating and imaging contrast[250], dendrimer-functionalized SPIONs with superior MH characteristics and MRI contrast enhancement[251], and uniform peptide-bound active targeting SPIONs for improved intratumoral delivery, optimized for MH, MRI, and MPI[252]. Other MRI-theranostic modalities use magnetic targeting of SPIONs for delivery of CpG ODNs to tumors[253] and redox-mediated release and transfection of siRNA for silencing of human telomerase reverse transcriptase genes from SPION carriers to hepatocellular carcinoma cells[254], while acting as contrast agent for MRI. Many studies similarly first engineer a magnetic drug carrier and then demonstrate the ability of the system to also function for MRI. As expected, there are numerous examples of dual contrast agent-drug carriers delivering chemotherapeutic drugs[255–257], but their application to immunotherapy is likely to increase with time.

3.2. *Magnetic particle imaging*

MPI is a recently developed preclinical molecular imaging technique, first described in 2005 by Gleich and Weizenecker[258]. SPIONs are injected as magnetic tracers and are saturated by gradient magnetic fields, except for within a field-free region (FFR) created by their intersection. Magnetic materials within the FFR exhibit measurable harmonics, which enables creation of a topographic image *via* movement of the FFR across the region of interest. This method has sub-millimeter spatial resolution and high sensitivity. It has been used for applications such as cell tracking, lung perfusion, and cancer imaging since its development[259]. For cancer theranostics, the FFR size can be changed by altering the strength of the magnetic field gradient, and therefore it can be spatially tuned to the tumor area for enhanced imaging or treatment[260].

When MPI is applied to theranostics, MH is used almost exclusively as the therapeutic modality[252,260–263]. Several studies have shown the improvement of spatial heating specificity with MPI fields instead of an AMF, preventing damage to clearance organs such as the liver and spleen during tumor MH treatment due to SPION saturation[259,264,265]. Fig. 10 shows the use of MPI fields for precise *in vivo* tumor treatment, focused to a region of 4 mm[264]. Surface coils traditionally used for MH treatment localize magnetic excitation with decreasing specificity at deeper locations in the body, which may result in healthy tissue damage. Conversely, MPI gradient fields can selectively suppress SPION activity, resulting in significantly less off-target heating and lower toxicity[264]. Furthermore, MPI and MH can be performed simultaneously for live imaging of treatment as well as prediction of the MH thermal dose[259,264]. This technology is of great interest due to its advancements in precision upon imaging and MH treatment. Additionally, it has significantly higher resolution than luminescent imaging strategies such as *in vivo* imaging systems (IVIS), with the ability to accurately quantify cell number *in vivo*[248].

Beyond these initial examples, few other studies have demonstrated the use of MPI in theranostics. One such study by Jung et al.[266] developed a unique system of exosomes isolated from human breast cancer cells and loaded with SPIONs and a poly (adenosine diphosphate-ribose) polymerase (PARP) inhibitor. Exosomes were preferentially taken up by hypoxic cells, which arise in tumors through outgrowing the vascular oxygen supply, which is often an indicator of poor prognosis. In response to the engineered exosomes, tumor growth was inhibited, and MPI was successfully demonstrated *in vivo*[267]. Other studies include the binding of a ligand to magnetic-gold core−shell particles for active targeting and MPI[268] and magnetic drug carriers for MPI loaded with DOX[269], both of which have potential for application to immunotherapies. Overall, MPI is an extremely promising area of theranostics with significant room for continued growth in MH and other immunotherapies, resolving many of the issues with off-target effects often associated with systemic immunotherapy. While preclinical MPI scanners for small animals are in use, continued work developing larger scanners for human use are necessary for future clinical adaptation[102].

4. Considerations for clinical translation

Nanomedicine is a steadily growing field with a range of products in development, in clinical trials, and in use commercially[270]. However, despite the success of magnetic nanomaterials *in vitro* and *in vivo*, there remains a significant lag in their clinical translation. SPIONs are the most likely candidate for clinical adoption in immunotherapies, due to the FDA approval of several iron oxide nanoparticles for use in applications other than cancer treatment or imaging[271]. MH is the most well-studied magnetic system in preclinical investigations, however, control over the biodistribution of administered drugs using magnetic targeting may be the most clinically relevant technology due to its ability to be used with approved drugs and applied to existing delivery systems for reduced off-target effects. However, the many improvements upon spatiotemporal control that systems such as active targeting and responsive biomaterials have made upon free drug delivery are not yet sufficient to eliminate concerns of magnetic particle toxicity due to functionalization, concentration, unintended bioactivity[271], or lack of efficacy due to cancer-specific and patient-specific responses.

While SPIONs are generally biocompatible[102,271–274], toxicity and biodistribution of each new system must be evaluated prior to clinical testing due to their variable size, magnetic content, and surface coatings[271,272]. These parameters, which are often carefully tuned to elicit the desired magnetic response, make it difficult to establish universal toxicity limits. Common coating types include polymers, silicates, and biological or organic molecules, which can result in increased circulation time and the ability for controlled drug release[176,178,272]. SPIONs lacking surface coatings often experience aggregation, absorption of plasma proteins, and rapid clearance from the bloodstream[273,275]. Coated SPIONs, therefore, are generally preferred for *in vivo* applications[272]. However, the use of coated SPIONs or magnetic moieties embedded within larger polymer vehicles (*e.g.*, ferrogels) complicates the ability to analyze systemic toxicity. Additional obstacles for using coated or embedded SPIONs include toxicity from residual catalysts used for covalent drug binding[272], reduced saturation magnetization by non-magnetic materials[276], and the potential for coating or vehicle materials to break down less easily than SPIONs. Several studies have shown that SPIONs can safely degrade in the lysosomes of macrophages[273] and that iron can be taken up by hemoglobin[277,278] or excreted through the endogenous iron metabolic pathway[279], depending on their surface coatings[102]; this is in contrast to the limited biodegradability and elimination of other common drug delivery systems such as liposomes or micelles[272].

Despite the complications associated with evaluating the safety of magnetic materials on a universal basis, toxicity studies have been performed on several types of SPIONs. In hamsters, the median lethal dose ranged from 300 to 600 mg_{Fe}/kg body weight (BW) for uncoated SPIONs and 2000−6000 mg_{Fe}/kg BW for dextran-coated SPIONs[280]; however, the median lethal dose may no longer be the best measure of toxicity, which is discussed elsewhere[281]. Non-cytotoxicity has also been demonstrated for uncoated SPIONs at concentrations under 100 μg/mL[282]. While issues such as cell cycle arrest and autophagy-mediated cytotoxicity have been observed in response to SPION treatment at high concentrations[274,283], there is a lack of toxicity at the quantities required for most therapeutic applications. Certain SPIONs in clinical trials for use as MRI agents have prompted dosage limits from 0.6 to 4.0 mg_{Fe}/kg BW, with half-life values from 0.5 to 30 h[102], which varied greatly with size and coating type. Biodistribution studies have shown that untargeted SPIONs primarily accumulate in the liver and spleen after intravenous injection prior to organ clearance and degradation[273,284]. While the short-term toxicity of iron oxides and long-term degradation and clearance is well-studied, other long-term effects such as iron imbalance or cell cycle regulation warrant further investigation[102,117].

Theranostics may serve as another route for magnetic materials to reach the clinic as imaging has played an increasingly important role in clinical oncology in recent years[237]. Gaining approval of SPIONs for MH and imaging that have been previously approved

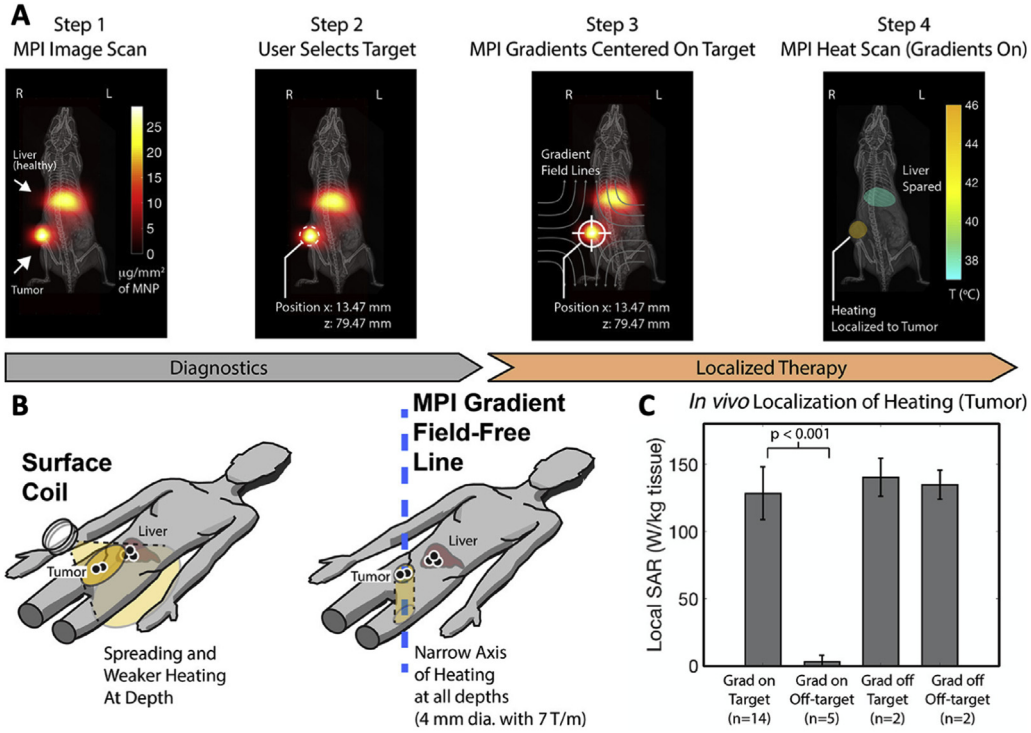


Figure 10 MPI gradient field improves specificity of MH *in vivo*. (A) 20 kHz, 20 mT MPI fields enable high contrast visualization of SPIONs in the tumor and liver of a U87MG xenograft mouse (diagnosis). The tumor is selected for localization of MH treatment by centering the FFR on the tumor target (therapy). MH conducted at 354 kHz, 13 mT heats the tumor only with the maintenance of MPI gradients. (B) Schematic illustration of the field created by coils traditionally used for MH treatment compared to the highly precise MPI gradient field. (C) Specific absorption rate (SAR) of SPIONs in the tumor is negligible when the MPI gradient is off. Reproduced with permission from Ref. 264. Copyright © 2018 American Chemical Society.

for other uses, such as anemia treatment, may accelerate this process. Similarly, combination therapy of established drugs and MH may allow for integration of magnetic materials into clinical use. Application of existing clinical technologies in magnetic therapies, such as MRI scanners, may help to accelerate clinical adoption of magnetic materials due to ease of use. While the advancements of MPI for MH and imaging may be more clinically beneficial than MRI due to higher precision and reduced off-target effects, adoption of both the magnetic stimulus and material may be difficult due to the extent of testing necessary and the cost of implementation. While there is much work to be done to move magnetic technologies into broader clinical use for immunotherapy, the potential for improving patient outcomes is clear.

5. Conclusions and future perspectives

Immunotherapy has strikingly positive outcomes for a subset of patients, but many patients or cancer types have little responsiveness to certain treatments[8]. Combatting immunosuppression by tumors and amplifying anti-tumor immunity while avoiding immune overstimulation or the generation of a cytokine storm are among the most critical challenges in immunotherapy[285]. As such, immunotherapy necessitates precise administration and spatiotemporal control to generate potent antitumoral responses without off-target effects. The intersection of magnetics and immunotherapy is uniquely positioned to fill this gap through the precision, versatility, and biocompatibility of magnetic materials. As the field of immunotherapy continues to come of age and our understanding of new clinical targets and treatment pathways increases, there is significant opportunity to apply existing magnetic systems to novel immunomodulatory drugs in place of chemotherapeutic drugs. Additionally, the use of SPIONs and other magnetic particles provides the opportunity for exploitation of multifunctionality through simultaneous spatial targeting, timed release, MH treatment, and imaging with MRI or MPI.

While the potential of magnetic systems to improve cancer immunotherapies is immense, there remain significant barriers both to fundamental advancements and clinical adoption of these techniques. In particular, poor stability of some immunomodulatory drugs may limit their incorporation into delivery vehicles due to the use of harsh solvents during fabrication or thermal instability. Furthermore, the toxicity and clearance of magnetic particles vary greatly with properties such as size, coating type, and magnetization, which prevents universal dosing standards and necessitates case-by-case decisions. Use of predicate technologies and methodologies (*e.g.*, approved immunotherapies, biocompatible polymer coatings, commonly used magnetic stimuli) is the most promising approach for expediting the introduction of magnetic materials into the clinic.

While many studies involving magnetic systems use tumor burden as a standalone metric for success, increased attention should be given to considerations such as biodistribution and toxicity of magnetic materials, metabolism, immune response, as well as composition and phenotype of the tumor immune microenvironment. Greater understanding of these properties will provide metrics that are currently lacking for comparison against traditional techniques, such as radiotherapy and chemotherapy. In order to continue developing these systems for clinical applications, there must be compelling data that can report both their improvement upon currently used techniques and also demonstrate thorough characterization of the materials. Then, informed decisions on treatment can be made specifically for individual patient and tumor types. By addressing these gaps in knowledge over time, research in magnetic drug delivery systems hold promise for the advancement of cancer immunotherapy.

Author contributions

Nicole B. Day and C. Wyatt Shields IV conceived the manuscript. Nicole B. Day and William C. Wixson wrote the manuscript. Nicole B. Day, William C. Wixson, and C. Wyatt Shields IV revised the manuscript. All of the authors have read and approved the final manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

References

1. Grippin AJ, Sayour EJ, Mitchell DA. Translational nanoparticle engineering for cancer vaccines. *OncoImmunology* 2017;**6**. e1290036.
2. Sui X, Jin T, Liu T, Wu S, Wu Y, Tang Z, et al. Tumor immune microenvironments (TIMEs): responsive nanoplatforms for antitumor immunotherapy. *Front Chem* 2020;**8**:804.
3. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. *N Engl J Med* 2016;**375**:1823−33.
4. Waldman AD, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: from T cell basic science to clinical practice. *Nat Rev Immunol* 2020;**20**:651−68.
5. Sarfaty M, Hall PS, Chan KK, Virik K, Leshno M, Gordon N, et al. Cost-effectiveness of pembrolizumab in second-line advanced bladder cancer. *Eur Urol* 2018;**74**:57−62.
6. Verma V, Sprave T, Haque W, Simone II CB, Chang JY, Welsh JW, et al. A systematic review of the cost and cost-effectiveness studies of immune checkpoint inhibitors. *J Immunother Cancer* 2018;**6**:128.
7. Tartari F, Santoni M, Burattini L, Mazzanti P, Onofri A, Berardi R. Economic sustainability of anti-PD-1 agents nivolumab and pembrolizumab in cancer patients: recent insights and future challenges. *Cancer Treat Rev* 2016;**48**:20−4.
8. Sharma P, Allison JP. The future of immune checkpoint therapy. *Science* 2015;**348**:56−61.
9. Remon J, Besse B, Soria JC. Successes and failures: what did we learn from recent first-line treatment immunotherapy trials in non-small cell lung cancer?. *BMC Med* 2017;**15**:55.
10. Wang S, Sun Z, Hou Y. Engineering nanoparticles toward the modulation of emerging cancer immunotherapy. *Adv Healthc Mater* 2021;**10**:2000845.
11. El-Sawy HS, Al-Abd AM, Ahmed TA, El-Say KM, Torchilin VP. Stimuli-responsive nano-architecture drug-delivery systems to solid tumor micromilieu: past, present, and future perspectives. *ACS Nano* 2018;**12**:10636−64.
12. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 2010;**363**:711−23.
13. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med* 2012;**366**:2455−65.
14. Liu J, Zhang R, Xu ZP. Nanoparticle-based nanomedicines to promote cancer immunotherapy: recent advances and future directions. *Small* 2019;**15**:1900262.

15. Goldberg MS. Improving cancer immunotherapy through nanotechnology. *Nat Rev Cancer* 2019;**19**:587−602.
16. Gamboa L, Zamat AH, Kwong GA. Synthetic immunity by remote control. *Theranostics* 2020;**10**:3652−67.
17. Oupicky D, Bisht HS, Manickam DS, Zhou QH. Stimulus-controlled delivery of drugs and genes. *Expet Opin Drug Deliv* 2005;**2**:653−65.
18. Sun T, Dasgupta A, Zhao Z, Nurunnabi M, Mitragotri S. Physical triggering strategies for drug delivery. *Adv Drug Deliv Rev* 2020;**158**: 36−62.
19. Kim Y, Choi H, Shin JE, Bae G, Thangam R, Kang H. Remote active control of nanoengineered materials for dynamic nanobiomedical engineering. *View* 2020;**1**:20200029.
20. Markides H, Rotherham M, El Haj AJ. Biocompatibility and toxicity of magnetic nanoparticles in regenerative medicine. *J Nanomater* 2012;**2012**:614094.
21. Dobson J. Remote control of cellular behaviour with magnetic nanoparticles. *Nat Nanotechnol* 2008;**3**:139−43.
22. Kozissnik B, Bohorquez AC, Dobson J, Rinaldi C. Magnetic fluid hyperthermia: advances, challenges, and opportunity. *Int J Hyperther* 2013;**29**:706−14.
23. Marincek B. Diagnostic improvement in MRI of gynecological neoplasms. *J Belge Radiol* 1996;**79**:13−7.
24. El-Sherbiny IM, Elbaz NM, Sedki M, Elgammal A, Yacoub MH. Magnetic nanoparticles-based drug and gene delivery systems for the treatment of pulmonary diseases. *Nanomedicine* 2017;**12**:387−402.
25. El-Boubbou K. Magnetic iron oxide nanoparticles as drug carriers: clinical relevance. *Nanomedicine* 2018;**13**:953−71.
26. Stepien G, Moros M, Pérez-Hernández M, Monge M, Gutiérrez L, Fratila RM, et al. Effect of surface chemistry and associated protein corona on the long-term biodegradation of iron oxide nanoparticles *in vivo*. *ACS Appl Mater Interfaces* 2018;**10**:4548−60.
27. Assa F, Jafarizadeh-Malmiri H, Ajamein H, Vaghari H, Anarjan N, Ahmadi O, et al. Chitosan magnetic nanoparticles for drug delivery systems. *Crit Rev Biotechnol* 2017;**37**:492−509.
28. Papageorgiou I, Brown C, Schins R, Singh S, Newson R, Davis S, et al. The effect of nano- and micron-sized particles of cobalt-chromium alloy on human fibroblasts *in vitro*. *Biomaterials* 2007; **28**:2946−58.
29. Klostergaard J, Seeney CE. Magnetic nanovectors for drug delivery. *Nanomedicine* 2012;**8 Suppl 1**:S37−50.
30. Ding B, Zheng P, Ma P, Lin J. Manganese oxide nanomaterials: synthesis, properties, and theranostic applications. *Adv Mater* 2020; **32**:1905823.
31. Dulińska-Litewka J, Łazarczyk A, Hałubiec P, Szafrański O, Karnas K, Karewicz A. Superparamagnetic iron oxide nanoparticles—current and prospective medical applications. *Materials* 2019;**12**:617.
32. Maldonado-Camargo L, Unni M, Rinaldi C. Magnetic characterization of iron oxide nanoparticles for biomedical applications. In: Petrosko SH, Day ES, editors. *Biomedical nanotechnology.* New York: Humana Press; 2017. p. 47−71.
33. Nikitin A, Khramtsov M, Garanina A, Mogilnikov P, Sviridenkova N, Shchetinin I, et al. Synthesis of iron oxide nanorods for enhanced magnetic hyperthermia. *J Magn Magn Mater* 2019;**469**:443−9.
34. Lemal P, Balog S, Geers C, Taladriz-Blanco P, Palumbo A, Hirt AM, et al. Heating behavior of magnetic iron oxide nanoparticles at clinically relevant concentration. *J Magn Magn Mater* 2019;**474**: 637−42.
35. Cho NH, Cheong TC, Min JH, Wu JH, Lee SJ, Kim D, et al. A multifunctional core−shell nanoparticle for dendritic cell-based cancer immunotherapy. *Nat Nanotechnol* 2011;**6**:675−82.
36. Veiseh O, Gunn JW, Zhang M. Design and fabrication of magnetic nanoparticles for targeted drug delivery and imaging. *Adv Drug Deliv Rev* 2010;**62**:284−304.
37. Anderson SD, Gwenin VV, Gwenin CD. Magnetic functionalized nanoparticles for biomedical, drug delivery and imaging applications. *Nanoscale Res Lett* 2019;**14**:188.
38. Kudr J, Haddad Y, Richtera L, Heger Z, Cernak M, Adam V, et al. Magnetic nanoparticles: from design and synthesis to real world applications. *Nanomaterials* 2017;**7**:243.
39. Feng Q, Liu Y, Huang J, Chen K, Huang J, Xiao K. Uptake, distribution, clearance, and toxicity of iron oxide nanoparticles with different sizes and coatings. *Sci Rep* 2018;**8**:2082.
40. Barrera C, Herrera A, Zayas Y, Rinaldi C. Surface modification of magnetite nanoparticles for biomedical applications. *J Magn Magn Mater* 2009;**321**:1397−9.
41. Wu W, Wu Z, Yu T, Jiang C, Kim WS. Recent progress on magnetic iron oxide nanoparticles: synthesis, surface functional strategies and biomedical applications. *Sci Technol Adv Mater* 2015;**16**. 023501.
42. Shaterabadi Z, Nabiyouni G, Soleymani M. Physics responsible for heating efficiency and self-controlled temperature rise of magnetic nanoparticles in magnetic hyperthermia therapy. *Prog Biophys Mol Biol* 2018;**133**:9−19.
43. Gilchrist RK, Gilchrist RK, Medal R, Shorey WD, Hanselman RC, Parrott JC, et al. Selective inductive heating of lymph nodes. *Ann Surg* 1957;**146**:596−606.
44. Cazares-Cortes E, Cabana S, Boitard C, Nehlig E, Griffete N, Fresnais J, et al. Recent insights in magnetic hyperthermia: from the "hot-spot" effect for local delivery to combined magneto-photothermia using magneto-plasmonic hybrids. *Adv Drug Deliv Rev* 2019;**138**:233−46.
45. Dutz S, Hergt R. Magnetic nanoparticle heating and heat transfer on a microscale: basic principles, realities and physical limitations of hyperthermia for tumour therapy. *Int J Hyperther* 2013;**29**:790−800.
46. Huang H, Delikanli S, Zeng H, Ferkey DM, Pralle A. Remote control of ion channels and neurons through magnetic-field heating of nanoparticles. *Nat Nanotechnol* 2010;**5**:602−6.
47. Grillone A, Ciofani G. Magnetic nanotransducers in biomedicine. *Chem Eur J* 2017;**23**:16109−14.
48. Rivera-Rodriguez A, Chiu-Lam A, Morozov VM, Ishov AM, Rinaldi C. Magnetic nanoparticle hyperthermia potentiates paclitaxel activity in sensitive and resistant breast cancer cells. *Int J Nanomed* 2018;**13**:4771−9.
49. Obaidat I, Issa B, Haik Y. Magnetic properties of magnetic nanoparticles for efficient hyperthermia. *Nanomaterials* 2015;**5**:63−89.
50. Jun YW, Lee JH, Cheon J. Chemical design of nanoparticle probes for high-performance magnetic resonance imaging. *Angew Chem Int Ed* 2008;**47**:5122−35.
51. Mahmoudi K, Bouras A, Bozec D, Ivkov R, Hadjipanayis C. Magnetic hyperthermia therapy for the treatment of glioblastoma: a review of the therapy's history, efficacy and application in humans. *Int J Hyperther* 2018;**34**:1316−28.
52. Engelmann UM, Seifert J, Mues B, Roitsch S, Ménager C, Schmidt AM, et al. Heating efficiency of magnetic nanoparticles decreases with gradual immobilization in hydrogels. *J Magn Magn Mater* 2019;**471**:486−94.
53. Issa B, Obaidat IM, Albiss BA, Haik Y. Magnetic nanoparticles: surface effects and properties related to biomedicine applications. *Int J Mol Sci* 2013;**14**:21266−305.
54. He S, Zhang H, Liu Y, Sun F, Yu X, Li X, et al. Maximizing specific loss power for magnetic hyperthermia by hard−soft mixed ferrites. *Small* 2018;**14**:1800135.
55. Frey B, Weiss EM, Rubner Y, Wunderlich R, Ott OJ, Sauer R, et al. Old and new facts about hyperthermia-induced modulations of the immune system. *Int J Hyperther* 2012;**28**:528−42.
56. Toraya-Brown S, Fiering S. Local tumour hyperthermia as immunotherapy for metastatic cancer. *Int J Hyperther* 2014;**30**:531−9.
57. Ito A, Honda H, Kobayashi T. Cancer immunotherapy based on intracellular hyperthermia using magnetite nanoparticles: a novel concept of "heat-controlled necrosis" with heat shock protein expression. *Cancer Immunol Immunother* 2006;**55**:320−8.
58. Liu X, Zhang Y, Wang Y, Zhu W, Li G, Ma X, et al. Comprehensive understanding of magnetic hyperthermia for improving antitumor therapeutic efficacy. *Theranostics* 2020;**10**:3793−815.

59. Saeed M, Ren W, Wu A. Therapeutic applications of iron oxide based nanoparticles in cancer: basic concepts and recent advances. *Biomater Sci* 2018;**6**:708−25.
60. Hildebrandt B, Wust P, Ahlers O, Dieing A, Kerner T, Felix R, et al. The cellular and molecular basis of hyperthermia. *Crit Rev Oncol Hematol* 2002;**43**:33−56.
61. Spirou SV, Basini M, Lascialfari A, Sangregorio C, Innocenti C. Magnetic hyperthermia and radiation therapy: radiobiological principles and current practice. *Nanomaterials* 2018;**8**:401.
62. Hurwitz MD. Hyperthermia and immunotherapy: clinical opportunities. *Int J Hyperther* 2019;**36**:4−9.
63. Curley SA. Radiofrequency ablation of malignant liver tumors. *Oncol* 2001;**6**:14−23.
64. Brace C. Thermal tumor ablation in clinical use. *IEEE Pulse* 2011;**2**: 28−38.
65. Dudek AM, Garg AD, Krysko DV, De Ruysscher D, Agostinis P. Inducers of immunogenic cancer cell death. *Cytokine Growth Factor Rev* 2013;**24**:319−33.
66. Galluzzi L, Buqué A, Kepp O, Zitvogel L, Kroemer G. Immunogenic cell death in cancer and infectious disease. *Nat Rev Immunol* 2017; **17**:97−111.
67. Adkins I, Sadilkova L, Hradilova N, Tomala J, Kovar M, Spisek R. Severe, but not mild heat-shock treatment induces immunogenic cell death in cancer cells. *OncoImmunology* 2017;**6**. e1311433.
68. Lepock JR. Cellular effects of hyperthermia: relevance to the minimum dose for thermal damage. *Int J Hyperther* 2003;**19**:252−66.
69. Harmon BV, Corder AM, Collins RJ, Gobé GC, Allen J, Allan DJ, et al. Cell death induced in a murine mastocytoma by 42–47 °C heating *in vitro*: evidence that the form of death changes from apoptosis to necrosis above a critical heat load. *Int J Radiat Biol* 1990;**58**:845−58.
70. Li Z, Deng J, Sun J, Ma Y. Hyperthermia targeting the tumor microenvironment facilitates immune checkpoint inhibitors. *Front Immunol* 2020;**11**:595207.
71. Srivastava P. Interaction of heat shock proteins with peptides and antigen presenting cells: chaperoning of the innate and adaptive immune responses. *Annu Rev Immunol* 2002;**20**:395−425.
72. Ito A, Shinkai M, Honda H, Yoshikawa K, Saga S, Wakabayashi T, et al. Heat shock protein 70 expression induces antitumor immunity during intracellular hyperthermia using magnetite nanoparticles. *Cancer Immunol Immunother* 2003;**52**:80−8.
73. Suzue K, Zhou X, Eisen HN, Young RA. Heat shock fusion proteins as vehicles for antigen delivery into the major histocompatibility complex class I presentation pathway. *Proc Natl Acad Sci U S A* 1997;**94**:13146−51.
74. Asea A, Rehli M, Kabingu E, Boch JA, Baré O, Auron PE, et al. Novel signal transduction pathway utilized by extracellular HSP70: role of toll-like receptor (TLR) 2 and TLR4. *J Biol Chem* 2002;**277**: 15028−34.
75. Vabulas RM, Ahmad-Nejad P, Ghose S, Kirschning CJ, Issels RD, Wagner H. HSP70 as endogenous stimulus of the toll/interleukin-1 receptor signal pathway. *J Biol Chem* 2002;**277**:15107−12.
76. Multhoff G, Botzler C, Jennen L, Schmidt J, Ellwart J, Issels R. Heat shock protein 72 on tumor cells: a recognition structure for natural killer cells. *J Immunol* 1997;**158**:4341−50.
77. Tanaka K, Ito A, Kobayashi T, Kawamura T, Shimada S, Matsumoto K, et al. Intratumoral injection of immature dendritic cells enhances antitumor effect of hyperthermia using magnetic nanoparticles. *Int J Cancer* 2005;**116**:624−33.
78. Subjeck JR, Sciandra JJ, Chao CF, Johnson RJ. Heat shock proteins and biological response to hyperthermia. *Br J Cancer Suppl* 1982;**5**: 127−31.
79. Subjeck JR, Sciandra JJ, Johnson RJ. Heat shock proteins and thermotolerance; a comparison of induction kinetics. *Br J Radiol* 1982; **55**:579−84.
80. Duff GW, Durum SK. Fever and immunoregulation: hyperthermia, interleukins 1 and 2, and T-cell proliferation. *Yale J Biol Med* 1982; **55**:437−42.
81. Ostberg JR, Repasky EA. Emerging evidence indicates that physiologically relevant thermal stress regulates dendritic cell function. *Cancer Immunol Immunother* 2006;**55**:292−8.
82. Mace TA, Zhong L, Kokolus KM, Repasky EA. Effector $CD8^+$ T cell IFN-γ production and cytotoxicity are enhanced by mild hyperthermia. *Int J Hyperther* 2012;**28**:9−18.
83. Zheng H, Benjamin IJ, Basu S, Li Z. Heat shock factor 1-independent activation of dendritic cells by heat shock: implication for the uncoupling of heat-mediated immunoregulation from the heat shock response. *Eur J Immunol* 2003;**33**:1754−62.
84. Pritchard MT, Li Z, Repasky EA. Nitric oxide production is regulated by fever-range thermal stimulation of murine macrophages. *J Leukoc Biol* 2005;**78**:630−8.
85. Kalber T, Ordidge KL, Southern P, Loebinger MR, Kyrtatos PG, Pankhurst QA, et al. Hyperthermia treatment of tumors by mesenchymal stem cell-delivered superparamagnetic iron oxide nanoparticles. *Int J Nanomed* 2016;**11**:1973−83.
86. Ito A, Tanaka K, Kondo K, Shinkai M, Honda H, Matsumoto K, et al. Tumor regression by combined immunotherapy and hyperthermia using magnetic nanoparticles in an experimental subcutaneous murine melanoma. *Cancer Sci* 2003;**94**:308−13.
87. Cornel AM, Mimpen IL, Nierkens S. MHC Class I downregulation in cancer: underlying mechanisms and potential targets for cancer immunotherapy. *Cancers* 2020;**12**:1760.
88. Ostberg JR, Dayanc BE, Yuan M, Oflazoglu E, Repasky EA. Enhancement of natural killer (NK) cell cytotoxicity by fever-range thermal stress is dependent on NKG2D function and is associated with plasma membrane NKG2D clustering and increased expression of MICA on target cells. *J Leukoc Biol* 2007;**82**:1322−31.
89. Chen T, Guo J, Yang M, Zhu X, Cao X. Chemokine-containing exosomes are released from heat-stressed tumor cells *via* lipid raft-dependent pathway and act as efficient tumor vaccine. *J Immunol* 2011;**186**:2219−28.
90. Dai S, Wan T, Wang B, Zhou X, Xiu F, Chen T, et al. More efficient induction of HLA-A*0201-restricted and carcinoembryonic antigen (CEA)-Specific CTL response by immunization with exosomes prepared from heat-stressed CEA-positive tumor cells. *Clin Cancer Res* 2005;**11**:7554−63.
91. de Jong OG, Verhaar MC, Chen Y, Vader P, Gremmels H, Posthuma G, et al. Cellular stress conditions are reflected in the protein and RNA content of endothelial cell-derived exosomes. *J Extracell Vesicles* 2012;**1**:18396.
92. Yang Y, Chen Y, Zhang F, Zhao Q, Zhong H. Increased anti-tumour activity by exosomes derived from doxorubicin-treated tumour cells *via* heat stress. *Int J Hyperther* 2015;**31**:498−506.
93. King HW, Michael MZ, Gleadle JM. Hypoxic enhancement of exosome release by breast cancer cells. *BMC Cancer* 2012;**12**:421.
94. Lancaster GI, Febbraio MA. Exosome-dependent trafficking of HSP70: a novel secretory pathway for cellular stress proteins. *J Biol Chem* 2005;**280**:23349−55.
95. Guo D, Chen Y, Wang S, Yu L, Shen Y, Zhong H, et al. Exosomes from heat-stressed tumour cells inhibit tumour growth by converting regulatory T cells to Th17 cells *via* IL-6. *Immunology* 2018;**154**: 132−43.
96. Zhong H, Yang Y, Ma S, Xiu F, Cai Z, Zhao H, et al. Induction of a tumour-specific CTL response by exosomes isolated from heat-treated malignant ascites of gastric cancer patients. *Int J Hyperther* 2011;**27**:604−11.
97. Meyer RE, Braun RD, Rosner GL, Dewhirst MW. Local 42 °C hyperthermia improves vascular conductance of the R3230Ac rat mammary adenocarcinoma during sodium nitroprusside infusion. *Radiat Res* 2000;**154**:196−201.
98. Song CW, Shakil A, Griffin RJ, Okajima K. Improvement of tumor oxygenation status by mild temperature hyperthermia alone or in combination with carbogen. *Semin Oncol* 1997;**24**:626−32.
99. Kong G, Braun RD, Dewhirst MW. Characterization of the effect of hyperthermia on nanoparticle extravasation from tumor vasculature. *Cancer Res* 2001;**61**:3027−32.

100. Toraya-Brown S, Sheen MR, Zhang P, Chen L, Baird JR, Demidenko E, et al. Local hyperthermia treatment of tumors induces $CD8^+$ T cell-mediated resistance against distal and secondary tumors. *Nanomedicine* 2014;**10**:1273−85.
101. Kolosnjaj-Tabi J, Di Corato R, Lartigue L, Marangon I, Guardia P, Silva AKA, et al. Heat-generating iron oxide nanocubes: subtle "destructurators" of the tumoral microenvironment. *ACS Nano* 2014;**8**:4268−83.
102. Chung EJ, Leon L, Rinaldi C. *Nanoparticles for biomedical applications*. Amsterdam: Elsevier; 2019.
103. Riley RS, June CH, Langer R, Mitchell MJ. Delivery technologies for cancer immunotherapy. *Nat Rev Drug Discov* 2019;**18**:175−96.
104. Kumar CS, Mohammad F. Magnetic nanomaterials for hyperthermia-based therapy and controlled drug delivery. *Adv Drug Deliv Rev* 2011;**63**:789−808.
105. Yatvin MB, Weinstein JN, Dennis WH, Blumenthal R. Design of liposomes for enhanced local release of drugs by hyperthermia. *Science* 1978;**202**:1290−3.
106. Weiss RB. The anthracyclines: will we ever find a better doxorubicin?. *Semin Oncol* 1992;**19**:670−86.
107. Chen KJ, Chaung EY, Wey SP, Lin KJ, Cheng F, Lin CC, et al. Hyperthermia-mediated local drug delivery by a bubble-generating liposomal system for tumor-specific chemotherapy. *ACS Nano* 2014;**8**:5105−15.
108. N'Guyen TTT, Duong HTT, Basuki J, Montembault V, Pascual S, Guibert C, et al. Functional iron oxide magnetic nanoparticles with hyperthermia-induced drug release ability by using a combination of orthogonal click reactions. *Angew Chem* 2013;**125**:14402−6.
109. Hardiansyah A, Huang LY, Yang MC, Liu TY, Tsai SC, Yang CY, et al. Magnetic liposomes for colorectal cancer cells therapy by high-frequency magnetic field treatment. *Nanoscale Res Lett* 2014;**9**:497.
110. Jose G, Lu YJ, Chen HA, Hsu HL, Hung JT, Anilkumar TS, et al. Hyaluronic acid modified bubble-generating magnetic liposomes for targeted delivery of doxorubicin. *J Magn Magn Mater* 2019;**474**:355−64.
111. Zahn D, Weidner A, Nosrati Z, Wöckel L, Dellith J, Müller R, et al. Temperature controlled camptothecin release from biodegradable magnetic PLGA microspheres. *J Magn Magn Mater* 2019;**469**:698−703.
112. Hardiansyah A, Yang MC, Liu TY, Kuo CY, Huang LY, Chan TY. Hydrophobic drug-loaded pegylated magnetic liposomes for drug-controlled release. *Nanoscale Res Lett* 2017;**12**:355.
113. Alawak M, Mahmoud G, Dayyih AA, Duse L, Pinnapireddy SP, Engelhardt K, et al. Magnetic resonance activatable thermosensitive liposomes for controlled doxorubicin delivery. *Mater Sci Eng C* 2020;**115**:111116.
114. Zhang Y, Li N, Suh H, Irvine DJ. Nanoparticle anchoring targets immune agonists to tumors enabling anti-cancer immunity without systemic toxicity. *Nat Commun* 2018;**9**:6.
115. Zhang ZQ, Song SC. Multiple hyperthermia-mediated release of TRAIL/SPION nanocomplex from thermosensitive polymeric hydrogels for combination cancer therapy. *Biomaterials* 2017;**132**:16−27.
116. Moy AJ, Tunnell JW. Combinatorial immunotherapy and nanoparticle mediated hyperthermia. *Adv Drug Deliv Rev* 2017;**114**:175−83.
117. Singh N, Jenkins GJ, Asadi R, Doak SH. Potential toxicity of superparamagnetic iron oxide nanoparticles (SPION). *Nano Rev* 2010;**1**:5358.
118. Beji Z, Hanini A, Smiri LS, Gavard J, Kacem K, Villain F, et al. Magnetic properties of Zn-substituted $MnFe_2O_4$ nanoparticles synthesized in polyol as potential heating agents for hyperthermia. Evaluation of their toxicity on Endothelial cells. *Chem Mater* 2010;**22**:5420−9.
119. Bischof JC, He X. Thermal stability of proteins. *Ann N Y Acad Sci* 2005;**1066**:12−33.
120. Hoopes PJ, Mazur CM, Osterberg B, Song A, Gladstone DJ, Steinmetz NF, et al. Effect of intra-tumoral magnetic nanoparticle hyperthermia and viral nanoparticle immunogenicity on primary and metastatic cancer. In: *Proceedings of SPIE 10066, Energy-based treatment of tissue and assessment IX*. San Francisco: SPIE; 2017. 100660G.
121. Ito A, Shinkai M, Honda H, Kobayashi T. Heat-inducible *TNF-α* gene therapy combined with hyperthermia using magnetic nanoparticles as a novel tumor-targeted therapy. *Cancer Gene Ther* 2001;**8**:649−54.
122. Anjum A, Fleischmann Jr WR. Effect of hyperthermia on the antitumor actions of interferons. *J Biol Regul Homeost Agents* 1992;**6**:75−86.
123. Tanaka K, Ito A, Kobayashi T, Kawamura T, Shimada S, Matsumoto K, et al. Heat immunotherapy using magnetic nanoparticles and dendritic cells for T-lymphoma. *J Biosci Bioeng* 2005;**100**:112−5.
124. Wust P, Hildebrandt B, Sreenivasa G, Rau B, Gellermann J, Riess H, et al. Hyperthermia in combined treatment of cancer. *Lancet Oncol* 2002;**3**:487−97.
125. Babincová M, Altanerova V, Altaner C, Bergemann C, Babinec P. *In vitro* analysis of cisplatin functionalized magnetic nanoparticles in combined cancer chemotherapy and electromagnetic hyperthermia. *IEEE Trans NanoBioscience* 2008;**7**:15−9.
126. Qu Y, Li J, Ren H, Leng J, Lin C, Shi D. Enhanced synergism of thermo-chemotherapy by combining highly efficient magnetic hyperthermia with magnetothermally-facilitated drug release. *Nanoscale* 2014;**6**:12408−13.
127. Chao Y, Chen G, Liang C, Xu J, Dong Z, Han X, et al. Iron nanoparticles for low-power local magnetic hyperthermia in combination with immune checkpoint blockade for systemic antitumor therapy. *Nano Lett* 2019;**19**:4287−96.
128. Tian Z, Yu X, Ruan Z, Zhu M, Zhu Y, Hanagata N. Magnetic mesoporous silica nanoparticles coated with thermo-responsive copolymer for potential chemo- and magnetic hyperthermia therapy. *Microporous Mesoporous Mater* 2018;**256**:1−9.
129. Estelrich J, Busquets MA. Iron oxide nanoparticles in photothermal therapy. *Molecules* 2018;**23**:1567.
130. Wang C, Xu L, Liang C, Xiang J, Peng R, Liu Z. Immunological responses triggered by photothermal therapy with carbon nanotubes in combination with Anti-CTLA-4 therapy to inhibit cancer metastasis. *Adv Mater* 2014;**26**:8154−62.
131. Almeida JP, Drezek RA, Foster AE. Controlling melanoma at local and systemic levels: is a combination of ablative therapy and immunotherapy the way forward?. *Immunotherapy* 2014;**6**:109−11.
132. Pan J, Hu P, Guo Y, Hao J, Ni D, Xu Y, et al. Combined magnetic hyperthermia and immune therapy for primary and metastatic tumor treatments. *ACS Nano* 2020;**14**:1033−44.
133. Wang Z, Zhang F, Shao D, Chang Z, Wang L, Hu H, et al. Janus nanobullets combine photodynamic therapy and magnetic hyperthermia to potentiate synergetic anti-metastatic immunotherapy. *Adv Sci* 2019;**6**:1901690.
134. Brero F, Albino M, Antoccia A, Arosio P, Avolio M, Berardinelli F, et al. Hadron therapy, magnetic nanoparticles and hyperthermia: a promising combined tool for pancreatic cancer treatment. *Nanomaterials* 2020;**10**:1919.
135. Mai BT, Balakrishnan PB, Barthel MJ, Piccardi F, Niculaes D, Marinaro F, et al. Thermoresponsive iron oxide nanocubes for an effective clinical translation of magnetic hyperthermia and heat-mediated chemotherapy. *ACS Appl Mater Interfaces* 2019;**11**:5727−39.
136. Shields IV CW, Wang LL, Evans MA, Mitragotri S. Materials for immunotherapy. *Adv Mater* 2020;**32**:1901633.
137. Ulbrich K, Holá K, Šubr V, Bakandritsos A, Tuček J, Zbořil R. Targeted drug delivery with polymers and magnetic nanoparticles: covalent and noncovalent approaches, release control, and clinical studies. *Chem Rev* 2016;**116**:5338−431.
138. Owen J, Pankhurst Q, Stride E. Magnetic targeting and ultrasound mediated drug delivery: benefits, limitations and combination. *Int J Hyperther* 2012;**28**:362−73.

139. Li J, Mooney DJ. Designing hydrogels for controlled drug delivery. *Nat Rev Mater* 2016;**1**:16071.
140. Vangijzegem T, Stanicki D, Laurent S. Magnetic iron oxide nanoparticles for drug delivery: applications and characteristics. *Expet Opin Drug Deliv* 2019;**16**:69–78.
141. Wilhelm S, Tavares AJ, Dai Q, Ohta S, Audet J, Dvorak HF, et al. Analysis of nanoparticle delivery to tumours. *Nat Rev Mater* 2016;**1**:16014.
142. Bourguignon LY, Shiina M, Li JJ. Hyaluronan–CD44 interaction promotes oncogenic signaling, microrna functions, chemoresistance, and radiation resistance in cancer stem cells leading to tumor progression. *Adv Cancer Res* 2014;**123**:255–75.
143. Zhang H, Liu XL, Zhang YF, Gao F, Li GL, He Y, et al. Magnetic nanoparticles based cancer therapy: current status and applications. *Sci China Life Sci* 2018;**61**:400–14.
144. Zheng H, Wen S, Zhang Y, Sun Z. Organosilane and polyethylene glycol functionalized magnetic mesoporous silica nanoparticles as carriers for CpG immunotherapy *in vitro* and *in vivo*. *PLoS One* 2015;**10**. e0140265.
145. Minchinton AI, Tannock IF. Drug penetration in solid tumours. *Nat Rev Cancer* 2006;**6**:583–92.
146. Trédan O, Galmarini CM, Patel K, Tannock IF. Drug resistance and the solid tumor microenvironment. *JNCI J Natl Cancer Inst* 2007;**99**:1441–54.
147. Parveen S, Misra R, Sahoo SK. Nanoparticles: a boon to drug delivery, therapeutics, diagnostics and imaging. *Nanomed Nanotechnol Biol Med* 2012;**8**:147–66.
148. Huang YS, Lu YJ, Chen JP. Magnetic graphene oxide as a carrier for targeted delivery of chemotherapy drugs in cancer therapy. *J Magn Magn Mater* 2017;**427**:34–40.
149. Yang X, Wen Y, Wu A, Xu M, Amano T, Zheng L, et al. Polyglycerol mediated covalent construction of magnetic mesoporous silica nanohybrid with aqueous dispersibility for drug delivery. *Mater Sci Eng C* 2017;**80**:517–25.
150. Albinali KE, Zagho MM, Deng Y, Elzatahry AA. A perspective on magnetic core-shell carriers for responsive and targeted drug delivery systems. *Int J Nanomed* 2019;**14**:1707–23.
151. Giri S, Trewyn BG, Stellmaker MP, Lin VS. Stimuli-responsive controlled-release delivery system based on mesoporous silica nanorods capped with magnetic nanoparticles. *Angew Chem* 2005;**117**:5166–72.
152. Sungsuwan S, Yin Z, Huang X. Lipopeptide-coated iron oxide nanoparticles as potential glycoconjugate-based synthetic anticancer vaccines. *ACS Appl Mater Interfaces* 2015;**7**:17535–44.
153. Diller E, Giltinan J, Sitti M. Independent control of multiple magnetic microrobots in three dimensions. *Int J Robot Res* 2013;**32**:614–31.
154. Ceylan H, Yasa IC, Kilic U, Hu W, Sitti M. Translational prospects of untethered medical microrobots. *Prog Biomed Eng* 2019;**1**. 012002.
155. Mahoney AW, Sarrazin JC, Bamberg E, Abbott JJ. Velocity control with gravity compensation for magnetic helical microswimmers. *Adv Robot* 2011;**25**:1007–28.
156. Garcia-Gradilla V, Orozco J, Sattayasamitsathit S, Soto F, Kuralay F, Pourazary A, et al. Functionalized ultrasound-propelled magnetically guided nanomotors: toward practical biomedical applications. *ACS Nano* 2013;**7**:9232–40.
157. Dreyfus R, Baudry J, Roper ML, Fermigier M, Stone HA, Bibette J. Microscopic artificial swimmers. *Nature* 2005;**437**:862–5.
158. Ghosh A, Fischer P. Controlled propulsion of artificial magnetic nanostructured propellers. *Nano Lett* 2009;**9**:2243–5.
159. Gao W, Sattayasamitsathit S, Manesh KM, Weihs D, Wang J. Magnetically powered flexible metal nanowire motors. *J Am Chem Soc* 2010;**132**:14403–5.
160. Liu E, Zhang M, Cui H, Gong JB, Huang YZ, Wang J, et al. Tat-functionalized Ag-Fe_3O_4 nano-composites as tissue-penetrating vehicles for tumor magnetic targeting and drug delivery. *Acta Pharm Sin B* 2018;**8**:956–68.
161. Han J, Zhen J, Nguyen VD, Go G, Choi Y, Ko SY, et al. Hybrid-actuating macrophage-based microrobots for active cancer therapy. *Sci Rep* 2016;**6**:28717.
162. Wang X, Cai J, Sun L, Zhang S, Gong D, Li X, et al. Facile fabrication of magnetic microrobots based on *spirulina* templates for targeted delivery and synergistic chemo-photothermal therapy. *ACS Appl Mater Interfaces* 2019;**11**:4745–56.
163. Li J, Li X, Luo T, Wang R, Liu C, Chen S, et al. Development of a magnetic microrobot for carrying and delivering targeted cells. *Sci Robot* 2018;**3**. eaat8829.
164. Li D, Ren Y. High-gradient magnetic field for magnetic nanoparticles drug delivery system. *IEEE Trans Appl Supercond* 2018;**28**:4402107.
165. Sharma S, Katiyar VK, Singh U. Mathematical modelling for trajectories of magnetic nanoparticles in a blood vessel under magnetic field. *J Magn Magn Mater* 2015;**379**:102–7.
166. McBain SC, Yiu HH, Dobson J. Magnetic nanoparticles for gene and drug delivery. *Int J Nanomed* 2008;**3**:169–80.
167. Liu JF, Lan Z, Ferrari C, Stein JM, Higbee-Dempsey E, Yan L, et al. Use of oppositely polarized external magnets to improve the accumulation and penetration of magnetic nanocarriers into solid tumors. *ACS Nano* 2020;**14**:142–52.
168. Shapiro B, Kulkarni S, Nacev A, Sarwar A, Preciado D, Depireux DA. Shaping magnetic fields to direct therapy to ears and eyes. *Annu Rev Biomed Eng* 2014;**16**:455–81.
169. Grief AD, Richardson G. Mathematical modelling of magnetically targeted drug delivery. *J Magn Magn Mater* 2005;**293**:455–63.
170. Baun O, Blümler P. Permanent magnet system to guide superparamagnetic particles. *J Magn Magn Mater* 2017;**439**:294–304.
171. Vermesh O, Aalipour A, Ge TJ, Saenz Y, Guo Y, Alam IS, et al. An intravascular magnetic wire for the high-throughput retrieval of circulating tumour cells *in vivo*. *Nat Biomed Eng* 2018;**2**:696–705.
172. Cregg PJ, Murphy K, Mardinoglu A. Inclusion of interactions in mathematical modelling of implant assisted magnetic drug targeting. *Appl Math Model* 2012;**36**:1–34.
173. Kempe H, Kempe M. The use of magnetite nanoparticles for implant-assisted magnetic drug targeting in thrombolytic therapy. *Biomaterials* 2010;**31**:9499–510.
174. Chiang CS, Lin YJ, Lee R, Lai YH, Cheng HW, Hsieh CH, et al. Combination of fucoidan-based magnetic nanoparticles and immunomodulators enhances tumour-localized immunotherapy. *Nat Nanotechnol* 2018;**13**:746–54.
175. Mahoney KM, Rennert PD, Freeman GJ. Combination cancer immunotherapy and new immunomodulatory targets. *Nat Rev Drug Discov* 2015;**14**:561–84.
176. Mejías R, Pérez-Yagüe S, Gutiérrez L, Cabrera LI, Spada R, Acedo P, et al. Dimercaptosuccinic acid-coated magnetite nanoparticles for magnetically guided *in vivo* delivery of interferon gamma for cancer immunotherapy. *Biomaterials* 2011;**32**:2938–52.
177. Grifantini R, Taranta M, Gherardini L, Naldi I, Parri M, Grandi A, et al. Magnetically driven drug delivery systems improving targeted immunotherapy for colon-rectal cancer. *J Control Release* 2018;**280**:76–86.
178. Zhang F, Lu G, Wen X, Li F, Ji X, Li Q, et al. Magnetic nanoparticles coated with polyphenols for spatio-temporally controlled cancer photothermal/immunotherapy. *J Control Release* 2020;**326**:131–9.
179. Iacovacci V, Lucarini G, Ricotti L, Dario P, Dupont PE, Menciassi A. Untethered magnetic millirobot for targeted drug delivery. *Biomed Microdevices* 2015;**17**:63.
180. Steager EB, Selman Sakar M, Magee C, Kennedy M, Cowley A, Kumar V. Automated biomanipulation of single cells using magnetic microrobots. *Int J Robot Res* 2013;**32**:346–59.
181. Tottori S, Zhang L, Qiu F, Krawczyk KK, Franco-Obregón A, Nelson BJ. Magnetic helical micromachines: fabrication, controlled swimming, and cargo transport. *Adv Mater* 2012;**24**:811–6.
182. Makadia HK, Siegel SJ. Poly Lactic-*co*-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. *Polymers* 2011;**3**:1377–97.

183. Gasparini G, Holdich RG, Kosvintsev SR. PLGA particle production for water-soluble drug encapsulation: degradation and release behaviour. *Colloids Surf B Biointerfaces* 2010;**75**:557−64.
184. Mhanna R, Qiu F, Zhang L, Ding Y, Sugihara K, Zenobi-Wong M, et al. Artificial bacterial flagella for remote-controlled targeted single-cell drug delivery. *Small* 2014;**10**:1953−7.
185. Zhang X, Wu F, Men K, Huang R, Zhou B, Zhang R, et al. Modified Fe_3O_4 magnetic nanoparticle delivery of CpG inhibits tumor growth and spontaneous pulmonary metastases to enhance immunotherapy. *Nanoscale Res Lett* 2018;**13**:240.
186. Maurer T, Pournaras C, Aguilar-Pimentel JA, Thalgott M, Horn T, Heck M, et al. Immunostimulatory CpG-DNA and PSA-peptide vaccination elicits profound cytotoxic T cell responses. *Urol Oncol* 2013;**31**:1395−401.
187. Soema PC, Rosendahl Huber SK, Willems GJ, Jiskoot W, Kersten GF, Amorij JP. Influenza T-cell epitope-loaded virosomes adjuvanted with CpG as a potential influenza vaccine. *Pharm Res* 2015;**32**:1505−15.
188. Lou Y, Liu C, Lizée G, Peng W, Xu C, Ye Y, et al. Antitumor activity mediated by CpG: the route of administration is critical. *J Immunother* 2011;**34**:279−88.
189. Grippin AJ, Wummer B, Wildes T, Dyson K, Trivedi V, Yang C, et al. Dendritic cell-activating magnetic nanoparticles enable early prediction of antitumor response with magnetic resonance imaging. *ACS Nano* 2019;**13**:13884−98.
190. Reséndiz-Hernández PJ, Rodríguez-Fernández OS, García-Cerda LA. Synthesis of poly(vinyl alcohol)-magnetite ferrogel obtained by freezing−thawing technique. *J Magn Magn Mater* 2008;**320**:e373−6.
191. Liao J, Huang H. Review on magnetic natural polymer constructed hydrogels as vehicles for drug delivery. *Biomacromolecules* 2020;**21**:2574−94.
192. Li Y, Huang G, Zhang X, Li B, Chen Y, Lu T, et al. Magnetic hydrogels and their potential biomedical applications. *Adv Funct Mater* 2013;**23**:660−72.
193. Ketebu O, Cumpson P, Yu E. The effect of temperature and agitation on polyethyleneimine adsorption on iron oxide magnetic nanoparticles in the synthesis of iron oxide-Au core−shell nanoparticles. *Adv Sci Eng Med* 2014;**6**:531−7.
194. Hu SH, Liu TY, Liu DM, Chen SY. Nano-ferrosponges for controlled drug release. *J Control Release* 2007;**121**:181−9.
195. Liu TY, Hu SH, Liu TY, Liu DM, Chen SY. Magnetic-sensitive behavior of intelligent ferrogels for controlled release of drug. *Langmuir* 2006;**22**:5974−8.
196. Zhao X, Kim J, Cezar CA, Huebsch N, Lee K, Bouhadir K, et al. Active scaffolds for on-demand drug and cell delivery. *Proc Natl Acad Sci U S A* 2011;**108**:67−72.
197. Dunussi-Joannopoulos K, Zuberek K, Runyon K, Hawley RG, Wong A, Erickson J, et al. Efficacious immunomodulatory activity of the chemokine stromal cell-derived factor 1 (SDF-1): local secretion of SDF-1 at the tumor site serves as T-cell chemoattractant and mediates T-cell-dependent antitumor responses. *Blood* 2002;**100**:1551−8.
198. Yang B, Jeang J, Yang A, Wu TC, Hung CF. DNA vaccine for cancer immunotherapy. *Hum Vaccines Immunother* 2014;**10**:3153−64.
199. Dumortier H, van Mierlo GJ, Egan D, van Ewijk W, Toes RE, Offringa R, et al. Antigen presentation by an immature myeloid dendritic cell line does not cause CTL deletion *in vivo*, but generates $CD8^+$ central memory-like T cells that can be rescued for full effector function. *J Immunol* 2005;**175**:855−63.
200. Cezar CA, Kennedy SM, Mehta M, Weaver JC, Gu L, Vandenburgh H, et al. Biphasic ferrogels for triggered drug and cell delivery. *Adv Healthc Mater* 2014;**3**:1869−76.
201. Shin BY, Cha BG, Jeong JH, Kim J. Injectable macroporous ferrogel microbeads with a high structural stability for magnetically actuated drug delivery. *ACS Appl Mater Interfaces* 2017;**9**:31372−80.
202. Kennedy S, Roco C, Déléris A, Spoerri P, Cezar C, Weaver J, et al. Improved magnetic regulation of delivery profiles from ferrogels. *Biomaterials* 2018;**161**:179−89.
203. Hu SH, Liu TY, Liu DM, Chen SY. Controlled pulsatile drug release from a ferrogel by a high-frequency magnetic field. *Macromolecules* 2007;**40**:6786−8.
204. Lu Z, Prouty MD, Guo Z, Golub VO, Kumar CS, Lvov YM. Magnetic switch of permeability for polyelectrolyte microcapsules embedded with Co@Au nanoparticles. *Langmuir* 2005;**21**:2042−50.
205. Liu TY, Hu SH, Liu KH, Shaiu RS, Liu DM, Chen SY. Instantaneous drug delivery of magnetic/thermally sensitive nanospheres by a high-frequency magnetic field. *Langmuir* 2008;**24**:13306−11.
206. Hoare T, Santamaria J, Goya GF, Irusta S, Lin D, Lau S, et al. A magnetically triggered composite membrane for on-demand drug delivery. *Nano Lett* 2009;**9**:3651−7.
207. Hu SH, Chen SY, Liu DM, Hsiao CS. Core/single-crystal-shell nanospheres for controlled drug release *via* a magnetically triggered rupturing mechanism. *Adv Mater* 2008;**20**:2690−5.
208. Seth A, Gholami Derami H, Gupta P, Wang Z, Rathi P, Gupta R, et al. Polydopamine-mesoporous silica core−shell nanoparticles for combined photothermal immunotherapy. *ACS Appl Mater Interfaces* 2020;**12**:42499−510.
209. Tang J, Tong Z, Xia Y, Liu M, Lv Z, Gao Y, et al. Super tough magnetic hydrogels for remotely triggered shape morphing. *J Mater Chem B* 2018;**6**:2713−22.
210. Eqtami A, Felfoul O, Dupont PE. MRI-powered closed-loop control for multiple magnetic capsules. In: *Proceedings of 2014 IEEE/RSJ international conference on intelligent robots and systems*. Chicago: IEEE; 2014. p. 3536−42.
211. Yim S, Sitti M. Shape-programmable soft capsule robots for semi-implantable drug delivery. *IEEE Trans Robot* 2012;**28**:1198−202.
212. Golovin YI, Gribanovsky SL, Golovin DY, Klyachko NL, Majouga AG, Master AM, et al. Towards nanomedicines of the future: remote magneto-mechanical actuation of nanomedicines by alternating magnetic fields. *J Control Release* 2015;**219**:43−60.
213. Hauser AK, Wydra RJ, Stocke NA, Anderson KW, Hilt JZ. Magnetic nanoparticles and nanocomposites for remote controlled therapies. *J Control Release* 2015;**219**:76−94.
214. Xu T, Yu J, Yan X, Choi H, Zhang L. Magnetic actuation based motion control for microrobots: an overview. *Micromachines* 2015;**6**:1346−64.
215. Lee SH, Kim BH, Park CG, Lee C, Lim BY, Choy YB. Implantable small device enabled with magnetic actuation for on-demand and pulsatile drug delivery. *J Control Release* 2018;**286**:224−30.
216. Pirmoradi FN, Jackson JK, Burt HM, Chiao M. A magnetically controlled MEMS device for drug delivery: design, fabrication, and testing. *Lab Chip* 2011;**11**:3072−80.
217. Yasa IC, Ceylan H, Bozuyuk U, Wild AM, Sitti M. Elucidating the interaction dynamics between microswimmer body and immune system for medical microrobots. *Sci Robot* 2020;**5**. eaaz3867.
218. Parham P. In: *The immune system*. 4th ed. New York: Garland Science; 2015.
219. Ebrahimian H, Giesguth M, Dietz KJ, Reiss G, Herth S. Magnetic tweezers for manipulation of magnetic particles in single cells. *Appl Phys Lett* 2014;**104**. 063701.
220. Lee K, Yi Y, Yu Y. Remote control of t cell activation using magnetic Janus particles. *Angew Chem Int Ed* 2016;**55**:7384−7.
221. Wilson BS, Pfeiffer JR, Oliver JM. Observing Fcεri signaling from the inside of the mast cell membrane. *J Cell Biol* 2000;**149**:1131−42.
222. Segal DM, Taurog JD, Metzger H. Dimeric immunoglobulin E serves as a unit signal for mast cell degranulation. *Proc Natl Acad Sci U S A* 1977;**74**:2993−7.
223. Mannix RJ, Kumar S, Cassiola F, Montoya-Zavala M, Feinstein E, Prentiss M, et al. Nanomagnetic actuation of receptor-mediated signal transduction. *Nat Nanotechnol* 2008;**3**:36−40.
224. Reber LL, Hernandez JD, Galli SJ. The pathophysiology of anaphylaxis. *J Allergy Clin Immunol* 2017;**140**:335−48.
225. Smith-Garvin JE, Koretzky GA, Jordan MS. T cell activation. *Annu Rev Immunol* 2009;**27**:591−619.
226. Perica K, Tu A, Richter A, Bieler JG, Edidin M, Schneck JP. Magnetic field-induced T cell receptor clustering by nanoparticles

enhances T cell activation and stimulates antitumor activity. *ACS Nano* 2014;**8**:2252−60.
227. Reichel D, Tripathi M, Perez JM. Biological effects of nanoparticles on macrophage polarization in the tumor microenvironment. *Nanotheranostics* 2019;**3**:66−88.
228. Zanganeh S, Hutter G, Spitler R, Lenkov O, Mahmoudi M, Shaw A, et al. Iron oxide nanoparticles inhibit tumour growth by inducing pro-inflammatory macrophage polarization in tumour tissues. *Nat Nanotechnol* 2016;**11**:986−94.
229. Riegler J, Wells JA, Kyrtatos PG, Price AN, Pankhurst QA, Lythgoe MF. Targeted magnetic delivery and tracking of cells using a magnetic resonance imaging system. *Biomaterials* 2010;**31**:5366−71.
230. Arbab AS, Jordan EK, Wilson LB, Yocum GT, Lewis BK, Frank JA. *In vivo* trafficking and targeted delivery of magnetically labeled stem cells. *Hum Gene Ther* 2004;**15**:351−60.
231. Wu L, Zhang F, Wei Z, Li X, Zhao H, Lv H, et al. Magnetic delivery of Fe_3O_4@polydopamine nanoparticle-loaded natural killer cells suggest a promising anticancer treatment. *Biomater Sci* 2018;**6**:2714−25.
232. Sanz-Ortega L, Rojas JM, Marcos A, Portilla Y, Stein JV, Barber DF. T cells loaded with magnetic nanoparticles are retained in peripheral lymph nodes by the application of a magnetic field. *J Nanobiotechnol* 2019;**17**:14.
233. Weissleder R, Stark DD, Engelstad BL, Bacon BR, Compton CC, White DL, et al. Superparamagnetic iron oxide: pharmacokinetics and toxicity. *Am J Roentgenol* 1989;**152**:167−73.
234. Jin H, Qian Y, Dai Y, Qiao S, Huang C, Lu L, et al. Magnetic enrichment of dendritic cell vaccine in lymph node with fluorescent-magnetic nanoparticles enhanced cancer immunotherapy. *Theranostics* 2016;**6**:2000−14.
235. Muthana M, Kennerley AJ, Hughes R, Fagnano E, Richardson J, Paul M, et al. Directing cell therapy to anatomic target sites *in vivo* with magnetic resonance targeting. *Nat Commun* 2015;**6**:8009.
236. Jang ES, Shin JH, Ren G, Park MJ, Cheng K, Chen X, et al. The manipulation of natural killer cells to target tumor sites using magnetic nanoparticles. *Biomaterials* 2012;**33**:5584−92.
237. Gobbo OL, Sjaastad K, Radomski MW, Volkov Y, Prina-Mello A. Magnetic nanoparticles in cancer theranostics. *Theranostics* 2015;**5**:1249−63.
238. Elzoghby AO, Hemasa AL, Freag MS. Hybrid protein-inorganic nanoparticles: from tumor-targeted drug delivery to cancer imaging. *J Control Release* 2016;**243**:303−22.
239. Datta NR, Krishnan S, Speiser DE, Neufeld E, Kuster N, Bodis S, et al. Magnetic nanoparticle-induced hyperthermia with appropriate payloads: Paul Ehrlich's "magic (nano)bullet" for cancer theranostics?. *Cancer Treat Rev* 2016;**50**:217−27.
240. Li X, Wei J, Aifantis KE, Fan Y, Feng Q, Cui FZ, et al. Current investigations into magnetic nanoparticles for biomedical applications. *J Biomed Mater Res* 2016;**104**:1285−96.
241. Zhu L, Zhou Z, Mao H, Yang L. Magnetic nanoparticles for precision oncology: theranostic magnetic iron oxide nanoparticles for image-guided and targeted cancer therapy. *Nanomedicine* 2017;**12**:73−87.
242. Cole AJ, Yang VC, David AE. Cancer theranostics: the rise of targeted magnetic nanoparticles. *Trends Biotechnol* 2011;**29**:323−32.
243. Singh A, Sahoo SK. Magnetic nanoparticles: a novel platform for cancer theranostics. *Drug Discov Today* 2014;**19**:474−81.
244. Yan X, Zhou Q, Vincent M, Deng Y, Yu J, Xu J, et al. Multifunctional biohybrid magnetite microrobots for imaging-guided therapy. *Sci Robot* 2017;**2**. eaaq1155.
245. Yang R, Sarkar S, Yong VW, Dunn JF. *In vivo* MR imaging of tumor-associated macrophages: the next frontier in cancer imaging. *Magn Reson Insights* 2018;**11**:1−8.
246. Lohrke J, Frenzel T, Endrikat J, Alves FC, Grist TM, Law M, et al. 25 Years of contrast-enhanced MRI: developments, current challenges and future perspectives. *Adv Ther* 2016;**33**:1−28.
247. Wahsner J, Gale EM, Rodríguez-Rodríguez A, Caravan P. Chemistry of MRI contrast agents: current challenges and new frontiers. *Chem Rev* 2019;**119**:957−1057.
248. Saritas EU, Goodwill PW, Croft LR, Konkle JJ, Lu K, Zheng B, et al. Magnetic particle imaging (MPI) for NMR and MRI researchers. *J Magn Reson* 2013;**229**:116−26.
249. Chen L, Wu Y, Wu H, Li J, Xie J, Zang F, et al. Magnetic targeting combined with active targeting of dual-ligand iron oxide nanoprobes to promote the penetration depth in tumors for effective magnetic resonance imaging and hyperthermia. *Acta Biomater* 2019;**96**:491−504.
250. Umut E, Coşkun M, Pineider F, Berti D, Güngüneş H. Nickel ferrite nanoparticles for simultaneous use in magnetic resonance imaging and magnetic fluid hyperthermia. *J Colloid Interface Sci* 2019;**550**:199−209.
251. Esmaeili E, Khalili M, Sohi AN, Hosseinzadeh S, Taheri B, Soleimani M. Dendrimer functionalized magnetic nanoparticles as a promising platform for localized hyperthermia and magnetic resonance imaging diagnosis. *J Cell Physiol* 2019;**234**:12615−24.
252. Du Y, Liu X, Liang Q, Liang XJ, Tian J. Optimization and design of magnetic ferrite nanoparticles with uniform tumor distribution for highly sensitive MRI/MPI performance and improved magnetic hyperthermia therapy. *Nano Lett* 2019;**19**:3618−26.
253. Guo Y, Ran Y, Wang Z, Cheng J, Cao Y, Yang C, et al. Magnetic-responsive and targeted cancer nanotheranostics by PA/MR bimodal imaging-guided photothermally triggered immunotherapy. *Biomaterials* 2019;**219**:119370.
254. Kubovcikova M, Koneracka M, Strbak O, Molcan M, Zavisova V, Antal I, et al. Poly-L-lysine designed magnetic nanoparticles for combined hyperthermia, magnetic resonance imaging and cancer cell detection. *J Magn Magn Mater* 2019;**475**:316−26.
255. Santra S, Kaittanis C, Grimm J, Perez JM. Drug/dye-loaded, multifunctional iron oxide nanoparticles for combined targeted cancer therapy and dual optical/magnetic resonance imaging. *Small* 2009;**5**:1862−8.
256. Das M, Mishra D, Dhak P, Gupta S, Maiti TK, Basak A, et al. Biofunctionalized, phosphonate-grafted. ultrasmall iron oxide nanoparticles for combined targeted cancer therapy and multimodal imaging. *Small* 2009;**5**:2883−93.
257. Gao N, Bozeman EN, Qian W, Wang L, Chen H, Lipowska M, et al. Tumor penetrating theranostic nanoparticles for enhancement of targeted and image-guided drug delivery into peritoneal tumors following intraperitoneal delivery. *Theranostics* 2017;**7**:1689−704.
258. Gleich B, Weizenecker J. Tomographic imaging using the nonlinear response of magnetic particles. *Nature* 2005;**435**:1214−7.
259. Hensley D, Tay ZW, Dhavalikar R, Zheng B, Goodwill P, Rinaldi C, et al. Combining magnetic particle imaging and magnetic fluid hyperthermia in a theranostic platform. *Phys Med Biol* 2017;**62**:3483−500.
260. Chandrasekharan P, Tay ZW, Hensley D, Zhou XY, Fung BK, Colson C, et al. Using magnetic particle imaging systems to localize and guide magnetic hyperthermia treatment: tracers, hardware, and future medical applications. *Theranostics* 2020;**10**:2965−81.
261. Hensley D, Tay ZW, Dhavalikar R, Goodwill P, Zheng B, Rinaldi C, et al. A theranostic platform for localized magnetic fluid hyperthermia and magnetic particle imaging. In: *Proceedings of SPIE 10066, energy-based treatment of tissue and assessment IX*. San Francisco: SPIE; 2017.
262. Wells J, Twamley S, Sekar A, Ludwig A, Paysen H, Kosch O, et al. Lissajous scanning magnetic particle imaging as a multifunctional platform for magnetic hyperthermia therapy. *Nanoscale* 2020;**12**:18342−55.
263. Murase K, Aoki M, Banura N, Nishimoto K, Mimura A, Kuboyabu T, et al. Usefulness of magnetic particle imaging for predicting the therapeutic effect of magnetic hyperthermia. *Open J Med Imag* 2015;**5**:85−99.

264. Tay ZW, Chandrasekharan P, Chiu-Lam A, Hensley DW, Dhavalikar R, Zhou XY, et al. Magnetic particle imaging-guided heating *in vivo* using gradient fields for arbitrary localization of magnetic hyperthermia therapy. *ACS Nano* 2018;**12**:3699−713.
265. Bauer LM, Situ SF, Griswold MA, Samia AC. High-performance iron oxide nanoparticles for magnetic particle imaging−guided hyperthermia (hMPI). *Nanoscale* 2016;**8**:12162−9.
266. Krohn KA, Link JM, Mason RP. Molecular imaging of hypoxia. *J Nucl Med* 2008;**49**(Suppl 2). 129S−48S.
267. Jung KO, Jo H, Yu JH, Gambhir SS, Pratx G. Development and MPI tracking of novel hypoxia-targeted theranostic exosomes. *Biomaterials* 2018;**177**:139−48.
268. Tomitaka A, Ota S, Nishimoto K, Arami H, Takemura Y, Nair M. Dynamic magnetic characterization and magnetic particle imaging enhancement of magnetic-gold core−shell nanoparticles. *Nanoscale* 2019;**11**:6489−96.
269. Fuller EG, Scheutz GM, Jimenez A, Lewis P, Savliwala S, Liu S, et al. Theranostic nanocarriers combining high drug loading and magnetic particle imaging. *Int J Pharm* 2019;**572**:118796.
270. Bobo D, Robinson KJ, Islam J, Thurecht KJ, Corrie SR. Nanoparticle-based medicines: a review of FDA-approved materials and clinical trials to date. *Pharm Res* 2016;**33**:2373−87.
271. Revia RA, Zhang M. Magnetite nanoparticles for cancer diagnosis, treatment, and treatment monitoring: recent advances. *Mater Today* 2016;**19**:157−68.
272. Laurent S, Saei AA, Behzadi S, Panahifar A, Mahmoudi M. Superparamagnetic iron oxide nanoparticles for delivery of therapeutic agents: opportunities and challenges. *Expet Opin Drug Deliv* 2014;**11**:1449−70.
273. Weissleder R, Bogdanov A, Neuwelt EA, Papisov M. Long-circulating iron oxides for MR imaging. *Adv Drug Deliv Rev* 1995;**16**:321−34.
274. Mahmoudi M, Simchi A, Vali H, Imani M, Shokrgozar MA, Azadmanesh K, et al. Cytotoxicity and cell cycle effects of bare and poly(vinyl alcohol)-coated iron oxide nanoparticles in mouse fibroblasts. *Adv Eng Mater* 2009;**11**:B243−50.
275. Sun C, Du K, Fang C, Bhattarai N, Veiseh O, Kievit F, et al. PEG-mediated synthesis of highly dispersive multifunctional superparamagnetic nanoparticles: their physicochemical properties and function *in vivo*. *ACS Nano* 2010;**4**:2402−10.
276. Gómez-Lopera SA, Plaza RC, Delgado AV. Synthesis and characterization of spherical magnetite/biodegradable polymer composite particles. *J Colloid Interface Sci* 2001;**240**:40−7.
277. Cook JD, Hershko C, Finch CA. Storage iron kinetics. I. Measurement of the cellular distribution of 59 Fe in rat liver. *J Lab Clin Med* 1972;**80**:613−23.
278. Hershko C, Cook JD, Finch DA. Storage iron kinetics. 3. Study of desferrioxamine action by selective radioiron labels of RE and parenchymal cells. *J Lab Clin Med* 1973;**81**:876−86.
279. Anzai Y, Piccoli CW, Outwater EK, Stanford W, Bluemke DA, Nurenberg P, et al. Evaluation of neck and body metastases to nodes with ferumoxtran 10-enhanced MR imaging: phase III safety and efficacy study. *Radiology* 2003;**228**:777−88.
280. Wada S, Yue L, Tazawa K, Furuta I, Nagae H, Takemori S, et al. New local hyperthermia using dextran magnetite complex (DM) for oral cavity: experimental study in normal hamster tongue. *Oral Dis* 2001;**7**:192−5.
281. Arami H, Khandhar A, Liggitt D, Krishnan KM. *In vivo* delivery, pharmacokinetics, biodistribution and toxicity of iron oxide nanoparticles. *Chem Soc Rev* 2015;**44**:8576−607.
282. Karlsson HL, Cronholm P, Gustafsson J, Möller L. Copper oxide nanoparticles are highly toxic: a comparison between metal oxide nanoparticles and carbon nanotubes. *Chem Res Toxicol* 2008;**21**:1726−32.
283. Mahmoudi M, Simchi A, Imani M. Cytotoxicity of uncoated and polyvinyl alcohol coated superparamagnetic iron oxide nanoparticles. *J Phys Chem C* 2009;**113**:9573−80.
284. Yu Q, Xiong XQ, Zhao L, Xu TT, Bi H, Fu R, et al. Biodistribution and toxicity assessment of superparamagnetic iron oxide nanoparticles *in vitro* and *in vivo*. *Curr Med Sci* 2018;**38**:1096−102.
285. Jiang Q, Wang K, Zhang X, Ouyang B, Liu H, Pang Z, et al. Platelet membrane-camouflaged magnetic nanoparticles for ferroptosis-enhanced cancer immunotherapy. *Small* 2020;**16**:2001704.