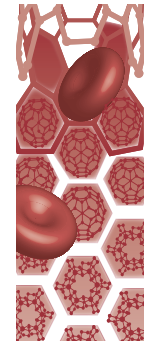
Review

For reprint orders, please contact: reprints@futuremedicine.com

Nanomedicine



Magnetic nanoparticles for precision oncology: theranostic magnetic iron oxide nanoparticles for image-guided and targeted cancer therapy

Recent advances in the development of magnetic nanoparticles (MNPs) have shown promise in the development of new personalized therapeutic approaches for clinical management of cancer patients. The unique physicochemical properties of MNPs endow them with novel multifunctional capabilities for imaging, drug delivery and therapy, which are referred to as theranostics. To facilitate the translation of those theranostic MNPs into clinical applications, extensive efforts have been made on designing and improving biocompatibility, stability, safety, drug-loading ability, targeted delivery, imaging signal and thermal- or photodynamic response. In this review, we provide an overview of the physicochemical properties, toxicity and theranostic applications of MNPs with a focus on magnetic iron oxide nanoparticles.

Lei Zhu[1,‡], Zhiyang Zhou[1,2,‡], Hui Mao[1] & Lily Yang*[,1]

[1]Departments of Surgery & Radiology & Imaging Sciences, Emory University School of Medicine, Atlanta, GA 30322, USA

[2]Xiangya School of Medicine, Central South University, Changsha, Hunan 410008, China

*Author for correspondence: lyang02@emory.edu

[‡]Authors contributed equally to this article

First draft submitted: 26 August 2016; Accepted for publication: 14 October 2016; Published online: 23 November 2016

Keywords: iron oxide nanoparticle • magnetic nanoparticles • theranostic

Cancer is characterized by the uncontrolled growth and spreading of abnormal cells. Human cancer is a heterogeneous disease with a wide range of genetic abnormalities and responses to therapeutics. The development of resistance to chemo- and radiotherapy has been the major issue in clinical management of cancer patients [1,2]. Although progress have been made in cancer diagnosis and treatment, survival of cancer patients remains low, especially for those diagnosed at the late stage. Therefore, there is an urgent need to develop sensitive diagnostic and effective therapeutic approaches for improvement of the prognosis of cancer patients [3].

Recent advances in nanomedicine have shown promises for targeted delivery of anticancer agents using engineered nanomaterials. These materials, usually in a nanoscale range, can be classified as organic nanoparticles (lipids, polymers, liposomes, polymeric micelles, dendrimers, and engineered peptides and nucleic acids) and inorganic nanoparticles (carbon nanoparticles, metal and metal oxide nanoparticles) [4–8] (Figure 1). In comparison with conventional chemotherapy, nanoparticle drug carriers offer the optimized drug formulations and improved pharmacokinetics to deliver drugs into tumors while reducing systemic toxicity. Unique physical, chemical and optical properties of nanomaterials provide the opportunity of imaging drug delivery into targeted tissues by noninvasive imaging approaches, and thermal or photo-controlled drug release. Such theranostic nanoparticles have the potential to develop targeted and image-guided cancer therapeutic protocols using a single nanoparticle platform.

Human tumors have heterogeneous blood vessel distributions and tumor stromal components that create barriers for drug delivery. The ability of timely monitoring drug delivery and response to therapy using theranostic nanoparticles allows the development of image-guided cancer therapeutic approach to evaluate drug delivery efficiency and intratumoral drug distribution, so that a precision

Future Medicine part of fsg

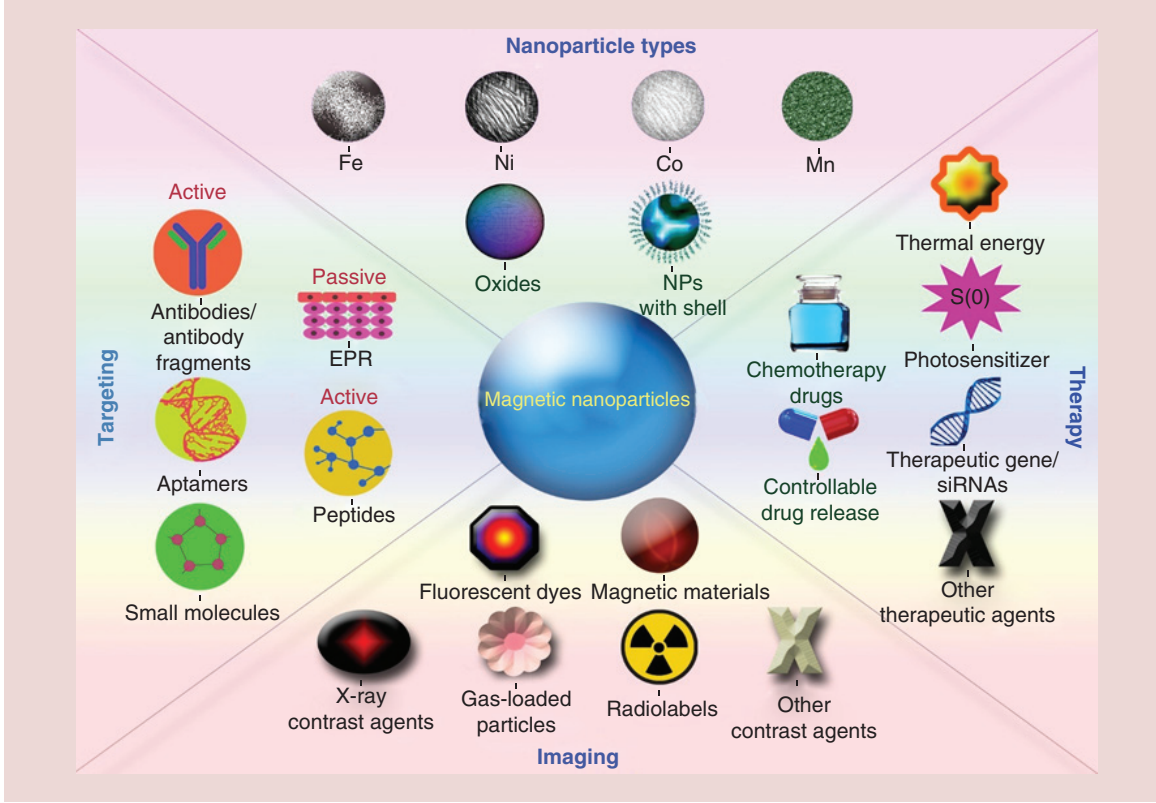


Figure 1. Types, modifications and functions of theranostic magnetic nanoparticles in biomedical applications.

oncology protocol can be applied to achieve effective therapeutic outcome for individual patients. This image-guided drug delivery is especially important for human tumors with enriched dense tumor stroma, such as pancreatic cancer and triple-negative breast cancer. At present, liposomes and polymeric nanoparticles are commonly used nanoparticle drug carriers and several of them have been approved by the US FDA for human use [9,10]. However, converting those nanoparticles into theranostic nanoparticles requires incorporation of imaging agents into the nanoparticles, such as radioactive, optical and MRI contrasts [6,11–13]. Following administration, those nanoparticle drug carriers loaded with imaging agents can be broken-down *in vivo* and subsequently release contrast agents, resulting in different tissue distributions for the imaging contrasts and the drug carriers. Therefore, to accurately assess biodistribution and tumor-targeted delivery of nanoparticle drug carriers, nanomaterials with imaging properties that are detectable by noninvasive imaging approach and drug-loading capacity are more suitable for the development of theranostic nanoparticles. Since cancer therapy often requires repeated administrations of high doses of drug in patients, it is essential to select drug-carrying nanomaterials that have low systemic toxicity and are biocompatible and biodegradable. Finally, for clinical translation, it is important to use nanoparticles with strong and long-lasting imaging signals that can be detected by a clinical available imaging modality. At present, magnetic iron oxide nanoparticle (IONP) or superparamagnetic iron oxide nanoparticle (SPION) is one of the few nanoparticle platforms that fit the above criteria for the development of theranostic agents for clinical applications [14–16]. As increasing need of image-guided cancer therapy for designing personalized therapeutic protocols for cancer patients, advances in the translational development of IONPs should have significant impact on the clinical management and prognosis of cancer patients.

During the last decade, extensive studies have been carried out to improve the chemical and physical properties of magnetic IONPs to make them suitable for biomedical applications in humans. Significant discoveries have been made in the production of IONPs with different core sizes, surface functions, MRI contrast properties and drug-loading capacity [15,17]. For example, various surface modifications have been developed to reduce nonspecific uptake of IONPs by macrophages in the reticuloendothelial system, such as polyethylene glycol (PEG) or antifouling polymer coating for altering the surface properties of IONPs [18,19]. Additionally, shape of nanoparticles also affects blood half-life, macrophage uptake, extravasation and internalization of nanoparticles by cells [20,21]. A marked feature of

magnetic IONPs is the ability of the production of controlled and uniform core size nanoparticles with a size-dependent magnetization. Ultrafine IONPs at a sub-5-nm core size have been developed for improved delivery efficiency and dual MRI contrast [14,18]. Such small-size IONPs can be efficiently delivered into tumors through the enhanced permeability and retention effect mediated by the leaking tumor vasculatures [22,23]. To further increase delivery efficiency, various targeting ligands have been conjugated to magnetic IONPs to enhance intratumoral delivery, distribution and retention of nanoparticles. Magnetic IONPs targeted to cell receptors highly expressed in tumor vasculatures, stromal cells and tumor cells have been developed [24].

Typically, targeting ligands include antibodies or engineered antibody fragments, natural ligands, peptides, structured DNA and RNA molecules, and small molecules [24] (Figure 1). Extensive preclinical studies have been conducted using different tumor-targeting ligand-conjugated IONP modified with therapeutic agents for disease detection and treatment applications [25,26]. The effects on tumor imaging and drug delivery of several targeted IONPs have been demonstrated in animal tumor models in mice [3,4,25–28]. At present, nontargeted and polymer-coated IONPs are one of the few FDA-approved nanomaterials that have been used in humans as MRI contrast agents (Table 1). With appropriate targeting ligands and surface modifications, an enhanced accumulation of IONPs in tumor tissues by passive or active targeting has been shown in animal tumor models while significantly reducing nonspecific accumulation in the liver and spleen [29,30]. Moreover, the unique magnetic properties of IONPs offer strong MRI contrast enhancing effect for MRI to monitor of drug delivery, evaluate treatment responses and external magnetic-field-controlled drug release or hyperthermia treatment [31]. Therefore, IONP is an excellent candidate for the development of new tumor imaging, targeted drug delivery and image-guided therapy that have the potential for novel clinical applications.

In this article, we provide an overview about recent progresses in the development of IONPs for cancer theranostics. First, we review factors affecting pharmacokinetics and biodistributions of IONPs. As examples of cancer theranostic applications, several recent cancer theranostic studies are highlighted here. Finally, challenges and considerations for clinical translation of theranostic IONPs are discussed.

Physical & chemical characteristics of IONPs

The magnetism of magnetic IONPs makes them applicable as MRI contrast agents. Sensitivity and specificity of cancer detection by MRI using nontargeted or targeted IONPs have been demonstrated in experimental animals as well as in human patients [32–34]. Results of those studies showed that core size, shape, surface property and functionalization of IONPs affect *in vivo* biodistribution, blood half-life, stability, pharmacokinetics, targeted delivery and MRI contrast intensity. Thus, those properties have to be taken into consideration for designing an IONP-based theranostic agent [35].

Size

In vivo applications of IONPs generally rely on transportation of nanoparticles to target organs and tissues via the blood circulation. Physicochemical properties of nanoparticles including particle size, shape and surface properties determine their behaviors in the blood, interactions with plasma proteins, uptake and clearance by macrophages, which consequently affect biodistribution and targeted delivery of payload drugs to the tumors [36–38]. It has been shown that size of IONPs has a significant effect on their *in vivo* behavior and uptake by cells. Changes in nanoparticle size and shape could lead to alterations in receptor cross-linking and cellular responses [38–40]. It is generally agreed that nanoparticles at diameters ranging from 10 to 100 nm are optimal for *in vivo* applications with acceptable pharmacokinetics. IONP sizes developed by different groups are all within the optimized size range for *in vivo* delivery [18,41,42] (Figure 2). Ultrasmall IONPs with core size (<10 nm) and ultrafine IONPs (<5 nm) have been synthesized and characterized [18,43]. Those small IONPs are promising IONPs because they can be excreted from the kidneys for body clearance. Small core size IONPs also have a short degradation time after being internalized into cells or reticuloendothelial system organs. More importantly, small IONPs can extravasate from the tumor blood vessels by the enhanced permeability and retention effect and then navigate through tumor stromal barriers more efficiently than that of the larger nanoparticles. Leaky and irregular tumor vessels allow extravasation of smaller size nanoparticles into the tumor tissues [44]. Furthermore, large nanoparticles have a tendency of aggregation under physiological conditions. The momentum force is stronger in larger particles, which lead to a high probability of wall collision and rapid clearance by mononuclear phagocytic system [38,45]. In an *in vivo* study, various sizes of IONPs (diameters of 10, 20, 30 and 40 nm) were delivered into mice to determine biodistribution and systemic toxicity [46]. Results showed the highest uptake of the smallest nanoparticles (10 nm) into the liver, while the spleen had the highest uptake of the largest nanoparticles (40 nm). The 10-nm IONPs were cleared faster from the kidney and liver compared with other groups.

Table 1. Current FDA-approved iron oxide nanoparticles for cancer imaging and therapy.

Name and formulation	Application	Status	Ref.
Feridex® Sterile aqueous colloid of superparamagnetic iron oxide coated with dextran	MRI	FDA-approved	[111–113]
Combidex® Ultrasmall superparamagnetic iron oxide covered with low-molecular weight dextran	MRI	Approved in Europe	[114,115]
Resovist® Superparamagnetic iron oxide nanoparticles coated with carboxyl dextran	MRI	Approved in Europe	[116–118]
Gastromark® Aqueous suspension of silicone-coated superparamagnetic iron oxide nanoparticle	MRI	FDA-approved	[28,119]
Feraheme® Nonstoichiometric superparamagnetic iron oxide coated with polyglucose sorbitol carboxymethylether	Iron-replacement therapy; MRI	FDA-approved	[120]
NanoTherm™ Aqueous dispersion of superparamagnetic iron oxide nanoparticles	Hyperthermia	Clinical trial	[121]
NCT01270139 Iron-bearing nanoparticles	Hyperthermia	Clinical trial	ClinicalTrials.gov
NCT01436123 Gold nanoparticles with iron oxide-silica shells	Hyperthermia	Clinical trial	ClinicalTrials.gov

In addition, shape also affects *in vivo* biodistribution of IONPs because attachment and subsequent cellular uptake of IONPs are shape-dependent [47]. IONPs can be prepared in different shapes including spheres, hexagons, cubes, rods and disks. Macrophages have different affinities to different types of IONPs and efficiently take up larger sized nanoparticles [38,48]. Inhibition of macrophage uptake prolonged the blood circulation time of nanoparticles [49].

A major application of magnetic IONPs in molecular imaging is the potential for the development as non-targeted or targeted MRI contrast agents. IONPs with higher saturation magnetization have better sensitivity for MRI detection, given stronger contrast enhancement, typically, sharply reducing the transverse relaxation time, or T_2, of the surrounding water. IONPs with a core size greater than 10 nm have been generally considered as T_2 contrasts, producing dark MRI contrast. Saturation magnetization is size-dependent until it reaches a threshold size [50,51]. Increasing IONP size leads to an increase in magnetic anisotropy energy per nanoparticle [52]. Relaxation rates are also size-dependent. Lager IONPs exhibit a higher transverse relaxivity [53,54], and consequently produce stronger MRI contrasts. When the nanoparticle core size is smaller (<5 nm), the T_2 contrast or darkening effect is reduced, while the effect on longitudinal relaxation time, or T_1 contrast, becomes more prominent. Thus, T_1 signal is dominated and gives rise to bright T_1 MRI contrast as demonstrated *in vitro* and *in vivo* by sub-5-nm core size ultrafine IONPs reported by Huang *et al.* [18]. Understanding the interplay between T_1 and T_2 contrast at different core sizes, one can use ultrashort echo time MRI sequence to obtain bright T_1 contrasts with larger sized IONPs as demonstrated in the study in which the accumulation of 10-nm core size IONPs can be observed with ultrashort TE MRI with bright T_1 contrast in orthotopic pancreatic tumors following systemic delivery [55]. To obtain the highest magnetism under size of 100 nm, a recent report showed that 65-nm Fe_3O_4 nanospheres presented the strongest saturation magnetization (89.24 emu/g) and microwave reflection loss (-30.37 dB at 9.68 GHz), compared with other IONPs at different sizes [56].

Surface properties

Surface charge of IONPs contributes to colloidal stability, circulation time, cellular uptake and toxicity of IONPs. IONPs have a low stability without surface modifications and easily form aggregates. Physical dimension and density of the aggregates significantly affect the reactive surface area, reactivity, bioavailability and toxicity [57]. Surface coating of IONPs increases stability and provides space and functional groups for

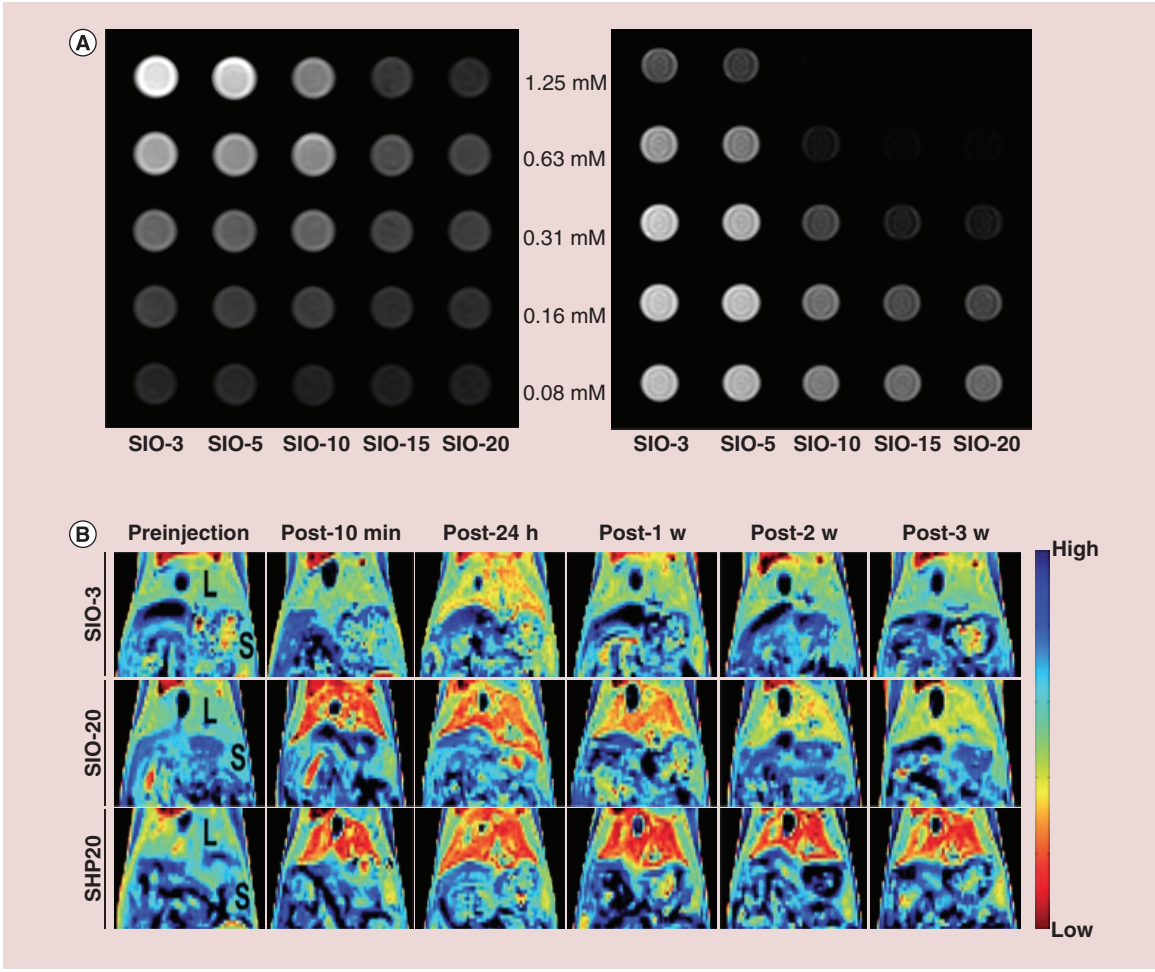


Figure 2. Size effects on MRI signals and biodistribution of iron oxide nanoparticle. (A) The MRI contrast enhancement effects of IONPs. T_1- (left) and T_2- (right) weighted MR images of the IONPs aqueous solutions with different Fe concentrations were demonstrated. SIO_3 exhibits the best T_1 contrast enhancement due to the highest surface-to-volume ratio. **(B)** Clearance studies of intravenously administered with different size of IONPs. A fast clearance of SIO_3 in the RES organs, such as the live and spleen, was found.
IONP: Iron oxide nanoparticle; RES: Reticuloendothelial system; SIO_3: 3.5-nm IONPs coated with oligosaccharides.
Reproduced with permission from [18] © the Royal Society of Chemistry (2014).

bioconjugation and drug loading. However, surface modifications of IONPs with different polymers led to an increase in the hydrodynamic particle size that may affect superparamagnetic properties [38]. Surface coating for IONPs includes dextran, PEG, carboxymethyldextran [58], PEI–PEG-chitosan-copolymer [59], amphiphilic copolymer [60], protein [61] and antifouling polymer [62]. Dextran-coated SPION was the first polymer-coated IONP that has been extensively investigated in experimental animal models and in humans as an MRI contrast. Systemic delivery of the long-circulating dextran-coated iron oxide particles led to intratumoral accumulation of 0.11% of the injected long-circulating dextran-coated iron oxide dose per gram of tissue in a rat brain tumor model and the signal was detectable by MRI [63]. In human studies, nontargeted dextran-coated IONPs have been used in cancer patients as MRI contrasts for the detection of liver tumors or lymph node metastases [64–66].

The effect of the surface charge on nanoparticle stability and nanoparticle–cell interaction was examined using different charged polyvinyl alcohol (PVA)-coated SPIONs. Results showed that positively charged aminated PVA SPIONs formed aggregates easily. On the other hand, negatively charged carboxylated PVA SPIONs and neutral PVA-coated SPIONs were less likely to form aggregates, implying that PVA-SPIONs had a lower colloidal stability than other groups. Additionally, nanoparticles with a positive-charged surface interact with the negative-charged cell surfaces to facilitate nanoparticle uptake by cells. Neutral and negatively charged nanoparticles have also been shown to prolong blood half-lives due to the reduced adsorption of serum proteins [67].

To develop biodegradable and functionalized IONPs, various proteins, such as albumin and milk protein [18,61], have been used to coat the nanoparticles. Milk protein, casein, has been shown to be a good protein to coat IONPs. Casein-coated IONPs not only stabilized the IONPs but also provided functional groups for drug conjugation. Following being internalized into cells, dissociation of casein protein coating from IONPs caused the formation of IONP aggregates and enhanced MRI T_2 contrast effect [18]. Targeting ligands, such as single-chain anti-EGFR antibody, could also be conjugated to the casein protein-coated IONPs and showed targeted accumulation of nanoparticles in a human breast cancer xenograft model in nude mice, allowing MRI detection of tumors using T_2-weighted MRI [18].

New surface modifications for IONPs have been shown to reduce nonspecific interactions with serum proteins and macrophage uptake. For instance, coating SPIONs with an anti-biofouling copolymeric system, named (trimethoxysilyl)propyl methacrylate-PEG-methacrylate increased biostability of SPIONs and reduced 'opsonization' process [62,68]. Resulting poly(trimethoxysilyl)propyl methacrylate-PEG-methacrylate-coated SPION had excellent biocompatibility, tumor-targeting ability and long-circulated time *in vivo*. Additionally, an anti-biofouling polymer-PEO-block-poly(γ-methacryloxypropyltrimethoxysilane) (PEO-*b*-PγMPS) has been developed to coat IONPs [69]. In comparison with other surface modifications, PEO-*b*-PγMPS-coated nanoparticles had significantly reduced nonspecific binding to serum proteins and uptake by macrophages in the liver and spleen. In a recent study, HER2 antibody and single-chain anti-EGFR antibody-conjugated PEO-*b*-PγMPS-IONPs showed a high level of the accumulation in tumors following systemic delivery, suggesting the potential for using this system to improve efficiency of tumor-targeted delivery [30].

Biodistribution, safety & degradation

The potential toxicity is the major concern for the most nanomaterials in clinical applications. The effect of any new formulations of IONPs should be evaluated thoroughly in experimental animals before human trials [70]. At a cellular level, induction of reactive oxygen species (ROS) after internalization of IONPs into cells has been found *in vitro* in various cell lines and *in vivo* in animal models [71]. It has been shown that internalization of SPIONs-COOH in cells increased the level of ROS that led to the alteration of the levels of expression of cell proliferative responsive genes. It has been shown that different cell types have different pathways to detoxify IONPs. Overall systemic toxicity following repeated *in vivo* administrations of IONPs has been shown to be very low. Following intravenous injection of pluronic polymer-coated IONPs into rats for 3 weeks, there was no long-term toxicity in the major organs. Using ^{59}Fe radiotracer-labeled superparamagnetic iron oxide preparation (AMI-25), it was possible to follow-up distribution, degradation, bioavailability and excretion of the nanoparticles *in vivo*. Results showed that the majority of the delivered nanoparticles accumulated in the liver (86%) and spleen (6.2%) within 1 h following intravenous injection. ^{59}Fe-labeled iron nanoparticles could be cleared out from the liver and spleen with a half-life of 3–4 days. ^{59}Fe was then found to be incorporated into hemoglobin of erythrocytes. Even with a high dose of 3000 μmol Fe/kg that is 150-times over the *in vivo* imaging dose, there was no acute or subacute toxic effects in rats or beagle dogs [70].

However, it has been shown that enhanced nonspecific uptake of positively charged IONPs into cells led to toxicity in normal tissues and cells [72]. Repeated systemic delivery of IONPs coated with positively charged polyethyleneimine or poly(acrylic acid) polymer coating in pregnant CD-1 mice resulted in decreased maternal weight gain, increased fetal deaths and accumulation of iron in the fetal liver and placenta [73]. Therefore, neutral and negatively charged nanoparticles are suitable for the production of targeted nanoparticle-imaging probes and drug carriers for *in vivo* applications.

Recently, the effect of systemic delivery of urokinase plasminogen activator receptor (uPAR)-targeted magnetic IONPs was examined in rhesus monkeys. Results showed that two monkeys tolerated well with intravenous injection of 5 mg/kg iron equivalent dose of uPAR-targeting ligand-conjugated amphiphilic polymer-coated IONPs, with or without PEG modification. There was no apparent systemic toxicity during acute phase (3 days) and long-term (up to 3 months). There was only a transient increase in serological levels of liver functional enzymes within 72 h following injection but returned to the normal in 20 days. Liver biopsy at 3 day or 3 months reveal no microscopic tissue damage [74].

Safety, biodistribution and pharmacokinetics of IONPs in humans have also been evaluated in patients. Ferumoxytol (Feraheme), is an FDA-approved SPIO-based MRI contrast agent as well as for the treatment of iron deficiency anemia in patients with chronic kidney disease. As intravenous doses for iron-deficient therapy, up to 510 mg/dose of Ferumoxytol have been administered safely in more than 700 patients in a clinical study [75,76]. Results from a Phase II/III clinical trial in over 2000 patients for MRI detection of lymph

node metastasis showed that it is safe to administer 2.9 mg Fe/kg dose of magnetic SPIONs (30 nm size) in humans as SPIONs were taken up by the macrophages in the spleen, liver and lymph nodes [77,78]. In general, a clinical dose of IONPs in humans (0.56–3 mg Fe/kg) will be much less than the normal blood iron concentration (≈33 mg Fe/kg body weight) and total body iron (≈3500 mg) [14]. Thus, it is likely that magnetic IONP-based drug carriers have less safety concern than other nanomaterials for the translational development of new theranostic agents for image-guided and targeted cancer therapeutic approaches. However, novel IONPs may give very different effects with respect to commercial ones since many factors affect the *in vivo* behavior of IONPs as we discussed above. Additionally, a low-cost and uniformed IONP complex production method is required for further development of INOPs as theranostic agents into clinical trials.

Theranostic applications of magnetic nanoparticles in cancer

Taking advantage of the unique physical and chemical properties of magnetic IONPs, extensive studies have been carried out to develop new and improved theranostic IONPs in preclinical studies [79]. To accurately assess the efficiency of intratumoral drug delivery, especially nanoparticle-mediated drug delivery, noninvasive imaging is preferred in individual patient since human tumors have highly heterogeneous tumor blood vessel distributions and stromal drug delivery barriers. Such a precision oncology approach using image-guided drug delivery system, or theranostic nanoparticles, should allow timely assessment and adjustment of treatment strategies for cancer patients. In addition to the promising imaging property, theranostic nanoparticles have been produced to carry a single therapeutic agent or the combination of two or more drugs [80,81], including chemotherapy drugs, small-molecule agents, photosensitizers and siRNAs. Significant advantages of nanoparticle-formulated drug delivery include: first, increasing the effective drug dose by selective delivery of a large amount of drug molecules, especially highly insoluble drug-loaded nanoparticles, into the tumor while reducing systemic side effects [82]; second, protecting drug molecules or biological therapeutic agents (siRNAs or peptides) from degradation before reaching target tissues and cells [83] and third, targeted delivery through cell receptors that bypasses multidrug-resistant mechanisms on the tumor cell membrane [84]. For example, gold-coated IONPs with cisplatin were used to treat human ovarian cancer cell lines and tested for the drug delivery efficiency [85]. The gold-coated IONPs with cisplatin exhibited up to 110-fold higher toxicity than cisplatin in those cancer cell lines. Moreover, in response to a laser irradiation, the PEGylated iron, iron/iron oxide core/shell nanoparticles (Fe@Fe_3O_4 NPs) were able to generate a stronger effect in tumor cell killing both *in vitro* and *in vivo* by photothermal therapy [86]. Furthermore, photodynamic therapy involved in nanoparticles conjugated with photosensitizers that could be activated by specific wavelength of light and then produced ROS to kill tumor cells [87]. Obviously, the development of theranostic nanoparticles with the ability of targeted drug delivery and imaging has the potential to contribute significantly to the personalized and effective cancer treatment.

Cancer diagnostics

In the past decades, the ability of IONPs or SPIONs as MRI contrast agents have attracted great interests in further development of those nanoparticles as cancer theranostics [12,88,89]. MRI has high-imaging resolution, 3D-imaging capability and anatomical information in soft tissues for the detection of intratumoral nanoparticle-drug delivery and distribution. Various targeted IONPs have been developed and evaluated for the feasibility as targeted MRI contrasts. Targeted IONPs are produced by covalently or noncovalently modifications with molecular-targeting ligands that bind to specific cancer biomarkers or overexpressed surface moieties on cancer cells. For example, peptides, antibodies or antibody fragments that specifically bind to receptors overexpressed in tumor cells, such as MUC-1, αvβ3 integrin, EGFR, HER2/neu, uPAR and prostate-specific membrane antigen, were conjugated to the surface of IONPs, leading to the targeted accumulation and retention of the IONPs in tumor tissues, and resulting in T_2 contrast for the detection of tumors by MRI [90–94]. Furthermore, receptor-mediated endocytosis increased intratumoral delivery of nanoparticles and relatively long-term retention of the nanoparticles in tumors for sensitive imaging of drug delivery and tumor responses to the therapy [95].

Another growing trend in IONP-mediated cancer diagnostics is the use of multimodal imaging agents. Up to now, various single or multiple imaging modality IONPs have been developed for optical, PET, SPECT, MRI and photoacoustic imaging. Several recently developed IONPs with optical imaging property have been investigated. For example, Ling *et al.* developed tumor pH-sensitive magnetic nanogrenades that were composed of self-assembled IONPs and pH-responsive ligands. These pH-sensitive magnetic nanogrenades could target to tumor cells and disassembled into active compartments under acid conditions to produce MRI and fluorescent signals, which led to the detection of small tumors with a diameter of 3 mm [96]. In addition,

IONP-based hybrid nanoparticles coated with gold or quantum dots required surface plasmon resonance effect [97]. Overall, IONPs with multimodal imaging capabilities could provide clinicians with a powerful probe for both cancer detection and monitoring of intervention efficacy.

Chemotherapeutic agent delivery

Chemotherapeutics consist of a broad category of small organic drug formulations, which have been developed to initiate therapeutic responses via cytotoxic, cytostatic or antineoplastic effects. Depending on chemical properties of therapeutic agents, they can either be conjugated to the surface active groups of IONPs or be encapsulated inside polymer coating [98,24]. So far, several chemical drugs, including paclitaxel, doxorubicin (DOX) and methotrexate, have been loaded to IONPs for cancer therapy. For the development of theranostic IONP-carrying drugs, it is necessary to consider loading, conjugation efficiency and mechanism of release of drug molecules. Quan *et al.* reported human serum albumin-stabilized IONPs for the delivery of DOX into tumors guided by MRI. A significant increase in the blood half-life of DOX and drug accumulation in tumors was observed in this study [99]. Recently, anti-CD44 antibody-targeted IONPs loaded with gemcitabine (Gem) have been produced and showed target specificity and growth inhibition of tumor cells [100].

It is well known that tumor stroma plays important roles in the development, biology and therapeutic response of human tumors. Although the presence of tumor stromal cells creates a physical barrier that limits invasion and migration of tumor cells, it is clear that active tumor stromal cells also produce cytokines and factors that promote tumor cell growth, invasion and metastasis. Tumor stroma also serves as a drug delivery barrier. Without the ability to overcome the tumor stromal barrier, the majority of delivered nanoparticles are sequestered at the perivascular areas [101]. To overcome the physical barrier of the stroma in drug delivery, theranostic nanoparticles targeting uPAR that is highly expressed in both cancer cells and tumor-associated stromal cells have been developed and their antitumor effects have been examined in a human pancreatic cancer xenograft model in nude mice. uPAR-targeted ligand, derived from the amino-terminal fragment (ATF) peptide of urokinase plasminogen activator was conjugated onto amphiphilic polymer-coated IONPs carrying conditional release chemotherapy drug, Gem (ATF-IONP-Gem) [93]. Systemic delivery of uPAR-targeted ATF-IONP-Gem resulted in a significant growth inhibition of pancreatic tumors. Nanoparticle–drug delivery and changes in MRI contrast and tumor size could be detected by MRI. To detect drug-resistant residual tumors, an ultrashort TE MRI scan method was developed and produced MR images with bright T_1 contrast in drug-resistant tumors containing delivered ATF-IONP-Gem [93].

Resistance to chemotherapy is a major and unmet challenge. Recent studies have demonstrated the ability of overcoming drug-resistant mechanism on the tumor cell membrane by nanoparticle-mediated internalization of nanoparticle–drug complexes. It has been shown that hollow IONPs modified with human serum albumin and incorporated with DOX had a significantly higher level of intratumoral cell nanoparticle–DOX delivery compared with conventional DOX treatment in a multidrug-resistant human ovarian cancer OVCAR8-ADR cell line [84]. This might be a result of decreased efflux of nanoparticle-drugs by P-glycoprotein that locates on the cellular membrane and transports drug molecules out of cells.

Increasing evidence shows that IGF1R is highly expressed in drug-resistant tumor cells and tumor stromal cells [92]. Recombinant human IGF1 has been used as a targeting ligand to be conjugated to theranostic INOP-carrying DOX. The effect of the theranostic IONPs was evaluated in an orthotopic human pancreatic cancer patient tissue-derived xenograft model that recapitulated heterogeneous tumor cells and enriched tumor stroma in human pancreatic cancer [92]. Results of this study showed that IGF1R targeted IGF1-IONP-DOX theranostic nanoparticles efficiently targeted to pancreatic tumors and were detectable by optical imaging and MRI. Repeated delivery of IGF1-IONP-DOX led to breaking tumor stromal drug delivery barrier and significant tumor growth inhibition in this human pancreatic cancer patient tissue-derived xenograft model in nude mice (Figure 3). Histological analysis also showed inhibition of cell proliferation and induction of cell apoptosis in pancreatic cancer cells following IGF1-IONP-DOX treatment.

Magnetic hyperthermia

Unlike chemotherapy, hyperthermia induces cancerous cells undergoing apoptosis under high temperature conditions [102]. It also sensitizes cancer cells to radiation therapy or chemotherapy. Specifically, IONPs could locally convert external high-frequency field energy to thermal energy, which is called magnetic hyperthermia [102–105]. For example, magnetic hyperthermia mediated by IONPs could increase the temperature at the tumor center to greater than 40°C after exposing to alternating magnetic field [106], resulting in tumor growth inhibition in a human head and neck tumor xenograft model. IONP-facilitated magnetic hyperthermia has been translated in human patients in clinical trials [107]. After received IONP-induced

hyperthermia and low-dosage radiotherapy, a significant increase in survival of the patients with recurrent glioblastoma was observed in the combination treatment group comparing with control groups. No serious complications were found in the clinical trials, suggesting the combination of thermotherapy and radiotherapy is safe and effective approach. As aforementioned, IONP-mediated magnetic hyperthermia triggered by the external magnetic field not only ablated cancer cells but also increased the effectiveness of other treatments. In comparison with laser-triggered photothermal therapy, magnetic hyperthermia therapy is more promising in translation due to the unlimited tissue penetration ability and reduced skin damage. Although there may be some magnetic–thermal conversion efficient concerns, it can be solved by radio/magnetic hyperthermia or chemo/magnetic hyperthermia-combined therapy.

siRNA

RNA interference, or gene silencing, is referred to utilization of siRNA molecules that specifically turn off the expression of a target gene. It has great therapeutic

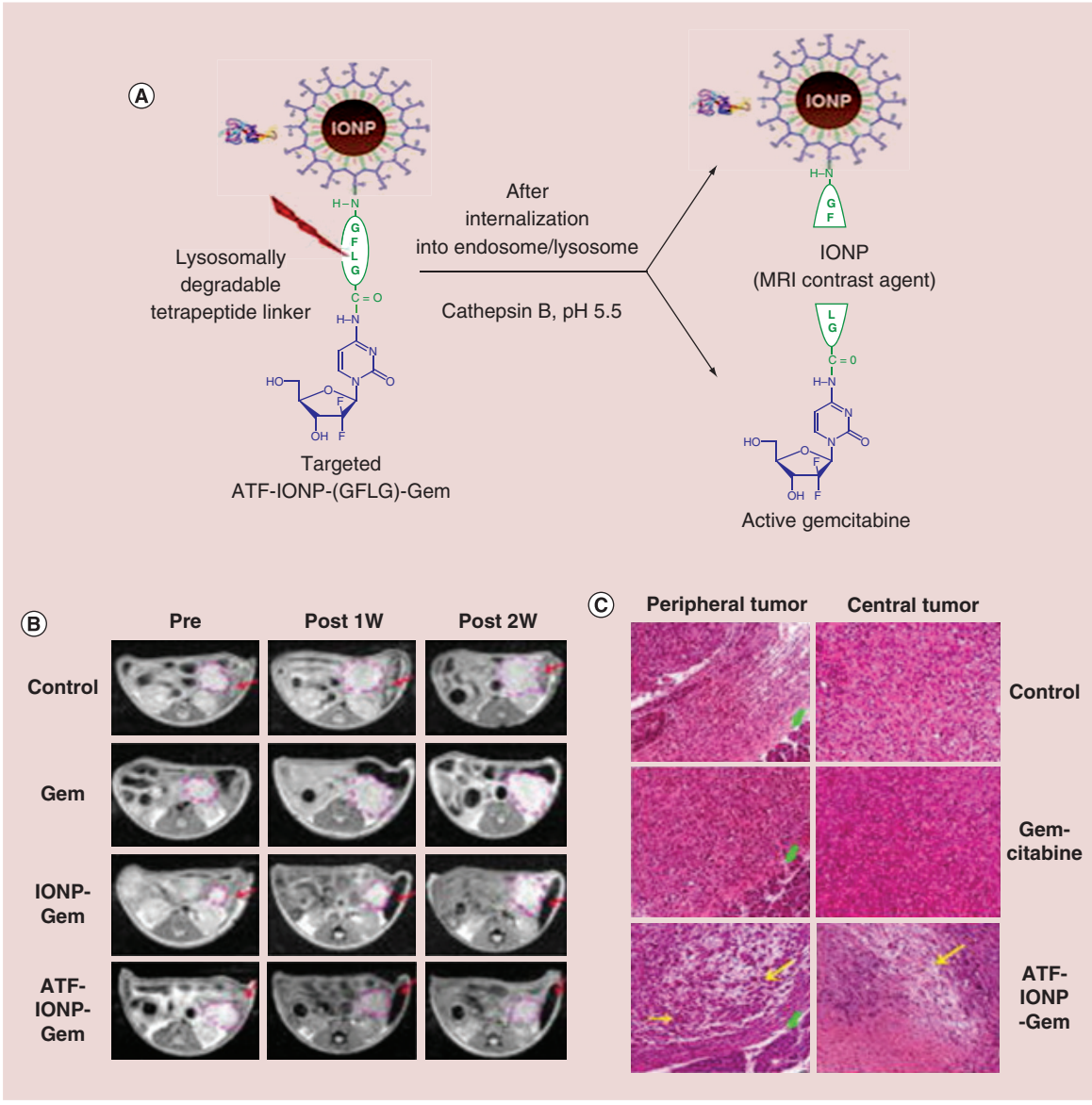


Figure 3. Theranostic iron oxide nanoparticles for MRI-guided chemotherapy of pancreatic cancer. **(A)** Design of activatable IONP theranostic complex. Gemcitabine is conjugated onto IONP via a cathespin B substrate and will be released in endosome and lysosome. The amino-terminal fragment (ATF) peptides of uPA is used for targeting pancreatic tumor. **(B)** MRI monitoring the therapeutic response of controlled released gemcitabine. **(C)** Histologic staining confirms the targeted therapeutic of pancreatic tumor.
IONP: Iron oxide nanoparticle; uPA: Urokinase plasminogen activator.
Adapted with permission from [93]. © American Chemical Society (2016).

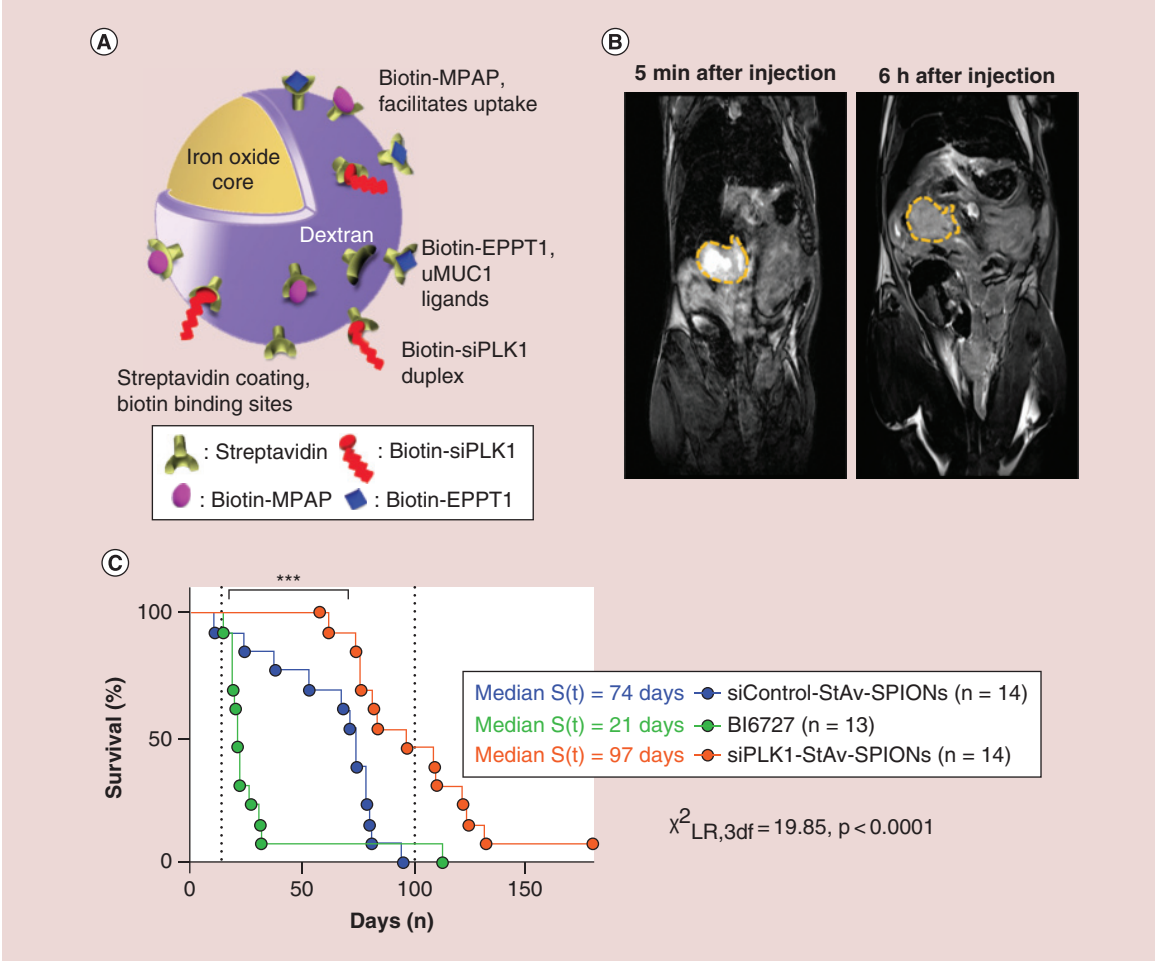


Figure 4. Theranostic iron oxide nanoparticles for MRI-guided gene therapy of pancreatic cancer. (A) Schematic representation of si*PLK1*-coupled streptavidin-conjugated dextran-coated SPIONs (si*PLK1*-StAv-SPIONs) conjugated to the membrane translocation peptide for mediating transportation to the cytoplasm (myristoylated polyarginine peptides), the underglycosylated MUC1 (uMUC1)-specific peptide (EPPT1) and siRNA molecules targeting PLK1 (si*PLK1*). **(B)** MRI visualization of the accumulation of si*PLK1*-StAv-SPIONs in pancreatic tumor. **(C)** Kaplan–Meier survival analysis from the time of enrolment to treatment with siControl-StAv-SPIONs (n = 14), BI6727 (n = 13) or siPLK1-StAv-SPIONs (n = 14). Dotted line window indicates maximum duration of therapy. Median survival time of siPLK1-StAv-SPION treatment (96 days) was significantly different to the siControl-StAv-SPION treatment (74 days). EPTT1: Palmitoyl-protein thioesterase 1; MUC1: Mucin 1; PLK1: Polo-like kinase-1; SPION: Superparamagnetic iron oxide nanoparticle.
Reproduced with permission from [110] © BMJ Publishing Group Ltd. (2016).

potential for diseases caused by abnormal gene expressions or genetic mutations. However, the main barrier for safe and effective gene therapy is a low efficiency in delivery of those macromolecules. Due to the excellent biocompatibility and biodegradable ability, IONP has been used as a carrier for gene delivery. In addition, magnetic property of IONPs allows MRI monitoring and targeted delivery of functional genes by utilizing an external magnetic field. For example, an NIR dye/IONP/siRNA complex was produced to successfully track the tumor uptake of this siRNA delivery system by MRI and optical imaging [108]. The silencing process could also be visualized, providing a reliable siRNA delivery and imaging approach that is important for the development of cancer theranostic agents. Sun *et al.* developed a PEG-coated IONP as a miRNA delivery system for overcoming drug resistance in gastric cancer cells by enforcing miR16 expression in tumor cells. The combination of *miR16* delivery with adriamycin treatment increased sensitivity of tumor cells to drug treatment and enhanced tumor growth inhibition in a drug-resistant gastric cancer mouse model [109]. More recently, a multifunctional SPION was used to deliver siRNAs that downregulated the cell cycle-specific serine-threonine-kinase, polo-like kinase-1 (PLK1) gene in pancreatic ductal adenocar-

cinoma [110]. A pancreatic cancer-targeting ligand and an endosomal escape peptide were also attached onto SPIONs for effective tumor recognition and intracellular delivery (Figure 4). *In vitro* and *in vivo* studies demonstrated that this SPION-based siRNA delivery system successfully decreased PLK1 expression and in turn, ablated tumor growth as visualized by MRI. This newly developed theranostic SPION system provided a simultaneous tumor-specific gene therapy and monitoring tumor response approach.

Conclusion & future perspective

In this article, we summarized current research progresses on the development, optimization, physicochemical characterization, biodistribution, safety and theranostic application of IONPs or SPIONs. We discussed strategies of surface modifications, functionalization and drug loading for the development of theranostic IONPs. Imaging properties of IONPs have also been discussed in the review. Examples are presented to support the potential applications of theranostic IONPs in cancer imaging and therapy. By labeling with additional imaging agents, intratumoral accumulation of theranostic nanoparticles could be detected using a single or multimodal noninvasive imaging. As an imaging nanoparticle with high drug-loading capacity and low toxicity, IONPs have become an attractive theranostic nanoparticle platform for translational development of novel image-guided cancer therapeutic agents. It is believed that targeted cancer therapy in combination with image-guided drug delivery and monitoring tumor response to therapy using the advanced theranostic nanoparticles offer a powerful and integrated cancer therapeutic approach for effective treatment of highly heterogeneous human cancers. The ability of noninvasive detection of tumor location and intratumoral accumulation of theranostic nanoparticles is extremely useful for image-guided cancer phototherapy, in order to apply the therapy precisely on the tumor site and at the best time point.

Currently, investigations concerning various theranostic IONPs are still at the preclinical stage. To bring IONP-based theranostic agents into clinical applications, several key issues have to be addressed. The production of targeted theranostic IONPs at the GMP grade for human use is one of the major challenges. In addition to demonstration of targeted imaging ability and strong effect of targeted cancer therapy in several animal tumor models, short- and long-term toxicity, biodistribution and clearance have to be evaluated in animal models. Those preclinical results are required for the Investigational New Drug application to receive an approval of a clinical trial from the FDA. Additionally, it is also necessary to determine the optimal dose of theranostic IONPs to be administered in humans to achieve sensitive and specific tumor imaging while producing a strong therapeutic effect. Finally, new approaches to significantly improve intratumoral delivery of theranostic nanoparticles and to break tumor stromal drug delivery barriers are needed for effective treatment of stroma-rich human tumors. Although significant progresses have been made, it is still too early to predict the success of nanotechnology in cancer therapy. Additional investigations have to be conducted to answer some fundamental questions concerning the application of theranostic IONPs for clinical applications.

Financial & competing interests disclosure

This research project was supported by the NIH/NCI grants U01CA151810, 1U01CA198913, R01CA154846 and the Nancy Panoz Endowed Chair Funds. The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- Magnetic iron oxide nanoparticles (IONPs) have unique physical and chemical properties for the development of new cancer diagnostic and therapeutic approaches.
- Various theranostic IONPs with high drug-loading capacity and MRI or multimodal imaging ability have been developed. Those promising theranostic platforms are under extensive preclinical developments for future clinical translation of image-guided and targeted cancer therapy that allows personalized cancer treatment.
- IONP is one of the few imaging nanoparticles that have been used in human patients as an imaging contrast for cancer detection or treatment of anemia of chronic kidney disease.
- Extensive studies have been conducted for the improvement of biocompatibility, stability, safety, drug loading, targeted delivery, imaging signal and thermal- or photodynamic response of IONPs for translation into clinical applications as theranostic agents.
- Targeted theranostic IONPs have the potential to address the major challenge in the treatment of highly heterogeneous human cancers through MRI detection of biomarker expression in tumors, image-guided drug delivery and monitoring therapeutic responses.

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1 Anand P, Kunnumakara AB, Sundaram C *et al.* Cancer is a preventable disease that requires major lifestyle changes. *Pharm. Res. Dord.* 25(9), 2097–2116 (2008).

2 Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J. Clin.* 65(1), 5–29 (2015).

3 Johnson L, Gunasekera A, Douek M. Applications of nanotechnology in cancer. *Discov. Med.* 47, 374–379 (2010).

4 Buzea C, Pacheco II, Robbie K. Nanomaterials and nanoparticles: sources and toxicity. *Biointerphases* 2(4), Mr17–Mr71 (2007).

5 Xie L, Wang G, Zhou H *et al.* Functional long-circulating single-walled carbon nanotubes for fluorescent/photoacoustic imaging-guided enhanced phototherapy. *Biomaterials* 103, 219–228 (2016).

6 Zhang LW, Gao S, Zhang F, Yang K, Ma QJ, Zhu L. Activatable hyaluronic acid nanoparticle as a theranostic agent for optical/photoacoustic image-guided photothermal therapy. *ACS Nano* 8(12), 12250–12258 (2014).

7 Xie J, Lee S, Chen XY. Nanoparticle-based theranostic agents. *Adv. Drug Deliv. Rev.* 62(11), 1064–1079 (2010).

8 Gao S, Zhang LW, Wang GH *et al.* Hybrid graphene/Au activatable theranostic agent for multimodalities imaging guided enhanced photothermal therapy. *Biomaterials* 79, 36–45 (2016).

9 Puri A, Loomis K, Smith B *et al.* Lipid-based nanoparticles as pharmaceutical drug carriers: from concepts to clinic. *Crit. Rev. Ther. Drug* 26(6), 523–580 (2009).

10 Perez-Herrero E, Fernandez-Medarde A. Advanced targeted therapies in cancer: drug nanocarriers, the future of chemotherapy. *Eur. J. Pharm. Biopharm.* 93, 52–79 (2015).

11 Zhou H, Fan Z, Deng J *et al.* Hyaluronidase embedded in nanocarrier PEG shell for enhanced tumor penetration and highly efficient antitumor efficacy. *Nano. Lett.* 16(5), 3268–3277 (2016).

12 Fu G, Zhu L, Yang K, Zhuang R, Xie J, Zhang F. Diffusion-weighted magnetic resonance imaging for therapy response monitoring and early treatment prediction of photothermal therapy. *ACS Appl. Mater. Interfaces* 8(8), 5137–5147 (2016).

13 Makino A, Kimura S. Solid tumor-targeting theranostic polymer nanoparticle in nuclear medicinal fields. *Scientific World J.* 2014, 424513 (2014).

14 Huang J, Li YC, Orza A *et al.* Magnetic nanoparticle facilitated drug delivery for cancer therapy with targeted and image-guided approaches. *Adv. Funct. Mater.* 26(22), 3818–3836 (2016).

15 Laurent S, Forge D, Port M *et al.* Magnetic iron oxide nanoparticles: synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications. *Chem. Rev.* 108(6), 2064–2110 (2008).

16 Laurent S, Boutry S, Mahieu I, Vander Elst L, Muller RN. Iron oxide based MR contrast agents: from chemistry to cell labeling. *Curr. Med. Chem.* 16(35), 4712–4727 (2009).

17 Boni A, Marinone M, Innocenti C *et al.* Magnetic and relaxometric properties of Mn ferrites. *J. Phys. D Appl. Phys.* 41(13), 134021 (2008).

18 Huang J, Wang LY, Zhong XD, Li YC, Yang LL, Mao H. Facile non-hydrothermal synthesis of oligosaccharide coated sub-5 nm magnetic iron oxide nanoparticles with dual MRI contrast enhancement effects. *J. Mater. Chem. B* 2(33), 5344–5351 (2014).

19 Li YC, Lin R, Wang LY *et al.* PEG-b-AGE polymer coated magnetic nanoparticle probes with facile functionalization and anti-fouling properties for reducing non-specific uptake and improving biomarker targeting. *J. Mater. Chem. B* 3(17), 3591–3603 (2015).

•• **New surface coating for reducing iron oxide nanoparticle (IONP) nonspecific uptake.**

20 Toy R, Peiris PM, Ghaghada KB, Karathanasis E. Shaping cancer nanomedicine: the effect of particle shape on the *in vivo* journey of nanoparticles. *Nanomedicine* 9(1), 121–134 (2014).

• **Shape affects IONP biodistribution.**

21 Liu Y, Tan J, Thomas A, Ou-Yang D, Muzykantov VR. The shape of things to come: importance of design in nanotechnology for drug delivery. *Ther. Deliv.* 3(2), 181–194 (2012).

22 Aslan B, Ozpolat B, Sood AK, Lopez-Berestein G. Nanotechnology in cancer therapy. *J. Drug Target* 21(10), 904–913 (2013).

23 Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer-chemotherapy – mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. *Cancer Res.* 46(12), 6387–6392 (1986).

24 Markman JL, Rekechenetskiy A, Holler E, Ljubimova JY. Nanomedicine therapeutic approaches to overcome cancer drug resistance. *Adv. Drug Deliv. Rev.* 65(14), 1866–1879 (2013).

25 Wolfbeis OS. An overview of nanoparticles commonly used in fluorescent bioimaging. *Chem. Soc. Rev.* 44(14), 4743–4768 (2015).

26 Petros RA, Desimone JM. Strategies in the design of nanoparticles for therapeutic applications. *Nat. Rev. Drug. Discov.* 9(8), 615–627 (2010).

27 Stockhofe K, Postema JM, Schieferstein H, Ross TL. Radiolabeling of nanoparticles and polymers for PET imaging. *Pharmaceuticals (Basel)* 7(4), 392–418 (2014).

28 Bonnemain B. Superparamagnetic agents in magnetic resonance imaging: physicochemical characteristics and clinical applications. A review. *J. Drug Target* 6(3), 167–174 (1998).

29 Yu MK, Park J, Jon S. Targeting strategies for multifunctional nanoparticles in cancer imaging and therapy. *Theranostics* 2(1), 3–44 (2012).

30 Chen HW, Wang LY, Yu QQ *et al.* Anti-HER2 antibody and ScFvEGFR-conjugated antifouling magnetic iron oxide nanoparticles for targeting and magnetic resonance imaging of breast cancer. *Int. J. Nanomed.* 8, 3781–3794 (2013).

31 Wang C, Sun XQ, Cheng L *et al.* Multifunctional theranostic red blood cells for magnetic-field-enhanced *in vivo* combination therapy of cancer. *Adv. Mater.* 26(28), 4794 (2014).

32 Nandwana V, De M, Chu S *et al.* Theranostic magnetic nanostructures (MNS) for cancer. *Cancer Treat. Res.* 166, 51–83 (2015).

33 Muthiah M, Park IK, Cho CS. Surface modification of iron oxide nanoparticles by biocompatible polymers for tissue imaging and targeting. *Biotechnol. Adv.* 31(8), 1224–1236 (2013).

34 Issa B, Obaidat IM, Albiss BA, Haik Y. Magnetic nanoparticles: surface effects and properties related to biomedicine applications. *Int. J. Mol. Sci.* 14(11), 21266–21305 (2013).

35 Wahajuddin, Arora S. Superparamagnetic iron oxide nanoparticles: magnetic nanoplatforms as drug carriers. *Int. J. Nanomedicine* 7, 3445–3471 (2012).

36 Albanese A, Tang PS, Chan WC. The effect of nanoparticle size, shape, and surface chemistry on biological systems. *Annu. Rev. Biomed. Eng.* 14, 1–16 (2012).

37 Toy R, Peiris PM, Ghaghada KB, Karathanasis E. Shaping cancer nanomedicine: the effect of particle shape on the *in vivo* journey of nanoparticles. *Nanomedicine (Lond.)* 9(1), 121–134 (2014).

38 Duan XP, Li YP. Physicochemical characteristics of nanoparticles affect circulation, biodistribution, cellular internalization, and trafficking. *Small* 9(9–10), 1521–1532 (2013).

39 Herd H, Daum N, Jones AT, Huwer H, Ghandehari H, Lehr CM. Nanoparticle geometry and surface orientation influence mode of cellular uptake. *ACS Nano* 7(3), 1961–1973 (2013).

40 Issa B, Obaidat IM, Albiss BA, Haik Y. Magnetic nanoparticles: surface effects and properties related to biomedicine applications. *Int. J. Mol. Sci.* 14(11), 21266–21305 (2013).

41 Xie J, Chen K, Lee HY *et al.* Ultrasmall c(RGDyK)-coated Fe_3O_4 nanoparticles and their specific targeting to integrin alpha(v)beta3-rich tumor cells. *J. Am. Chem. Soc.* 130(24), 7542–7543 (2008).

42 Sun SH, Zeng H. Size-controlled synthesis of magnetite nanoparticles. *J. Am. Chem. Soc.* 124(28), 8204–8205 (2002).

43 Wang LN, Tang W, Zhen ZP, Chen HM, Xie J, Zhao Q. Improving detection specificity of iron oxide nanoparticles (IONPs) using the SWIFT sequence with long T-2 suppression. *Magn. Reson. Imaging* 32(6), 671–678 (2014).

44 Schleich N, Po C, Jacobs D *et al.* Comparison of active, passive and magnetic targeting to tumors of multifunctional paclitaxel/SPIO-loaded nanoparticles for tumor imaging and therapy. *J. Control. Release* 194, 82–91 (2014).

45 Davis ME, Chen Z, Shin DM. Nanoparticle therapeutics: an emerging treatment modality for cancer. *Nat. Rev. Drug Discov.* 7(9), 771–782 (2008).

46 Yang L, Kuang HJ, Zhang WY *et al.* Size dependent biodistribution and toxicokinetics of iron oxide magnetic nanoparticles in mice. *Nanoscale* 7(2), 625–636 (2015).

• **Size affects IONP biodistribution.**

47 Park JH, Von Maltzahn G, Zhang LL *et al.* Magnetic iron oxide nanoworms for tumor targeting and imaging. *Adv. Mater.* 20(9), 1630–+ (2008).

48 Veiseh O, Gunn JW, Zhang MQ. Design and fabrication of magnetic nanoparticles for targeted drug delivery and imaging. *Adv. Drug Deliv. Rev.* 62(3), 284–304 (2010).

49 Liu Z, Cai W, He L *et al. In vivo* biodistribution and highly efficient tumour targeting of carbon nanotubes in mice. *Nat. Nanotechnol.* 2(1), 47–52 (2007).

50 Kolhatkar AG, Jamison AC, Litvinov D, Willson RC, Lee TR. Tuning the magnetic properties of nanoparticles. *Int. J. Mol. Sci.* 14(8), 15977–16009 (2013).

51 Guardia P, Labarta A, Batlle X. Tuning the size, the shape, and the magnetic properties of iron oxide nanoparticles. *J. Phys. Chem. C* 115(2), 390–396 (2011).

52 Kolhatkar AG, Jamison AC, Litvinov D, Willson RC, Lee TR. Tuning the magnetic properties of nanoparticles. *Int. J. Mol. Sci.* 14(8), 15977–16009 (2013).

53 Huang J, Zhong X, Wang L, Yang L, Mao H. Improving the magnetic resonance imaging contrast and detection methods with engineered magnetic nanoparticles. *Theranostics* 2(1), 86–102 (2012).

54 Bjornerud A, Johansson L. The utility of superparamagnetic contrast agents in MRI: theoretical consideration and applications in the cardiovascular system. *NMR Biomed.* 17(7), 465–477 (2004).

55 Wang LY, Zhong XD, Qian WP *et al.* Ultrashort echo time (UTE) imaging of receptor targeted magnetic iron oxide nanoparticles in mouse tumor models. *J. Magn. Reson. Imaging* 40(5), 1071–1081 (2014).

56 Jia K, Zhang JD, Huang X, Liu XB. Size dependent electromagnetic properties of Fe_3O_4 nanospheres. *Chem. Phys. Lett.* 614, 31–35 (2014).

57 Zhang W. Nanoparticle aggregation: principles and modeling. *Adv. Exp. Med. Biol.* 811, 19–43 (2014).

58 Bullivant JP, Zhao S, Willenberg BJ, Kozissnik B, Batich CD, Dobson J. Materials characterization of feraheme/ferumoxytol and preliminary evaluation of its potential for magnetic fluid hyperthermia. *Int. J. Mol. Sci.* 14(9), 17501–17510 (2013).

59 Kievit FM, Veiseh O, Bhattarai N *et al.* PEI-PEG-chitosan-copolymer-coated iron oxide nanoparticles for safe gene delivery: synthesis, complexation, and transfection. *Adv. Funct. Mater.* 19(14), 2244–2251 (2009).

60 Yang LL, Peng XH, Wang YA *et al.* Receptor-targeted nanoparticles for *in vivo* imaging of breast cancer. *Clin. Cancer Res.* 15(14), 4722–4732 (2009).

•• **Targeted IONP for cancer diagnosis.**

61 Xie J, Wang JH, Niu G *et al.* Human serum albumin coated iron oxide nanoparticles for efficient cell labeling. *Chem. Commun.* 46(3), 433–435 (2010).

62 Lee H, Lee E, Kim DK, Jang NK, Jeong YY, Jon S. Antibiofouling polymer-coated superparamagnetic iron oxide nanoparticles as potential magnetic resonance contrast

agents for *in vivo* cancer imaging. *J. Am. Chem. Soc.* 128(22), 7383–7389 (2006).

63 Moore A, Marecos E, Bogdanov A, Weissleder R. Tumoral distribution of long-circulating dextran-coated iron oxide nanoparticles in a rodent model. *Radiology* 214(2), 568–574 (2000).

64 Harisinghani MG, Barentsz J, Hahn PF *et al.* Noninvasive detection of clinically occult lymph-node metastases in prostate cancer. *N. Engl. J. Med.* 348(25), U2491–U2495 (2003).

65 Schultz JF, Bell JD, Goldstein RM, Kuhn JA, Mccarty TM. Hepatic tumor imaging using iron oxide MRI: comparison with computed tomography, clinical impact, and cost analysis. *Ann. Surg. Oncol.* 6(7), 691–698 (1999).

66 Mouli SK, Zhao LC, Omary RA, Thaxton CS. Lymphotropic nanoparticle enhanced MRI for the staging of genitourinary tumors. *Nat. Rev. Urol.* 7(2), 84–93 (2010).

67 Blanco E, Shen H, Ferrari M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. *Nat. Biotechnol.* 33(9), 941–951 (2015).

68 Park SJ, Lee KB, Choi IS, Langer R, Jon SY. Dual functional, polymeric self-assembled monolayers as a facile platform for construction of patterns of biomolecules. *Langmuir* 23(22), 10902–10905 (2007).

69 Chen HW, Wang LY, Yeh J *et al.* Reducing non-specific binding and uptake of nanoparticles and improving cell targeting with an antifouling PEO-b-P gamma MPS copolymer coating. *Biomaterials* 31(20), 5397–5407 (2010).

70 Weissleder R, Stark DD, Engelstad BL *et al.* Superparamagnetic iron-oxide – pharmacokinetics and toxicity. *Am. J. Roentgenol.* 152(1), 167–173 (1989).

71 Mahmoudi M, Laurent S, Shokrgozar MA, Hosseinkhani M. Toxicity evaluations of superparamagnetic iron oxide nanoparticles: cell 'vision' versus physicochemical properties of nanoparticles. *ACS Nano* 5(9), 7263–7276 (2011).

72 Liu G, Gao J, Ai H, Chen X. Applications and potential toxicity of magnetic iron oxide nanoparticles. *Small* 9(9–10), 1533–1545 (2013).

73 Di Bona KR, Xu YL, Ramirez PA *et al.* Surface charge and dosage dependent potential developmental toxicity and biodistribution of iron oxide nanoparticles in pregnant CD-1 mice. *Reprod. Toxicol.* 50, 36–42 (2014).

74 Chen YS, Gong L, Gao N *et al.* Preclinical evaluation of a urokinase plasminogen activator receptor-targeted nanoprobe in rhesus monkeys. *Int. J. Nanomed.* 10, 6689–6698 (2015).

75 Schwenk MH. Ferumoxytol: a new intravenous iron preparation for the treatment of iron deficiency anemia in patients with chronic kidney disease. *Pharmacotherapy* 30(1), 70–79 (2010).

76 Hetzel D, Strauss W, Bernard K, Li Z, Urboniene A, Allen LF. A Phase III, randomized, open-label trial of ferumoxytol compared with iron sucrose for the treatment of iron deficiency anemia in patients with a history of unsatisfactory oral iron therapy. *Am. J. Hematol.* 89(6), 646–650 (2014).

77 Harisinghani M, Ross RW, Guimaraes AR, Weissleder R. Utility of a new bolus-injectable nanoparticle for clinical cancer staging. *Neoplasia* 9(12), 1160–1165 (2007).

78 European Medicines Agency (EMA). Superparamagnetic iron oxide nanoparticles stabilised with dextran and sodium citrate. Doc. Ref.: EMEA/CHMP/11527/2008 Procedure No. EMEA/H/C/801, 10–12 (2008). www.ema.europa.eu

79 Pankhurst QA, Thanh NTK, Jones SK, Dobson J. Progress in applications of magnetic nanoparticles in biomedicine. *J. Phys. D Appl. Phys.* 42(22), 224001 (2009).

80 Choi KY, Liu G, Lee S, Chen XY. Theranostic nanoplatforms for simultaneous cancer imaging and therapy: current approaches and future perspectives. *Nanoscale* 4(2), 330–342 (2012).

81 Schleich N, Sibret P, Danhier P *et al.* Dual anticancer drug/superparamagnetic iron oxide-loaded PLGA-based nanoparticles for cancer therapy and magnetic resonance imaging. *Int. J. Pharmaceut.* 447(1–2), 94–101 (2013).

82 Choi KY, Yoon HY, Kim JH *et al.* Smart nanocarrier based on PEGylated hyaluronic acid for cancer therapy. *ACS Nano* 5(11), 8591–8599 (2011).

83 Zuckerman JE, Gritli I, Tolcher A *et al.* Correlating animal and human Phase Ia/Ib clinical data with CALAA-01, a targeted, polymer-based nanoparticle containing siRNA. *Proc. Natl Acad. Sci. USA* 111(31), 11449–11454 (2014).

84 Xing R, Bhirde AA, Wang S *et al.* Hollow iron oxide nanoparticles as multidrug resistant drug delivery and imaging vehicles. *Nano Res.* 6(1), 1–9 (2012).

85 Wagstaff AJ, Brown SD, Holden MR *et al.* Cisplatin drug delivery using gold-coated iron oxide nanoparticles for enhanced tumour targeting with external magnetic fields. *Inorganica Chimica Acta* 393, 328–333 (2012).

86 Zhou Z, Sun Y, Shen J *et al.* Iron/iron oxide core/shell nanoparticles for magnetic targeting MRI and near-infrared photothermal therapy. *Biomaterials* 35(26), 7470–7478 (2014).

87 Wang DS, Fei BW, Halig LV *et al.* Targeted iron-oxide nanoparticle for photodynamic therapy and imaging of head and neck cancer. *ACS Nano* 8(7), 6620–6632 (2014).

88 Zhang F, Huang XL, Zhu L *et al.* Noninvasive monitoring of orthotopic glioblastoma therapy response using RGD-conjugated iron oxide nanoparticles. *Biomaterials* 33(21), 5414–5422 (2012).

89 Wang GH, Zhang F, Tian R *et al.* Nanotubes-embedded indocyanine green-hyaluronic acid nanoparticles for photoacoustic-imaging-guided phototherapy. *ACS Appl. Mater. Inter.* 8(8), 5608–5617 (2016).

90 Tse BW, Cowin GJ, Soekmadji C *et al.* PSMA-targeting iron oxide magnetic nanoparticles enhance MRI of preclinical prostate cancer. *Nanomedicine (Lond)* 10(3), 375–386 (2015).

91 Byrne JD, Betancourt T, Brannon-Peppas L. Active targeting schemes for nanoparticle systems in cancer therapeutics. *Adv. Drug Deliver. Rev.* 60(15), 1615–1626 (2008).

92 Zhou HY, Qian WP, Uckun FM *et al.* IGF1 receptor targeted theranostic nanoparticles for targeted and image-guided therapy of pancreatic cancer. *ACS Nano* 9(8), 7976–7991 (2015).

•• **Imaging guided cancer theranostic with targeted IONP nanocomplex.**

93 Lee GY, Qian WP, Wang LY *et al.* Theranostic nanoparticles with controlled release of gemcitabine for targeted therapy and MRI of pancreatic cancer. *ACS Nano* 7(3), 2078–2089 (2013).

94 Arosio P, Orsini F, Piras AM *et al.* MR imaging and targeting of human breast cancer cells with folate decorated nanoparticles. *RSC Adv.* 5(50), 39760–39770 (2015).

95 Peng XH, Qian XM, Mao H *et al.* Targeted magnetic iron oxide nanoparticles for tumor imaging and therapy. *Int. J. Nanomed.* 3(3), 311–321 (2008).

96 Ling D, Park W, Park SJ *et al.* Multifunctional tumor pH-sensitive self-assembled nanoparticles for bimodal imaging and treatment of resistant heterogeneous tumors. *J. Am. Chem. Soc.* 136(15), 5647–5655 (2014).

97 Xie J, Zhang F, Aronova M *et al.* Manipulating the power of an additional phase: a flower-like Au-Fe_3O_4 optical nanosensor for imaging protease expressions *in vivo*. *ACS Nano* 5(4), 3043–3051 (2011).

98 Mccarthy JR, Weissleder R. Multifunctional magnetic nanoparticles for targeted imaging and therapy. *Adv. Drug Deliv. Rev.* 60(11), 1241–1251 (2008).

99 Quan QM, Xie J, Gao HK *et al.* HSA coated iron oxide nanoparticles as drug delivery vehicles for cancer therapy. *Mol. Pharmaceut.* 8(5), 1669–1676 (2011).

100 Aires A, Ocampo SM, Simoes BM *et al.* Multifunctionalized iron oxide nanoparticles for selective drug delivery to CD44-positive cancer cells. *Nanotechnology* 27(6), 065103 (2016).

101 Bremnes RM, Donnem T, Al-Saad S *et al.* The role of tumor stroma in cancer progression and prognosis emphasis on carcinoma-associated fibroblasts and non-small cell lung cancer. *J. Thorac. Oncol.* 6(1), 209–217 (2011).

102 Lartigue L, Innocenti C, Kalaivani T *et al.* Water-dispersible sugar-coated iron oxide nanoparticles. an evaluation of their relaxometric and magnetic hyperthermia properties. *J. Am. Chem. Soc.* 133(27), 10459–10472 (2011).

103 Torres-Lugo M, Rinaldi C. Thermal potentiation of chemotherapy by magnetic nanoparticles. *Nanomedicine (Lond.)* 8(10), 1689–1707 (2013).

104 Hilger I. *In vivo* applications of magnetic nanoparticle hyperthermia. *Int. J. Hyperthermia* 29(8), 828–834 (2013).

105 Hilger I, Kaiser WA. Iron oxide-based nanostructures for MRI and magnetic hyperthermia. *Nanomedicine (Lond.)* 7(9), 1443–1459 (2012).

106 Zhao Q, Wang L, Cheng R *et al.* Magnetic nanoparticle-based hyperthermia for head & neck cancer in mouse models. *Theranostics* 2, 113–121 (2012).

107 Maier-Hauff K, Ulrich F, Nestler D *et al.* Efficacy and safety of intratumoral thermotherapy using magnetic iron-oxide nanoparticles combined with external beam radiotherapy on patients with recurrent glioblastoma multiforme. *J. Neurooncol.* 103(2), 317–324 (2011).

108 Medarova Z, Pham W, Farrar C, Petkova V, Moore A. *In vivo* imaging of siRNA delivery and silencing in tumors. *Nat. Med.* 13(3), 372–377 (2007).

109 Sun ZC, Song XX, Li XJ *et al. In vivo* multimodality imaging of miRNA-16 iron nanoparticle reversing drug resistance to chemotherapy in a mouse gastric cancer model. *Nanoscale* 6(23), 14343–14353 (2014).

110 Mahajan UM, Teller S, Sendler M *et al.* Tumour-specific delivery of siRNA-coupled superparamagnetic iron oxide nanoparticles, targeted against PLK1, stops progression of pancreatic cancer. *Gut* doi:10.1136/gutjnl-2016-311393 (2016) (Epub ahead of print).

111 Wen G, Zhang XL, Chang RM, Xia Q, Cang P, Zhang Y. Superparamagnetic iron oxide (Feridex)-enhanced MRI in diagnosis of focal hepatic lesions. *Di Yi Jun Yi Da Xue Xue Bao* 22(5), 451–452 (2002).

112 Clement O, Siauve N, Cuenod CA, Frija G. Liver imaging with ferumoxides (Feridex): fundamentals, controversies, and practical aspects. *Top. Magn. Reson. Imaging* 9(3), 167–182 (1998).

113 Johnson L, Pinder SE, Douek M. Deposition of superparamagnetic iron-oxide nanoparticles in axillary sentinel lymph nodes following subcutaneous injection. *Histopathology* 62(3), 481–486 (2013).

114 Kernstine KH, Stanford W, Mullan BF *et al.* PET, CT, and MRI with Combidex for mediastinal staging in non-small cell lung carcinoma. *Ann. Thorac. Surg.* 68(3), 1022–1028 (1999).

115 Harisinghani MG, Saini S, Hahn PF, Weissleder R, Mueller PR. MR imaging of lymph nodes in patients with primary abdominal and pelvic malignancies using ultrasmall superparamagnetic iron oxide (Combidex). *Acad. Radiol.* 5(Suppl. 1), S167–S169 (1998).

116 Reimer P, Balzer T. Ferucarbotran (Resovist): a new clinically approved RES-specific contrast agent for contrast-enhanced MRI of the liver: properties, clinical development, and applications. *Eur. Radiol.* 13(6), 1266–1276 (2003).

117 Vogl TJ, Hammerstingl R, Schwarz W *et al.* Magnetic resonance imaging of focal liver lesions. Comparison of the superparamagnetic iron oxide resovist versus gadolinium-DTPA in the same patient. *Invest. Radiol.* 31(11), 696–708 (1996).

118 Reimer P, Rummeny EJ, Daldrup HE *et al.* Clinical results with Resovist: a Phase 2 clinical trial. *Radiology* 195(2), 489–496 (1995).

119 Campbell JL, Arora J, Cowell SF *et al.* Quasi-cubic magnetite/silica core-shell nanoparticles as enhanced MRI contrast agents for cancer imaging. *PLoS ONE* 6(7), e21857 (2011).

120 Bullivant JP, Zhao S, Willenberg BJ, Kozissnik B, Batich CD, Dobson J. Materials characterization of Feraheme/ferumoxytol and preliminary evaluation of its potential for magnetic fluid hyperthermia. *Int. J. Mol. Sci.* 14(9), 17501–17510 (2013).

121 Maier-Hauff K, Ulrich F, Nestler D *et al.* Efficacy and safety of intratumoral thermotherapy using magnetic iron-oxide nanoparticles combined with external beam radiotherapy on patients with recurrent glioblastoma multiforme. *J. Neuro-Oncol.* 103(2), 317–324 (2011).