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REVIEW

Clinical advances of nanocarrier-based cancer therapy and diagnostics

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ABSTRACT

Introduction: Cancer is a leading cause of death worldwide and efficient new strategies are urgently needed to combat its high mortality and morbidity statistics. Fortunately, over the years, nanotechnology has evolved as a frontrunner in the areas of imaging, diagnostics and therapy, giving the possibility of monitoring, evaluating and individualizing cancer treatments in real-time.

Areas covered: Polymer-based nanocarriers have been extensively studied to maximize cancer treatment efficacy and minimize the adverse effects of standard therapeutics. Regarding diagnosis, nanomaterials like quantum dots, iron oxide nanoparticles or gold nanoparticles have been developed to provide rapid, sensitive detection of cancer and, therefore, facilitate early treatment and monitoring of the disease. Therefore, multifunctional nanosystems with both imaging and therapy functionalities bring us a step closer to delivering precision/personalized medicine in the cancer setting.

Expert opinion: There are multiple barriers for these new nanosystems to enter the clinic, but it is expected that in the near future, nanocarriers, together with new "targeted drugs", could replace our current treatments and cancer could become a nonfatal disease with good recovery rates. Joint efforts between scientists, clinicians, the pharmaceutical industry and legislative bodies are needed to bring to fruition the application of nanosystems in the clinical management of cancer.

KEYWORDS: cancer, nanotherapeutics, polymeric drug delivery systems, nanotheranostic

TABLE OF ABBREVIATIONS:

AIDS	Acquired Immunodeficiency Syndrome
Au-NP	Gold nanoparticles
C-dots	Cornell dots
CMC	Critical micelle concentration
CT	Computed tomography
DACH-Pt	Diaminocyclohexane-platinum
DDS	Drug delivery systems
DHAD	Dihydroxyanthracenedione
EPR	Enhanced permeability and retention
FDA	Food and Drug Administration
HPMA	N-(2-hydroxypropyl) methacrylamide)
MDR	Multidrug resistant
MRI	Magnetic resonance imaging
NCT	National Clinical Trial. ClinicalTrials.gov identifier
NIR	Near-infrared
NIRF	Near-infrared fluorescence
NP	Nanoparticles
OCM	Optical coherence microscopy
OCT	Optical coherence tomography
OI	Optical imaging
PAA	Poly (aspartic acid)
PACA	Poly (alkyl cyanoacrylate)
PBCA	Poly (butyl cyanoacrylate)
PDC	Polymer-drug conjugates
PEG	Poly (ethylene glycol)
PET	Positron-emission tomography
PGA	Poly (glutamic acid)
PIHCA	Poly (isohexyl cyanoacrylate)
PLA	Poly (lactic acid)
PLGA	Poly (lactic-co-glycolic) acid
PM	Polymeric micelles
PNP	Polymeric nanoparticles
PPO	Polypropylene oxide
PSMA	Prostate-specific membrane antigen
SPECT	Single photon emission computed tomography
SPIO	Superparamagnetic iron oxide
SPION	Superparamagnetic iron

oxide nanoparticles

USPIO Ultra-small superparamagnetic iron oxide

WHO World Health Organization

Article highlights box

- The clinical application of nanotechnology in cancer is changing the current diagnosis and therapy concepts and it is gradually reaching clinical use.
- Polymer-based nanoformulations, along with liposomes, are the most clinically available nanomaterials for human use. Some micelles are already available for clinical use and more ones, as well as, polymer-drug conjugates and nanoparticles, are under clinical development.
- Pharmaceutical research of nanosystems for the detection and monitoring of cancer is focused on different imaging techniques such as magnetic resonance, X-rays, computed tomography, positron-emission tomography or optical imaging. Some of these diagnosis nanosystems have also reached the market.
- There are some proof-of-principle in primary clinical trials of multifunctional nanosystems for the combination of diagnosis and therapy of cancer. They are showing the potential of nanotheranostic in the personalization of cancer treatments.

1. INTRODUCTION

Cancer is one of the most alarming diseases of all human disorders. According to the WHO World Cancer Report 2014, this disease was responsible for 8.2 million deaths in 2012, with 14 million new cases in the same year. In fact, it is expected that within the next 2 decades, annual cancer numbers will reach 22 million [1]. Cancer is a heterogeneous group of malignant diseases that begins when a DNA mutated cell that should die does not do so. With fatal consequences, this cell triggers abnormal cancer cell growth, forming a tumor (except in the case of hematologic cancers) that invades healthy tissues and then spreads to other parts of the body creating secondary tumors named metastases, which are the major cause of death from cancer [2, 3]. The methods globally used for cancer therapy are surgery, radiation, chemotherapy and immunotherapy. However, efficient new treatments are urgently needed to combat the high mortality and morbidity statistics. Regarding conventional chemotherapy, its inconveniences include high toxicity and the inadequate bio-distribution and pharmacokinetics profile of the cytostatic drugs [4, 5]. On the other hand, early detection of cancer significantly increases patient survival. Nonetheless, current diagnostic methods (biopsies, imaging procedures and detection of markers) are often invasive, present low sensitivity or detect cancer only in its later stages, which is the main reason for the high mortality rate. Although new biomarkers are being investigated, it is still necessary to develop new, faster, highly specific and more sensitive diagnostic technologies alongside new therapy strategies [6, 7]. At present, two main research lines are being developed to improve cancer management. The first one involves the use of genomics and proteomics studies for the identification of specific targets in order to synthesize therapeutically active drugs without side effects (“targeted drugs”). Several are already on the market and are producing good results, such as the tyrosine kinase

inhibitor Glivec (Gleevec in the USA). However, it is important not to forget the drug resistance that they induce [8, 9]. The second one, which will form the object of this review, is the design of nanomaterials to transport and deliver biomedical compounds through biological systems for the treatment, diagnosis, and for the theranostics of cancer (with the combination of diagnostic and therapeutic compounds into multifunctional nanoplatforms) [10]. The use of nanotechnology to develop these systems has been well established over the past decade, both in pharmaceutical research and the clinical setting. Nanosystems have tuneable size, shape and surface characteristics, and they offer two mechanisms to reach cancerous tissue: passive and active targeting. The passive accumulation of nanocarriers in solid tumors is based on the so-called enhanced permeability and retention (EPR) effect that consists in their retention due to increased leakiness of neovascularization as well as impaired lymphatic drainage in tumor tissues [5, 11]. On the other hand, active targeting is possible through the functionalization of the surface of the nanocarriers with biological targeting moieties (ligands). These biomolecules enable the selective targeting to specific receptors expressed on cancer cells, as well as, to tumor endotelial cells. [4, 12].

At present, one of the most frequent applications of biomedical nanotechnology is to enhance the efficacy of anticancer drugs already used in clinical settings by improving their bioavailability and safety, and their targeting at the cancer cells, without damaging healthy tissues. It is known that drugs carried by nanoparticles (NP) evade the efflux mechanism (over-expressed in tumors), maintain a high concentration within tumor cells, and therefore avoid drug resistance in the cells, which is one of the biggest challenges in cancer chemotherapy [5]. On the other hand, the application of diagnostic nanomedicines allows the early detection and identification of tumor cells which is indispensable to improve the prognosis of the disease. Therefore, theranostics

nanocarriers could personalize the treatment of cancer, avoiding the over- and underdosing that currently occurs as a result of the high interindividual variability of this disease [10, 13]. In fact, it is expected that these nanosystems accomplish significant improvements, offering early diagnosis, lower toxicity and reduced treatment costs [14]. To date, the medical use of nanomaterials in oncology has made good progress, with some nano-based products already on the market and others in various stages of preclinical and clinical development. This review highlights the clinical status and recent advances of nanotechnology based products in cancer, encompassing organic and inorganic-based systems.

2. CLINICAL STATUS OF POLYMER-BASED NANOCARRIERS FOR CANCER THERAPY

Nanomaterials designed for cancer therapy can be as diverse as micelles, dendrimers, inorganic NP, carbon NP and nanotubes, nanodiamonds, nanoemulsions, viral nanocarriers, peptide NP, solid lipid NP [15-18], etc., although most clinically available nanomaterials for human use are liposomes and polymer-based nanoformulations [11, 12]. In fact, the first nanotechnology-based cancer drugs on the market was a pegylated liposome with the drug doxorubicin encapsulated (Doxil) [5], which was approved in 1995 by the Food and Drug Administration (FDA) for the treatment of AIDS-related Kaposi's sarcoma, and in 1997 in Europe (now also indicated for the treatment of metastatic breast cancer, ovarian cancer and multiple myeloma) [5, 12]. However, despite the clinical progress made using liposomes, they present difficulties when it comes to modulate drug release *in vivo*, as well as stability problems and a limited capacity for drug loading [12]. Fortunately, polymer-based nanostructures have been

developed to overcome these problems [10, 12] and nowadays polymer therapeutics are being developed with a wide variety of architectures and chemical properties. Polymers used in drug delivery systems (DDS) can be synthetic, like poly(esters), poly(alkyl cyanoacrylates) and poly(ethers) or natural, like proteins (such as albumin) and polysaccharides [12, 19]. Synthetic polymers have the advantage of being prepared with tailored compositions and have properties that are easily adjustable to specific applications. Therefore, although there are some natural polymer-based DDS already on the market for cancer treatment, owing to the great versatility that synthetic polymers offer, this section will focus on the clinical status of the most relevant synthetic polymer-based DDS, including polymeric micelles (PM), polymer-drug conjugates (PDC) and polymeric nanoparticles (PNP) (Figure 1).

2.1 POLYMERIC MICELLES

PM are promising vehicles for the controlled delivery of poorly water soluble drugs, and therefore offer great potential to improve the therapeutic window of lipophilic antitumor drugs such taxanes or platinates. With a mean diameter ranging from 5 to 100 nm, PM are nano-sized supramolecular constructs made of amphiphilic block copolymers that self-assemble in an aqueous environment above a polymer concentration known as critical micelle concentration (CMC) [20]. They present a core-shell architecture in which the hydrophobic block of the copolymer forms a semi-solid core and the hydrophilic segment a coronal layer (see Figure 1 a). Within this structure, the active molecules can be physically entrapped in the hydrophobic core, avoiding the requirement of functional groups for drug encapsulation, or may also be chemically conjugated to the amphiphilic polymer, enhancing drug loading and preventing premature drug release. On the other hand, the hydrophilic corona provides good stability for the micellar structure as well as protection against rapid clearance from the body [21]. Regarding the polymers used for the formulation of PM, although alternatives are being explored, poly (ethylene glycol) (PEG) is the most frequent hydrophilic block in the copolymer structure**.** In fact, this polymer is widely used in the synthesis of nanosystems because it prevents recognition of the carrier as a foreign body by the mononuclear phagocyte system, increasing the blood circulation time. Conversely, there are various polymers used to form the micellar core, poly(ethers), poly(esters), poly(amino acid)s and N-(2- hydroxypropyl) methacrylamide (HPMA) being the ones that have a longer development track record.

PM have been under intense investigation for cancer therapy purposes during the past few decades, and some of them are currently undergoing clinical evaluation or are already on the market. A summary is presented in Table 1. To date, there are two PM on

the market: paclitaxel-PM, and docetaxel-PM, two monomethoxy PEG-b-poly(D,L, lactic acid) (PLA) formulations which were specifically designed to improve the solubility of paclitaxel and docetaxel, respectively, and avoid the need to use toxic solubilizing agents such polyoxyl 35 castor oil (Cremophor EL) or polysorbate 80. Paclitaxel-PM is available in South Korea and other Asian countries for the treatment of breast, non-small cell lung and ovarian cancer [22, 23] and is currently undergoing bioequivalence testing to gain marketing approval in the USA. Paclitaxel-PM will probably be registered in the USA and European markets as a bioequivalent to nab-paclitaxel [24, 25]. Regarding docetaxel-PM, which is also commercialized in South Korea, it is under clinical evaluation for pharmacokinetic equivalence with docetaxel injection concentrate as well as for safety and antitumor efficacy (NCT01336582 and NCT02639858).

Besides PLA micelles, other PM undergoing clinical trials are poloxamers and poly(amino acid) micelles. Poloxamers are amphiphilic PEG-poly(propylene oxide) (PPO)-PEG tri-block copolymers that present temperature dependent self-assembling and thermo-gelling behavior. Poloxamer 181 (PEG_2–PPO_{30}–PEG_2) is a potent Pgp inhibitor and sensitizer of multidrug resistant (MDR) cancer cells and poloxamer 407 (PEG_{100}–PPO_{65}–PEG_{100}) can improve the physical stability and increase the blood circulation time of the carrier due to its long PEG hydrophilic chain. SP1049C is a mixed micelle formulation ofpoloxamer 181 and 407, which physically encapsulates doxorubicin. It is particularly active in MDR and metastatic cancers and has successfully completed a phase II clinical trial demonstrating safety and efficacy in patients with advanced adenocarcinoma of the esophagus and gastroesophageal junction, and has achieved FDA orphan drug approval [20, 26]. Moreover, an international phase III study designed for this formulation has been reviewed and agreed

to with the FDA under a Special Protocol Assessment procedure [27]. On the other hand, micelles made of block copolymers of poly(amino acid)s are very attractive due to their high biocompatibility and flexibility to carry drugs by chemical conjugation to the polymer. There are two types of PEG-poly(amino acid) micelles that have been evaluated in clinical trials, PEG-poly (glutamic acid) (PGA) and PEG-poly (aspartic acid) (PAA) micelles. The first PEG-poly(amino acid) micelle to advance into clinical evaluation was NK911 [14, 21], a PEG-PAA micelle in which doxorubicin is chemically conjugated to increase the affinity of the core for physically encapsulated doxorubicin, improving the stability of the micellar structure and achieving high drug loading [28]. Similarly, in the paclitaxel containing NK105, the PEG-PAA copolymer was modified by an esterification reaction with 4-phenyl-1-butanol to increase its core hydrophobicity and enhance its affinity for the drug. This formulation is already far advanced in clinical studies in patients with metastatic or recurrent breast cancer (phase III) (NCT01644890) [29]. Along the same lines, before the self-assembly of the micelle, hydrophobic drugs can be conjugated to this type of PEG-poly(amino acid) copolymers via linkages dissociable under the desired conditions that trigger the drug delivery [14, 21]. Using this method, stimuli-responsive micellar systems are obtained. NK012, currently in phase II development, is prepared by conjugating the active metabolite of irinotecan hydrochloride SN-38 to the PGA copolymer segment via an ester bond that can be cleaved by hydrolysis under physiological conditions [30]. The same occurs with NC-6300, a pH sensitive micellar system. In this case, the cytostatic drug epirubicin has been covalently bonded to the copolymer through a hydrazone linkage to be selectively released at the low pH of intracellular and tumor environments [20, 31, 32]. In addition, PEG-poly(amino acid) micelles have also reached clinical trials using the major component in chemotherapy regimens, platinum drugs. After showing low bloodstream

stability with PAA, PGA was used as hydrophobic block in NC-4016 and NC-6004. These systems encapsulate diaminocyclohexane platinum (DACH-Pt, the active metabolite of oxaliplatin) and cisplatin, respectively, presenting a prolonged blood circulation time and a safer profile than non-encapsulated active molecules [33, 34].

Finally, another type of PM that have entered clinical trials are core-cross-linked PM, which have been designed to enhance micelle stability and prevent the premature dissociation of the micelle and consequent drug release at concentrations below CMC, as occur in the bloodstream [35]. Cripec-docetaxel is a PM composed of methoxy PEG-b-poly (HPMA lactate) thermosensitive block copolymers cross-linked through the conjugation of the core with the docetaxel itself by hydrolysis-sensitive covalent linkages. This core cross-linked PM is under clinical trial to find the highest safe dose in the treatment of solid tumors [35-38].

Therefore, even though there are still certain difficulties in controlling micelle dissociation and drug release rate, PM hold promise as effective DDS in cancer therapy [19, 20, 39]. Indeed, on the basis of the ongoing efforts, it is expected that in the coming years more PM will go on the market [21, 37].

2.2 POLYMER-DRUG CONJUGATES

Polymer-drug conjugates (PDC) are macromolecular prodrugs of 5-15 nm comprising a chemotherapeutic agent covalently attached, usually through a peptidyl or ester linkage, to a polymeric carrier used to improve the performance of the drug (see Figure 1 b). The PDC formed is a new entity with different solubility, toxicity and pharmacokinetic profile and with the ability to overpass drug resistance mechanisms and to accumulate in the tumor by the EPR effect [40, 41]. However, although, a large number of studies have been carried out in the field of PDC, unfortunately none has yet reached the market (Table 2). The most advanced PDC in clinical trials is paclitaxel-polyglumex, a paclitaxel-PGA conjugate which is being studied alone or in combination with others antineoplastics in phase III clinical trials. In this conjugate, paclitaxel is bound to PGA through a glycinate ester linkage and is only released by the action of cathepsin B, an intracellular lysosomal protease enzyme up-regulated in many tumor types [12]. Likewise, peptidyl linkages are stable in plasma and cleavage by lysosomal proteases. They are commonly used in the synthesis of HPMA copolymer–drug conjugates. Some examples include PK1, the first PDC to proceed to clinical trials in 1994. PK1 consists of a HPMA copolymer covalently conjugated to doxorubicin via a glycyl–phenylalanyl–leucyl–glycine linker [42] which is under two phase II clinical trials for the treatment of breast, lung and colorectal cancer [14]. The same conjugate with active targeting ability has also been developed under the name of PK2 (FCE28069), in which galactosamine moieties were added to target the asialoglycoprotein receptor present in hepatocytes and hepatoma cell lines [43]. Phase I studies of this conjugate have demonstrated liver-specific doxorubicin delivery [44], but the accumulation of PK2 in normal liver tissue is still a serious concern and therefore, currently, PK2 is not under an active development program. AP5280 is another HPMA polymer conjugated to cis-NH_3

platinum via a tetrapeptide linker that has provided promising phase I clinical results [45]; however, the company, Access Pharmaceuticals, focused on AP5346, discontinuing the development of AP5280. This conjugate has already completed a phase II clinical trial for advanced recurrent ovarian cancer and has been shown to release DACH-Pt from HPMA at acidic environments, such as the tumor microenvironment or the intracellular lysosomal compartment [46]. It is important to highlight the importance of using linkers that ensure the stability of the conjugate in the systemic circulation, as some PDC have failed in early clinical studies due to this issue. This is the case with the HPMA conjugate of camptothecin (PNU 166148), a conjugate with bladder toxicity due to its urine labile linker and high urinary excretion, or paclitaxel (PNU 166945), which caused the same neurotoxicity as the free drug due to the fast drug release from the conjugate [47, 48]. PEG is another polymer commonly used to synthesize PDC. PEG possesses two functional –OH groups suitable for conjugation and it can be modified to obtain more sites of drug binding, giving place to PDC with a higher drug loading capacity while the molecular weight is simultaneously increased. Most of the drugs in PEG-drug conjugates under clinical trials are from the camptothecin family (camptothecin, SN38 and irinotecan). Pegamotecan is a camptophecin-PEG conjugate whose development was discontinued because it had a similar toxicological profile to native drug due to quick *in vivo* hydrolysis of its alaninate ester linkage [49]. The company therefore focused on improving the formulation: the new conjugate, named EZN-2208, is made up of a camptothecin derivate SN38 and a 4-armPEG polymer, and has improved drug loading with slower hydrolysis of the ester linker. All these improvements allow the new formulation to accumulate in the tumor by the EPR effect. This last architecture of multi-arm PEGs was also exploited for the preparation of docetaxel-PEG (NKTR-105) conjugate,

currently under dose-escalation phase I study and irinotecan-PEG (NKTR-102) conjugate, which is highly advanced in phases I, II and III of clinical trials. As in the case of other polymer conjugates, there is a PEG conjugate with failed clinical development. This is the case of paclitaxel-PEG conjugate, which completed a phase I clinical trial, but the company Enzon unfortunately discontinued its development without apparent reasons [49, 50]. Similarly, more PDC studies appear to have been discontinued without sufficient information, such as the dextran bioconjugates of the topoisomerase I inhibitor exatecan (DE-310) and doxorubicin (AD-70, DOX-OXD) [51, 52].

Finally, XMT-1001 is a novel active camptothecin analogue conjugated to the biodegradable polyacetal polymer poly(1-hydroxymethylethylene hydroxymethylformal), which has successfully completed a phase I clinical trial and is currently in phase Ib clinical trial for the treatment of gastric and non-small cell lung cancer. Specifically, this conjugate is a polymeric pro-drug derivative of camptothecin with a dual release mechanism; first the active camptothecin analogue is released non-enzymatically and enters cells readily because of its lipophilicity.Then, mostly intracellularly, the analogue can be further converted into another active analogue or camptothecin through hydrolysis. Therefore, PDC enhance the efficacy of camptothecin by increasing accumulation of the drug and its active analogues in the tumor. Furthermore, due to the low level of camptothecin in blood, its urinary excretion is low and its bladder toxicity is avoided. In addition, the use of this analogue avoids the gastrointestinal toxicity associated with other camptothecin analogues such as irinotecan or SN-38 [53-55].

Although some PDC clinical trials failed, showing us the importance of a careful design of polymer-drug linkers, more than 10 anticancer conjugates are currently in clinical

development and it is expected that they will enter the market in the near future. Indeed, a future PDC generation will reach clinical development, meeting challenges such as the development of novel polymers with high molecular weight and the development of versatile conjugation chemistry to allow accurate control of therapy as well as the delivery of different or multiple drugs.

2.3 POLYMERIC NANOPARTICLES

Polymeric NP (PNP) are submicron-sized colloidal systems much larger than PM [50-500 nm) that have proved to be efficient carriers for the sustained and prolonged release of anti-cancer drugs (Figure 1 c). These carriers can be prepared using different biocompatible polymers. In fact, the release of anti-cancer drugs can be easily modulated by the type of polymer used [4, 19]. PNP are usually prepared by two main approaches; starting from initial monomers that are polymerized (e.g., by emulsion polymerization); or starting from pre-synthesized polymer (e.g., by nanoprecipitation, emulsification/solvent evaporation, etc.) [56]. These polymeric nanocarriers can be matrix systems in which the anticancer agent is dissolved or dispersed (nanospheres), or reservoir systems in which the anticancer agent is in a cavity surrounded by the polymer (nanocapsules) [19]; the conjugation of anticancer agent to the surface or core of the particle is also possible.

Among nanosystems made of natural polymer or biopolymers, nab-paclitaxel**,** used in the treatment of breast, lung and pancreatic cancer, is the only formulation currently on the market. This nanosystem consists of paclitaxel bound albumin NP which allows the administration of high drug doses [57]. On the other hand, there are no PNP made of synthetic polymers being marketed, and only a few are under clinical evaluation (Table

3), even though they are usually more stable in biological media than nanocarriers based on natural polymers [56].

As far as passive targeting is concerned, NP formed with the biodegradable polymer poly (alkyl cyanoacrylate) (PACA) have been extensively used for drug delivery based on their ability to encapsulate small hydrophobic drugs and to improve the oral bioavailability of small molecular weight drugs [58]. In fact, doxorubicin Transdrug is produced by the emulsion polymerization method using anionic surfactants and consists in a PIHCA ((poly (isohexyl cyanoacrylate)) nanosphere formulation loaded with doxorubicin. Currently, although only for one indication, it is the most advanced PNP in clinical evaluation. It is an orphan drug in Europe and the US and is in phase III for i.v. treatment of advanced hepatocellular carcinoma. Moreover Onxeo Company is already exploring new indications and its combination with other drugs to achieve a synergistic effect [59, 60]. Another PACA NP prepared by the emulsion polymerization method is DHAD-PBCA NP which consists of mitoxantrone (dihydroxyanthracenedione, DHAD) loaded into poly (butyl cyanoacrylate) (PBCA), a biodegradable polymer that has been used as a medical adhesive for decades. It is in phase II clinical trials and has slightly improved the survival rates in patients with hepatic cancer [61, 62]. Other polymers that have also been used for PNP preparation are poly(esters). This is the case with Docetaxel-PNP, a formulation comprised of a mixture of monovalent metal salts of PLA, amphiphilic diblock copolymers of monomethoxy PEG-PLA and the drug docetaxel. It is being developed by Samyang Pharmaceuticals and is under phase I clinical trials for advanced solid tumors in South Korea [63]. On the other hand, CRLX101, a camptothecin nanosystem used in various clinical trials, which is showing enhanced pharmacokinetic efficacy in various solid tumors, and CRLX301, a docetaxel

nanosystem in phase Ib/IIa, are both NP-drug conjugates. Between PDC and PNP, they are composed of a co-polymer, formed with β-cyclodextrins (a macrocyclic oligosaccharide) and PEG, which self-assembles into NP of 30-40 nm after its previous covalent glycinate linkage with the active drug [64-66].

Regarding active targeting, and following the pioneering work of Langer and Farokhzad, only BIND-014 has reached clinical development [67]. BIND-014 is a docetaxel PNP targeted to Prostate-Specific Membrane Antigen (PSMA), a tumor antigen expressed on prostate cancer cells and on the neovasculature of most non-prostate solid tumors. BIND-014 has a biodegradable polymeric core of PLA, PEG and PLGA, and a pseudo-mimetic dipeptide as the PSMA-targeting ligand. This formulation is in various phase II clinical trials for treatment of solid tumors and in phase I for advanced and metastatic cancer [21, 40].

Despite the poor clinical development of PNP, there are promising candidates currently under preclinical investigation which appear to offer prolonged and effective control of drug delivery. Indeed, despite their more complicated synthesis methods ,compared to micelles and conjugates, PNP show better stability and a more controlled drug release (via diffusion through the polymeric matrix or by the erosion and degradation of the particles) [2]. Moreover, like some PM, PNP can also overcome the mechanisms of chemo resistance developed by tumor cells that affect standard chemotherapy agents. Thus, although PNP provide promising new therapeutic properties [59], pharmaceutical companies are still cautious about the clinical study of these nanosystems with more complex production processes. Their arrival on the market is thus being delayed, as are their expected benefits in cancer therapy.

3. CANCER DIAGNOSIS AND NANOTECHNOLOGY

Imaging tumorous tissue is of paramount importance to early identify the tumor type, location and stage of cancer. A precise tumor depiction enables specialists to establish accurate judgments about the tumor distribution and its response to surgical removal and adjuvant therapies [68]. There is a wide variety of imaging modalities to depict cancer tissue, including positron-emission tomography (PET), X-ray computed tomography (CT) and magnetic resonance imaging (MRI).

3.1 MAGNETIC RESONANCE IMAGING

MRI is an essential imaging technique in medicine devised to achieve a detailed submillimetre-level spatial resolution and soft tissue contrast without the use of ionizing radiation or potentially harmful radiotracers [68]. MRI contrast agents contain paramagnetic or superparamagnetic metal ions that affect the MRI signal properties of surrounding tissue. The aim of these contrast agents is to increase the sensitivity of MRI for detecting various pathological processes and to characterize various pathologies. Superparamagnetic iron oxide nanoparticles (SPION) have generated great interest in the field of cancer diagnosis owing to their intrinsic magnetic property that enables them to be used as contrast agents in MRI (Figure 2 a and 3 a, b) SPION are extremely good enhancers of proton relaxation and do not self-aggregate when the external magnetic field is terminated. The longitudinal relaxation time (T1), and transverse relaxation time (T2) define the way that the protons revert back to their resting states after the radiofrequency pulse is applied. SPION are categorized as negative contrast agents, decreasing T_2 relaxation timeferidex and thus the signal intensity. Stability,

biocompatibility and blood half-life are the three key design considerations for SPION. Once SPION are administered (Figure 3 c) and cleared from blood by phagocytosis, they are metabolized in the lysosomes into a soluble and non-superparamagnetic form of iron that becomes part of the normal iron pool [69]. At present there are 18 nanoparticle formulations under clinical investigation for MRI imaging, which are producing notable results (see Table 4) [70]. For instance, the accuracy of SPIO-enhanced MRI imaging for the detection of local hepatic lesions is higher than that achieved with non-enhanced MRI [71]. The early marketed SPION based MRI contrast agents clinically available were ferumoxydes (Feridex and Endorem) and ferucarbotran (Resovist). Feridex is a SPIO colloid with a dextran coating and a particle size in the range 120-180 nm. Hypotension and lumbar pain/leg pain represent the most frequent symptoms associated with Feridex. On the other hand, Resovist is a carboxydextrane-coated SPIO colloid with a particle size between 40-60nm. Unlike Feridex, the incidence of cardiovascular adverse events and back pain is significantly less with Resovist. Although Feridex and Resovist were previously clinically approved, on-going concern was focused on the long term toxicity of these SPION based MRI contrast agents and they were withdrawn from use in humans [72]. Endorem, 5 nm SPION coated with dextran (hydrodynamic diameter 80-150 nm), is efficiently accumulated in the liver and spleen within minutes of administration and its blood, liver and spleen half-life is 6 min, 3 days and 4 days, respectively [73]. The recommended administration dose is 15 mmol/kg [71]. Oral SPIO preparations based on ferumoxsil such as Lumirem (300 nm), GastroMARK (300 nm) and Abdoscan (3.5 m) contain larger particles than the injectable contrast agents to prevent their being absorbed in the bowel [71]. These contrast agents enhance the ability to distinguish the loops of the bowel from other abdominal structures, as well as the bowel from adjacent tissues and organs in the upper

gastrointestinal tract [74]. The recommended clinical dose concentration is 1.5-3.9 mM [75]. Ultrasmall superparamagnetic iron oxide (USPIO) agents make it possible to prolong the blood half-life and cross the capillary wall in order to achieve more widespread tissue distribution (Figure 2 a and 3 d). Ferumoxtran-10, commercialized in Europe and USA, is a USPIO composed of 4-6 nm magnetic NP surrounded by a hydrophilic dextran coating (hydrodynamic diameter of 11 ± 5 nm) to promote wide circulation in the intravascular space. Postcontrast imaging is usually obtained 24 h after administration of the contrast agent [71]. Their clinical dose depends on the type of MIR imaging and can range from 13.8- 44.7 mmol/kg [69]. However, the significantly high number of false positives in the identification of lymph node metastases has stopped the clinical development [76]. NC100150 is also a type of USPIO surrounded by a carbohydrate-PEG coating and with a vascular half-life in the range of 3-4 h. The recommended clinical dose is 50-100 mmol/kg [77].

Finally, ferumoxytol is a 30 nm SPION formulation with a magnetite core covered by a polyglucose sorbitol carboxymethylether coating. It is an approved iron replacement therapy agent that has also shown potential for use as a contrast agent in imaging studies for tumors, especially involving lymph nodes that have been affected by cancer. Ferumoxytol is taken up by normal lymph nodes, but excluded from cancerous lymph node tissue [74].

3.2 POSITRON-EMISSION TOMOGRAPHY

PET is a highly sensitive (down to picomolar level) and non-invasive nuclear imaging tool widely applied for preclinical and clinical imaging of diseases. However, the resolution is relatively low (typically < 1 mm). Upon the injection of either a radiotracer

or a radiolabeled NP, PET can monitor its distribution and accumulation. Radiolabeled NP are paramount in the field of cancer imaging [78]. Beyond the development of radiolabeled nanoprobes suitable for PET alone, recent tendencies aim at the synthesis of bimodal imaging probes applicable in PET as well as optical imaging (OI) in order to exploit the potential of both imaging techniques [79]. The combination of PET and OI provides clinical advantages: 1) PET possesses a high tissue penetration, allowing quantitative imaging able to identify and visualize tumors and metastases in the whole body. 2) OI is based on light scattering and exhibits only a limited tissue penetration but enables the identification of tumor margins and infected lymph nodes during surgery without bearing a radiation burden for the surgeon [79]. Although an extensive number of fluorescent particle nanoplatforms have been investigated [80], only Cornell dots (C-dots) have received the first FDA-approved investigational new drug approval for human clinical trials (Figure 2 b and 3 e). This type of core-shell silica NP shows clear advantages in comparison with single fluorophore labeling in diagnostics and theranostics. In addition, they also provide higher brightness and photostability than the single fluorophore moieties, two key points in fluorescent imaging [80]. Most interestingly, these NP are non-toxic, have a fast cellular uptake and complete clearance. In addition, it is considered that complete renal clearance is achieved when the NP have a particle size under the effective renal glomerular filtration size cut-off (approx. 10 nm) [81]. The use of 6 nm C-dots was reported for the imaging of cancer in human clinical trials [82]. C-dots were labeled with ^{124}I for PET imaging and modified with cyclo-(Arg-Gly-Asp-Tyr) peptides, cRGDY, for sentinel lymph node mapping [83] and molecular targeting to cancer cells: melanoma, hepatic metastasis and pituitary adenoma. C-dot whole-body clearance half-time values range from 13 to 21 hours, which is smaller than for large NP ie, 90 nm liposomes which have median clearance

half-time values ranging from 40 to 103 hours [82]. *In vivo* PET imaging was able to accurately estimate the fraction of the injected particle load that accumulates at tumor sites, in addition to monitoring time-varying particle uptake and clearance.

Advanced imaging techniques such as single photon emission computed tomography (SPECT) coupled with additional techniques such as Near-infrared fluorescence (NIRF) make it possible to detect the sentinel lymph nodes to detect an image at the primary site of lymphatic metastasis [84]. ^{99m}Tc-labelled nanocolloids, ^{99m}Tc-labeled sulphur-colloid (USA) and ^{99m}Tc colloid albumin (Europe) were selected as tracer (see Figure 2 c). After subcutaneous injection, ^{99m}Tc-labelled colloid particles are filtered into lymphatic capillaries, then transported along the lymphatic vessels and trapped in functionary lymph nodes. This technique has been evaluated for tumor resection, showing improved and accurate sentinel lymph node identification in oral cancer patients.

3.3 X-RAY COMPUTED TOMOGRAPHY

X-ray computed tomography (CT) is one of the leading radiology technologies applied in the field of biomedical imaging. The basic process of CT is to detect the X-rays that pass through a sample. CT is among the most convenient imaging tools in terms of availability, efficiency and cost. CT, unlike PET and MRI, can provide three-dimensional (3D) anatomic details with high spatial and temporal resolution, even to capture cardiac motion [84]. The higher the atomic number of the CT contrast agent, the better the resulting CT contrast. As a result, iodinated contrast agents are widely used as CT contrast agents in clinical practice [85]. Gold nanoparticles (Au-NP) have demonstrated greater contrast than iodinated agents, as well as reduced toxicity and

prolonged circulation times [86]. Lanthanides with high atomic number can be also used as CT contrast agents, i.e. gadolinium. However, free lanthanide ions are toxic and must be chelated to obtain FDA-approval. Au-NP are by far the most widely investigated noble metal type NP as CT contrast agent (Figure 2 d and 3 f-i). In addition, Au-NP are used also in optical coherence tomography (OCT) and optical coherence microscopy (OCM). OCT can generate a signal based on refractive index mismatches and scattering events [86]. Au-NP make it possible to achieve an extra scattering because they possess both unique absorption and scattering properties in the near-infrared (NIR) region that have generated promise in differentiating normal from diseased tissue [86]. However, no Au-NP products have been clinically approved. Auroshell, silica-gold nanoshells coated with PEG (Figure 3 g), developed by Nanospectra to thermally ablate solid tumors, is also being considered for cancer imaging [87]. Auroshell still faces certain technical and biological challenges before clinical approval, such as determining the biological fate and long-term biocompatibility and proving that this nanosystem can be used intravenously utilizing the EPR effect [88]. The optical behavior of gold nanoshells in the NIR is noteworthy, as they show scattering and/or absorption cross-sections that are often several times higher than the particle geometric cross-section [87]. Gold nanoshells can efficiently lower the photon reflectance in comparison with gold colloid, enhancing reflectance signatures through absorption for spectroscopic detection modalities [87]. This considerable change in reflectance is observed with only a very small concentration of nanoshells and it is rarely observed with other type of Au-NP. In addition, gold nanoshells can be used in numerous bioconjugate applications as their surfaces are virtually chemically identical to universally used gold colloid [89]. This implies that gold nanoshells can selectively be targeted to cancer cells.

In the past decade, enormous advances have been made in the research of imaging sciences, and many new technologies (PET, CT and MRI) and imaging agents based on nanosystems have been applied to oncology research and clinical trials. The translation of these nanosystems and technologies from the laboratory to the clinic has been much slower than was initially hoped. The main reasons for this could be summarized as: 1) Lack of reliable technology to scale up the production of advanced nanomaterials [90], 2) Considerable regulatory hurdles and market forces [90, 91], 3) lower profit margins for imaging than for therapeutic drugs [90], 4) Low target selectivity (high number of false positives) for imaging and ultrasensitive detection of near and distant metastases and 5) Toxicity and side effects in patients. Despite these hurdles, several new nanosystems in clinical trials show that they are more robust and versatile, since they can enhance and improve current imaging and diagnostic techniques. For instance, PET nanoparticle tracers could complement the information that is not acquired by nonspecific radiopharmaceuticals.

4. CANCER THERANOSTICS AND NANOTECHNOLOGY

Originally introduced by Funkhouser in 2002, the term "theranostics" describes any "material that combines the modalities of therapy and diagnostic imaging" into a single package [92]. Nowadays, nanomedicine theranostics for cancer is progressing with the design of multifunctional platforms that consist of colloidal NP ranging in sizes from 10 to 1000 nm in which the diagnostic and therapeutic agents are adsorbed, conjugated, entrapped or encapsulated [91, 93]. The same therapeutic agent has not the same effect on all patients with the same diagnosis. The objective of nanotheranostic is therefore to achieve real-time traceable drug distribution and delivery, and monitor the therapeutic efficacy non-invasively. Therefore, with theranostic nanosystems, patients would have better treatment regimens based on each individual's responses and needs, which would enhance their quality of life by lowering the adverse side effects and the therapeutic efficacy of over- or under-dosed antitumor drugs [10, 94].

In the development of theranostic nanoplatforms it should be consider that the optimal concentration for the desired therapy is generally much higher than that required for imaging [95]. Furthermore, it is necessary to have an equilibrium between the desired long circulation time for therapeutic efficacy and the short time frame for the imaging agent, which is enough to evaluate the disease with low toxicity [96]. Consequently, to achieve clinical translation, increased regulatory barriers that depend on each function of the nanosystem need to be included [97]. In this way, despite the successful introduction of the therapeutic and diagnostic nanosystems already discussed into clinical trials and even onto the market, most of the results for theranostic nanomedicines reported in the literature are *in vitro* studies and only a few *in vivo* data are available to demonstrate their potential clinical application [94]. In this sense, it is important to highlight that there are some proof-of-principle clinical studies of

therapeutic NP in which biodistribution proofs have been obtained through their self-imaging properties (inherent or added), which have shown the promising possibilities of theranostic nanosystems.

Various nanocarriers are being investigated for sustained, controlled and targeted co-transport of diagnostic and therapeutic agents. Two different strategies are being investigated, each one with strengths and weaknesses. The first one is an "All in One" approach in which the nanosystem carries both agents (see Figure 4 a). The most commonly used are liposomes and polymer-based nanocarriers such as PMs, polymer conjugates, PNP or dendrimers [94, 95]. They carry, at the same time, the therapeutic drug and the diagnostic agent such as radionuclides, different NIR dyes, MRI agents or inorganic NP. They are excellent theranostic carriers owing to their biocompatibility, protection of loaded drug/diagnostic agent and controlled drug release. However, it should be borne in mind that physicochemical and drug loading properties could change after adding the imaging agent; and also, that the imaging agent could be lost from nanoplatforms during systemic circulation [96]. Nevertheless, there are already proof-of-principle clinical studies of "All in One" strategy in PK1 (doxorubicin–HPMA conjugate) and PK2 (hepatocellular carcinoma targeted doxorubicin–HPMA conjugate) clinical studies. Theranostic studies with the radiolabeled PK1 were carried out in phase I and II clinical studies. They showed a significant tumor accumulation of PK1, also in metastatic lesions, in a large number of patients. It should therefore be possible to visualize the efficacy of the treatment in real-time with a mixture of trace amounts of radiolabeled PK1 with regular PK1 [42, 98]. On the other hand, in similar studies with radiolabeled PK2, the conjugate was primarily accumulated in healthy liver tissue, rather than in the tumors. This result indicates that the targeting of PK2 may not be very effective. In fact, antitumor responses in patients were modest [only 3 out of 31 patients

with advanced liver cancer responded) [44, 96]. This study shows the usefulness of monitoring therapeutic NP to understand and explain the therapeutic efficacy of nanocarriers.

The second strategy used to produce theranostic nanosystems, is a “One for All” approach in which the nanocarrier, such as inorganic NP and carbon nanotubes, have inherent imaging properties and can transport the therapeutic agent, or can even also act as a therapeutic agent by photothermal (such as Au-NP or SPION) or photodynamic (such as silicon NP or quantum dots) therapy [94, 99, 100] (see Figure 4 b). As we have seen in the previous section, metallic and magnetic NP are excellent diagnostic tools for imaging applications. Nevertheless, they are commonly coated with organic polymers (dextran, chitosan, polysorbate, PEG, polyaniline), organic surfactants (oleate and dodecylamine) or other metallic materials (gold, silica or carbon), providing limited cargo space for therapeutic payloads within the protective coatings [10, 101]. However, if the nanosystem has both therapeutic and diagnostic functionalities, this drug loading problem is avoided. This occurs, for example, with Au-NP which, due to their unique surface characteristics, can act as CT imaging agents at the same time as they can act as radiotherapy sensitizers and photothermal agents [102]. However, although Au-NP show low toxicity [5] and the coating of SPION covers the oxidative sites and reduces their toxicity [94], it is believed that “hard” materials such as gold, silver, and ceramics (silica) formulation, are not biodegradable and may aggregate in the liver and lymph system causing long-term adverse effects [97]. Fortunately, the potential of theranostic nanomedicine in cancer using the strategy of “One for All” can be appreciated, as the proof-of-principle clinical study, in the CYT-6091 biodistribution studies. CYT-6091 was a first multifunctional NP system combining both imaging and therapeutic functionalities to progress into clinical trials. It is composed of a PEGylated colloidal-

Au-NP core conjugated to recombinant human tumor necrosis factor alpha as a tumor growth inhibitor. [103]. In phase 0 (NCT00436410) and I (NCT00356980) clinical trials the imaging properties of colloidal gold particles were used for the analysis of tumor biopsies. The detection of Au-NP in tissue biopsy samples via transmission electron microscopy was used as initial proof of concept of the tumor targeting ability of CYT-6091 [104].

Nanotheranostic technologies are therefore showing their potential to personalize the management of cancer through the monitoring, evaluation and individualization of treatments in real-time. Moreover, nanotheranostics can facilitate clinical efficacy and toxicity studies and a better understanding of various important aspects of the drug delivery process such as the efficacy of targeting or stimuli drug release. The employment of clinically validated nanomaterials could possibly accelerate the clinical translation of theranostic NP [95]. However, to achieve safe, efficacious clinical platforms, further *in vivo* research efforts are needed [105].

5. CONCLUSION

The application of nanotechnology in cancer, is changing current diagnosis and therapy concepts. The possibility of manipulating nanocarriers' properties, such as their size, shape, charge or surface functionality, is the best strategy to achieve the desired *in vivo* behavior. Polymer-based nanocarriers have shown excellent therapeutic potential in both preclinical and clinical development. In fact, owing to their favourable physicochemical properties, polymeric DDS have been shown to be excellent carriers of therapeutic agents, increasing the therapeutic efficacy with better pharmacokinetic profiles and fewer side effects. Regarding nanosystems for diagnosis, some are already on the market and there are several ongoing clinical trials, which suggests that other formulations will reach the market in the upcoming years. Moreover, theranostic nanomedicine opens up the door to personalized medicine. Some proof-of-principle in primary clinical trials of therapeutic nanocarriers have shown the possibility of monitoring, evaluating and individualizing cancer treatments in real-time. However, no theranostic nanosystem is currently undergoing clinical trials, and still further *in vivo* work will be required prior to clinical application. Indeed, despite the revolutionary impact of potential applications of nanosystems in medicine; their clinical translation is progressing slowly and only a few nanosystems have reached the marketplace.

6. EXPERT OPINION

Two of the major challenges in cancer therapy are the early diagnosis of cancer cells and their selective eradication. Both challenges could be met with nanomedicine. Nanocarriers have the potential for significant improvements in disease prevention, diagnosis and treatment. Nevertheless, in spite of the variety of nanosystems investigated, only a few, such as pegylated liposomal doxorubicin, nab-paclitaxel, paclitaxel-PM, docetaxel PM, Endorem and Lumirem, have been given approval for use in the treatment and diagnosis of cancer. The translation of oncological nanomedicines into clinical practice has been slow. As previously stated, some major reasons could be the lack of reliable technology to scale up the production of advanced nanomaterials and the regulatory hurdles and market forces. In fact, the challenge of ensuring the quality of the nanosystems, and our knowledge gaps about the disease, are delaying the development of new systems of this kind. A better understanding of the interaction between the NP and tumor microenvironment is urgently needed, especially of the internalization and trafficking of NP into tumor cells. In this sense, the identification of new molecular targets would advance the active targeting in nanomedicine in order to attain clinical success. Up to now, no targeted nanocarrier has come onto the market, and only a few clinical trials (such as the PM for gene therapy CALAA-01) are under development. This clinical failure can be attributed to various barriers that the nanosystems have to cross before they are recognized by the cells, which may explain why targeted and untargeted NP *in vivo* behave in the same way. On the other hand, in comparison to conventional therapeutics, the production of nanomedicine has a high cost and this is delaying its commercialization. Nonetheless, the positive cost-effectiveness of nanomedicine would justify its development and manufacturing costs due to its large clinical and economic benefits, concretely, more effectivity, less

mortality and adverse effect and reduction of hospitalization days and personnel cost. Among the different nanomedicines currently under investigation nanotheranostic systems are the one that present more clinical advantages and in which we should put our efforts. However, there are still many challenges which need to be addressed before their use in clinical practice, such as their more complex manufacturing in comparison to diagnosis and therapeutic nanosystems. Therefore, it is more likely that in the coming years nanosystems for cancer therapy or diagnosis will have more impact in the market than novel nanotheranostic formulations. Summing up, to ensure successful clinical evaluation and connect the needs of cancer medicine to the enormous potential of nanotechnology, we need to integrate a wide variety of disciplines (scientific, technological and legal) and to make rules for clinical studies and production of nanocarriers. All of this could speed up the progress of nanomedicine, and address concrete problems such as the prediction of new side effects not associated with either the drug or the carrier, as in the case of pegylated liposomal doxorubicin and certain cases of nab-paclitaxel, which have the dose-limiting “hand and foot syndrome” (or Palmar-Plantar erythrodysesthesia) because of their long circulation and their deposition in the peripheral tissues.

Overall, this is an exciting time in the field of nanotherapeutics, with advances being made in diagnostics, therapeutics and theranostics. There are multiple barriers for these new nanosystems to enter the clinic, but it is expected that in the near future, nanocarriers, together with new “targeted drugs”, could replace our current treatments and cancer could become a nonfatal disease with good recovery rates. Joint efforts between scientists, clinicians, the pharmaceutical industry and legislative bodies are needed to bring to fruition the application of new nanosystems in the clinical management of cancer.

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Figure 1. - Illustration of the most relevant synthetic polymer-based drug delivery systems in clinical trials.

PEG = Poly(ethylene glycol)

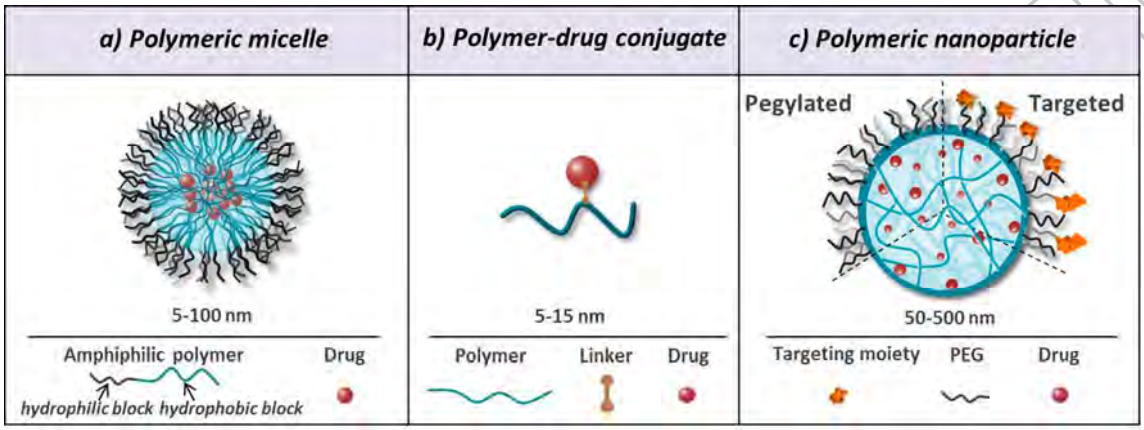


Figure 2. - Illustration of the most relevant nanosystems in clinical trials for cancer imaging.

cRGDY = Cyclic arginine–glycine–aspartic acid peptide; CT = X-ray computed tomography; MRI = Magnetic resonance imaging; PEG = Poly(ethylene glycol); PET = Positron-emission tomography; SPECT = Single photon emission computed tomography; SPION = Superparamagnetic iron oxide nanoparticles; USPION = Ultrasmall superparamagnetic iron oxide nanoparticles

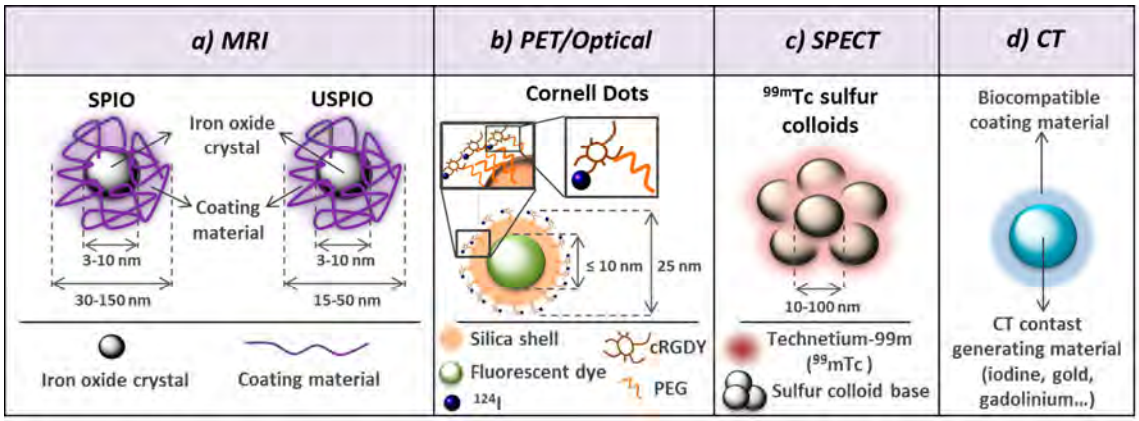


Figure 3.-Nanoparticles applied for cancer imaging. a) TEM image of SPION. b) Schematic representation to scale of SPION and the structure of different molecules used for their functionalization. Adapted from ref[110] under CC license. c) Routes of administration of marketed SPION: intrathecal, intratumor, intravenous and intramuscular or subcutaneous methods. Adapted from ref[110] under CC license. d) Common organ distribution of nanoparticles as a function of particle size. Most nanoparticles for *in vivo* use fall into the intermediate category (10–300 nm), where distribution to liver, spleen, lymph nodes and bone marrow is common. Bottom: CT images of nanoparticles used in a human patient (Tc-labelled NP) and mouse mode (Zr-labelled cross-linked dextran nanoparticles). Adapted from ref[111] with permission of Nature Publishing group. e) TEM image of Cornell dots. Adapted from ref[112] with permission of American Chemical Society. [][]f) Hollow gold nanoparticles. Adapted from ref[113] with permission of Royal Society of Chemistry. g) Au-SiO_2 nanoshells. Adapted from ref[114] with permission of Royal Society of Chemistry. h) Au nanorods. Adapted from ref[115] with permission of Royal Society of Chemistry. i) Au-NP loaded in polymeric PLGA NP. Adapted from ref[93] under CC license.

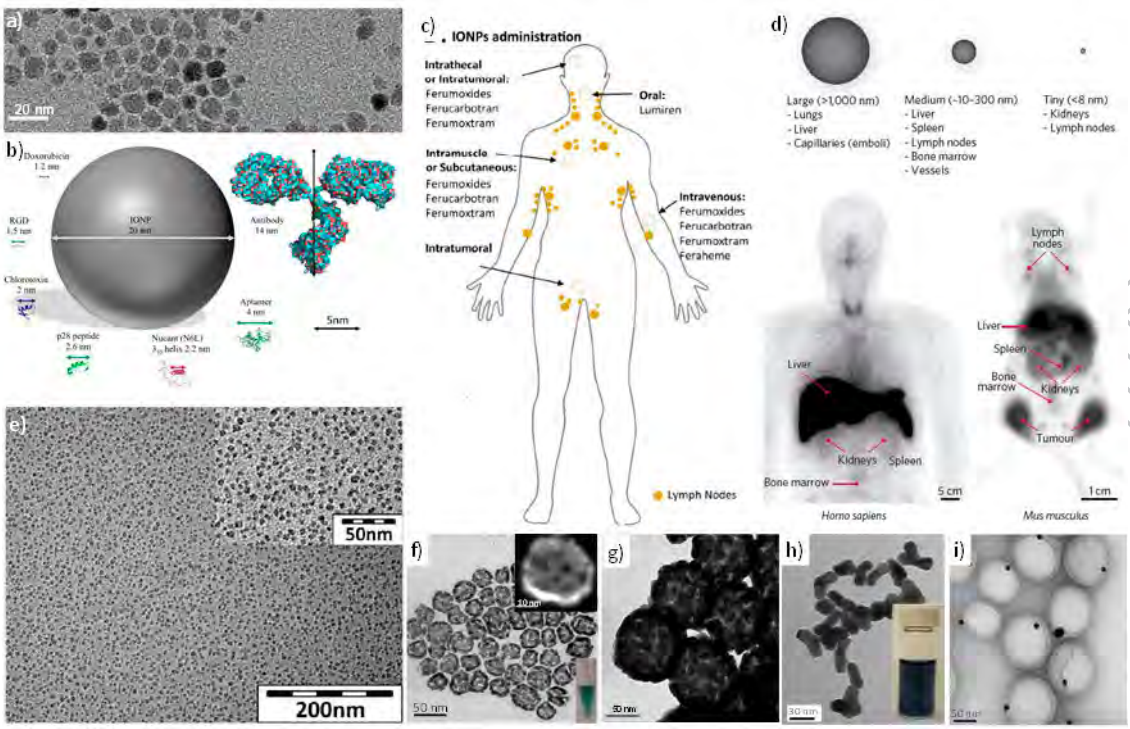
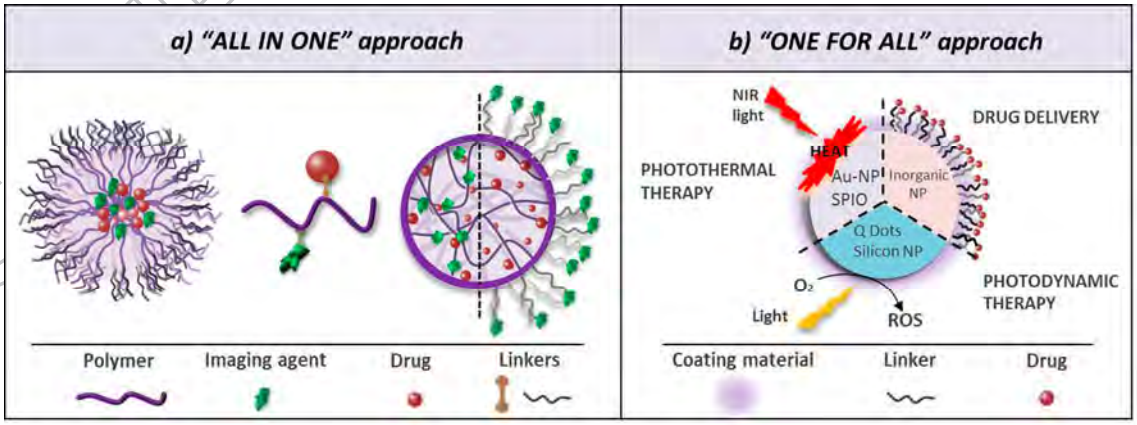


Figure 4. - Illustration of the most relevant strategies used to develop theranostic nanosystems for cancer imaging and therapy.

Au-NP = Gold nanoparticles; NIR = Near-infrared; NP = Nanoparticles; Q Dots = Quantum dots; ROS= Reactive oxygen species; SPIO = Superparamagnetic iron oxides



Product name	Company	Drug	Polymer	Indication	Clinical status
Paclitaxel-PM (IG-001)(Genexol-PM) (Paxus-PM) (Cynviloq)	Samyang Biopharmaceuticals Corporation	Paclitaxel	mPEG-PLA	Breast cancer, ovarian cancer and NSCLC	Marketed in South Korea and other Asian countries
				Metastatic or locally recurrent breast cancer	(NCT02064829) Bioequivalence study versus Nab-paclitaxel
				Recurrent or metastatic breast cancer	Phase III (NCT00876486)
				Taxane-pretreated recurrent breast cancer	Phase IV (NCT00912639)
				Unresectable locally advanced or metastatic pancreatic cancer	Phase II (NCT00111904)
				Advanced NSCLC in combination with carboplatin	Phase II (NCT01770795)
				Locally advanced HNSCC in combination with cisplatin	Phase II (NCT01689194)
				Advanced Urothelial Cancer	Phase II (NCT01426126)
				Advanced ovarian cancer in combination with carboplatin	Phase I (NCT00877253)
Docetaxel-PM (Nanoxel-PM) (Nanoxel M)	Samyang Biopharmaceuticals Corporation	Docetaxel	mPEG-PLA	Breast, NSCLC, prostate, ovarian, head and neck, gastric and esophageal cancer	Marketed in South Korea
				Advanced solid tumor or NSCLC, biliary tract, and bladder cancer	Phase I (NCT01336582)
				Recurrent or metastatic HNSCC	Phase II (NCT02639858)
SP1049C	Supratek Pharma Inc.	Doxorubicin	Pluronic® P-61 and F-127 block copolymers	Advanced refractory adenocarcinoma of the esophagus or GEJ	Phase II [26]
NK911	Nippon Kayaku Co., Ltd	Doxorubicin	PEG-poly(α,β-Asp)	Solid tumors	Phase I (Japan)[106]
				Metastatic pancreatic cancer	Phase II [14]
NK105	NanoCarrier Co., Ltd Nippon Kayaku Co., Ltd	Paclitaxel	PEG-modified poly(α,β-Asp)	Recurrent or metastatic breast cancer	Phase III (NCT01644890)
				Advanced gastric cancer	Phase II (JapicCTI-090769)
NC-6300 K-912	NanoCarrier Co., Ltd. Kowa Company, Ltd	Epirubicin	PEG-poly (aspartate-hydrazone)	Advanced or metastatic solid tumors	Phase I (JapicCTI-132221)
NK012	Nippon Kayaku Co., Ltd	SN38	PEG-modified PGA	Triple negative breast cancer	Phase II (NCT00951054)
				Refractory solid tumors	Phase I (NCT00542958)
				Relapsed SCLC	Phase II (NCT00951613)
				Metastatic colorectal cancer in combination with 5-fluorouracil	Phase II (NCT01238939)
				Unresectable advanced colorectal cancer	Phase II (JapicCTI-090780)
				Multiple myeloma	Phase I/II (JapicCTI-111652)
NC-4016	NanoCarrier Co., Ltd.	Oxaliplatin	mPEG-PGA	Advanced solid tumors or lymphoma	Phase I (NCT01999491)
NC-6004 (Nanoplatin)	NanoCarrier Co., Ltd.	Cisplatin	mPEG-PGA	Locally advanced or metastatic pancreatic cancer in combination with gemcitabine	Phase III (NCT02043288); Phase I/II (NCT00910741)
				Advanced solid tumor	Phase I/II (NCT02240238)
Cripec-docetaxel	Cristal Therapeutics	Docetaxel	Thermosensitive PEG-β-poly(N-(2-hydroxypropyl)-methacrylamide-	Solid tumors	Phase I (NCT02442531)

lactate)

Table 1: Polymeric micelles on the market or clinical trials for cancer therapy.

Asp = aspartic acid; GEJ = gastroesophageal junction; HNSCC = head and neck squamous cell carcinoma; JapicCTI# = clinicaltrials.jp registry number, clinical trials information of the Japan Pharmaceutical Information Centre; mPEG = methoxy-poly(ethylene glycol); NCT# = ClinicalTrials.gov registry number; NSCLC = non-small cell lung cancer; PGA = poly(L-glutamic acid); PLA = poly (D,L lactic acid); SCLC = small cell lung cancer

Product name	Company	Drug	Polymer (linker/spacer)-*targeting moiety*	Indication	Clinical status
(paclitaxel poliglumex) (CT-2103) (Opaxio) (Xyotax)	Cell Therapeutics, Inc	Paclitaxel	PGA (Ester)	Advanced NSCLC	Phase III (NCT00054197 and NCT00269828); Phase II (NCT00487669) (in combination with pemetrexed); Phase III (NCT00576225, NCT00054210 and NCT00551733)(in combination with carboplatin)
				Progressive NSCLC	Phase III (NCT00054184)
				Metastatic breast cancer	Phase I (NCT00270907) (in combination with gemcitabine); Phase II (NCT00148707); Phase II (NCT00265733) (in combination with capecitabine)
				Advanced HNSCC in combination with cetuximab	Phase I/II (NCT00660218)
				Epithelial ovarian, primary peritoneal, or fallopian tube carcinoma	Phase I/II (NCT00017017); Phase I (NCT00060359) (in combination with carboplatin)
				Maintenance therapy in advanced ovarian, primary peritoneal or fallopian tube cancer	Phase III (NCT00108745)
				Recurrent or persistent epithelial ovarian or primary peritoneal cancer	Phase II (NCT00045682); Phase II (NCT00069901) (in combination with carboplatin)
				Advanced hormone refractory prostate cancer	Phase II (NCT00446836)
				Androgen Independent Prostate Cancer	Phase II (NCT00459810) (in combination with transdermal estradiol)
				Esophageal cancer in combination with cisplatin and radiation	Phase II (NCT00522795)
				Metastatic colorectal cancer	Phase I (NCT00598247)
				newly diagnosed brain tumors in combination with temozolomide and radiation	Phase II (NCT00763750)
				Newly diagnosed glioblastoma multiforme in combination with radiation therapy	Phase II (NCT01402063)
CT-2106	Cell Therapeutics, Inc	Camptothecin	PGA (Ester)	Advanced ovarian cancer	Phase II (NCT00291837)
				Metastatic colorectal cancer in combination with 5-FU and folic acid	Phase I/II (NCT00291785)
				Unspecified adult advanced solid tumor	Phase I (NCT00059917)
PK1 (FCE28068)	Pharmacia and Upjohn	Doxorubicin	HPMA copolymer(Amide/ GFLG)	Advanced breast cancer	Phase II (NCT00003165)
				Breast, lung and colorectal cancer	Phase II [98]
PK2 (FCE 28069)	Pharmacia and Upjohn	Doxorubicin	HPMA copolymer (Amide/GFLG)-*galactosamine*	Liver cancer	Phase I[44]
AP5280	Access pharmaceuticals, inc	Platinum	HPMA copolymer (Aminomalonate/ GFLG)	Solid tumors	Phase I[45]
AP5346 (ProLindac)	Access pharmaceuticals, inc	DACH platinate	HPMA copolymer (Aminomalonate/ GGG)	Head and neck cancer	Pilot study (NCT00415298)
				Advanced recurrent ovarian cancer	Phase II (EudraCT Number: 2010-020030-25)
PNU 166148 (MAG-CPT)	Pfizer; Cancer Research Campaign UK	Camptothecin	HPMA copolymer (ester)	Solid tumors	Phase I (NCT00004076); discontinued
PNU 166945	Pfizer; Cancer Research Campaign UK	Paclitaxel	HPMA copolymer (ester)	Solid tumors	Phase I; discontinued [107]
Pegamotecan (EZ-246) (Prothecan)	Enzon Pharmaceuticals, Inc	Camptothecin	PEG (Ester)	Locally advanced or metastatic adenocarcinoma of the stomach or gastroesophageal junction	Phase II (NCT00080002); Discontinued
EZN-2208	Enzon	SN-38	4-arm PEG	Metastatic colorectal carcinoma in	Phase II (NCT00931840)

	Pharmaceuticals, Inc		(Glycinamidoester)	combination or not with Cetuximab	
				Metastatic breast cancer	Phase II (NCT01036113)
				Refractory solid tumors in combination with Bevacizumab	Phase I (NCT01251926)
				Pediatric patients with relapsed or refractory solid tumors	Phase I/II (NCT01295697)
				Advanced solid tumors or lymphoma	Phase I (NCT00520637, NCT00520390)
NKTR-105	Nektar Therapeutics	Docetaxel	4-arm PEG	Refractory solid cancers	Phase I
				Locally advanced or metastatic second-line colorectal cancer	Phase II (NCT00856375), (NCT00598975) (in combination with cetuximab)
				Metastatic or locally recurrent breast cancer	Phase III (NCT01492101)
Etirinotecan pegol (NKTR-102)	Nektar Therapeutics	Etirinotecan	4-arm PEG (Glycinamidoester)	Refractory brain metastases and advanced lung cancer or metastatic breast cancer	Phase II (NCT02312622)
				Relapsed SCLC	Phase II (NCT01876446)
				Advanced or metastatic solid tumors in patients with hepatic impairment	Phase I (NCT01991678)
				Metastatic or locally advanced platinum-resistant ovarian cancer	Phase II (NCT00806156)
				Bevacizumab-resistant high grade glioma	Phase II (NCT01663012)
				Metastatic and recurrent NSCLC	Phase II (NCT01773109)
PEG-paclitaxel	Enzon Pharmaceuticals, Inc	Paclitaxel	PEG (Ester)	Advanced solid tumors or lymphoma	Phase I (NCT00023166); discontinued
DE-310, DX-8951	Daiichi Pharmaceuticals, Japan	Exatecan mesylate	Carboxymethyldextran (GFLG)	Solid tumors	Phase I; discontinued [108]
AD-70; DOX-OXD	Mitsubishi Tanabe Pharma	Doxorubicin	Oxidized dextran (Schiff's base)	Refractory solid tumors	Phase I; discontinued [109]
XMT-1001	Mersana Therapeutics	Camptothecin	PHF (Succinamidoester)	Advanced solid tumors	Phase I (NCT00455052)

Table 2: Polymer-drug conjugates on clinical trials for cancer therapy.

DACH = diaminocyclohexane; EudraCT Number = Clinical trial registry number of the European Union Drug Regulatory Authorities Clinical Trial System; GFLG = Gly-Phe-Leu-Gly; HNSCC =Head and Neck Squamous Cell Carcinoma; HPMA = N-(2-Hydroxypropyl)methacrylamide; NCT = ClinicalTrials.gov registry number; NSCLC= non-small cell Lung Cancer; PEG = poly(ethylene glycol); PGA = Poly-L-glutamic acid; PHF = Poly(1-hydroxyl-methylethylene hydroxyl-methyl-formal); SCLC= small cell lung cancer

Product name	Company	Drug	Polymer/ targeting moiety	Indication	Clinical status
Non-targeted polymeric nanoparticles					
Doxorubicin Transdrug (Livatag)	Onxeo (BioAlliance Pharma)	Doxorubicin	PIHCA	Advanced hepatocellular carcinoma	Phase III (NCT01655693)
DHAD-PBCA-NP	-	Mitoxantrone	PBCA	Hepatocellular carcinoma	Phase II [61]
Docetaxel-PNP	Samyang Biopharmaceuticals	Docetaxel	mPEG-PLA	Advanced solid tumors	Phase I (NCT02274610; NCT01103791)
CRLX101 (IT-101)	Cerulean Pharma Inc.	Camptothecin	Cyclodextrin-PEG	NSCLC	Phase II (NCT01380769)
				SCLC	Phase II (NCT01803269)
				Locally advanced rectal cancer in combination with capecitabine and radiation therapy	Phase Ib/II (NCT02010567)
				Recurrent ovarian, tubal and peritoneal cancer	Phase II (NCT01652079) (with bevacizumab); Phase I (NCT02389985) (with paclitaxel)
				Solid tumors	Phase I (NCT02648711); Phase Ib/IIa (NCT00333502)
				Advanced or metastatic stomach, gastroesophageal, or esophageal cancer	Pilot study (NCT01612546)
				Metastatic renal cell carcinoma in combination with bevacizumab	Phase II (NCT02187302)
CRLX301	Cerulean Pharma Inc.	Docetaxel	Cyclodextrin-PEG	Advanced solid tumors	Phase I/IIa (NCT02380677)
Targeted polymeric nanoparticles					
BIND-014	Bind Therapeutics	Docetaxel	PEG-PLGA/ PSMA	Urothelial carcinoma, cholangiocarcinoma, cervical cancer and HNSCC	Phase II (NCT02479178)
				NSCLC	Phase II (NCT01792479)
				KRAS mutation positive or squamous cell NSCLC	Phase II (NCT02283320)
				Metastatic castration-resistant prostate cancer	Phase II (NCT01812746)
				Advanced or metastatic cancer	Phase I (NCT01300533)

Table 3: Polymeric nanoparticles on clinical trials for cancer therapy.

DHAD = dihydroxyanthracenedione (mitoxantrone); HNSCC = Head and neck squamous cell carcinoma; mPEG = methoxy-poly(ethylene glycol); NCT = ClinicalTrials.gov registry number; NSCLC = non-small cell lung cancer; PBCA = poly(butyl cyanoacrylate); PIHCA = poly(isohexyl cyanoacrylate); PLA = poly (D,L

lactic acid); PLGA = poly (D,L lactic-co-glycolic acid); PMSA = prostate-specific membrane antigen; SCLC = small cell lung cancer

Imaging modality	Agent	nanoplatform and composition	Trade name	Company	imaging indication	Status
MRI	Ferucarbotran (SHU-555)	SPIO NP coated with carboxydextran	Resovist	Bayer Schering Pharma AG	Liver/spleen malignancies	Approved in Europe; withdrawn
	Ferumoxyde (AMI-25)	SPIO NP coated with dextran	Feridex I.V.	Bayer Schering Pharma AG	Liver/spleen malignancies	FDA-approved; withdrawn
			Endorem	Guerbet	Liver/spleen malignancies	Approved in Europe
	Ferumoxsil (AMI-121)	SPIO NP	Sienna+	Endomagnetics Ltd	Sentinel nodes mapping in breast cancer	NCT01790399 (Feasibility study); NCT02336737 (comparison study)
			Lumirem	Guerbert	Gastrointestinal tract	FDA-approved
			GastroMARK	Advanced Magnetics	Gastrointestinal tract	FDA-approved
			Abdoscan	Nycomed (now GE Healthcare)	Gastrointestinal tract	Approved in Europe. Taken off the market
	Ferumoxtran-10 (AMI-227)	USPIO NP coated with dextran	Sinerem	Guerbet	Lymph node metastasis	Approved in Europe; withdrawn
			Combidex	AMAG Pharmaceuticals	Lymph node metastasis in different neoplasms	Phase I/II (NCT00188695)(uterine, cervix, bladder and prostatic neoplasms), (NCT00416455) (cervical or endometrial cancer); Phase II (NCT00107484) (breast cancer); Phase IV (NCT00185029) (prostatic neoplasms); discontinued
	NC100150	USPIO NP coated with carbohydrate-polyethylene glycol	Clariscan	Nycomed (now GE Healthcare)	Angiography-Perfusion	Clinical trials stopped
	Ferumoxytol (Code 7728)	USPIO NP coated with poly (glucose sorbitol carboxymethylether)	Feraheme (USA and Canada)/ Rienso (Europe)	AMAG Pharmaceuticals/ Takeda Pharmaceutical Company Ltd.	Brain neoplasms	Phase II (NCT00103038), (NCT00659126)
					Primary and nodal tumor in HNSCC	Phase 0 (NCT01895829)
					Lymph node metastasis in prostate cancer and GU cancers	Phase I (NCT01296139) (prostate cancer); Phase II (NCT02141490) (GU cancers)
					Pre-operative staging of pancreatic cancer	Phase IV (NCT00920023)
PET	124I	**124I-cRGDY-PEG-dots (Cornell dots, core-shell silica NP)**	C-dots	-	Melanoma, malignant brain tumors, pituitary adenoma and hepatic metastasis	NCT01266096, [82]
Optical imaging	Cy5.5	**cRGDY-PEG-Cy5.5-C-dots (Cornell dots, core-shell silica NP)**	C-dots	-	Sentinel Lymph Node Mapping in Head and Neck Melanoma, Breast and Cervical/ Uterine Cancer	NCT02106598

SPECT	Technetium Tc 99m	Technetium Tc 99m sulphur/albumin colloid NP	Nanocoll/ Nanocis	GE Health Care	sentinel lymph node mapping in invasive breast cancer	Preliminary clinical study (NCT00438477 (Breast cancer) NCT00070317 (cervical cancer)
CT	Au	Au-SiO2 colloid	Auroshell	Nanospectra Biosciences	Solid tumors	NCT02680535 (Phase I)

Table 4: Nanoparticles on the market or in clinical trials for cancer imaging.

C-dots=Cornell dots; cRGDY= cyclic arginine–glycine–aspartic acid peptide; CT= X-ray computed tomography; FDA= Food and Drug Administration in USA; GU= Genito-Urinary; MRI = Magnetic resonance imaging; NCT = ClinicalTrials.gov registry number; NIRF= Near-infrared fluorescence; NP=Nanoparticles; HNSCC= Head and Neck Squamous Cell Carcinomas; I.V=Intravenous; OCT= Optical coherence tomography; PEG= poly(ethylene glycol); PET= positron-emission tomography; SPECT= photon emission computed tomography; SPIO= Superparamagnetic Iron Oxides; USPIO= Ultrasmall Superparamagnetic Iron Oxides