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The Emergence of Nanopharmacy: From Biology to Nanotechnology and Drug Molecules to Nanodrugs

Marilena Hadjidemetriou, Zahraa Al-Ahmady, Mariarosa Mazza, and Kostas Kostarelos

Nanomedicine Lab, Faculty of Medical and Human Sciences, University of Manchester, AV Hill Building, Manchester M13 9PT, United Kingdom.

3.1
Introduction

Nanopharmaceuticals consist of a pharmaceutically active molecule or substance ("pharmacon") adsorbed onto, conjugated to, or encapsulated within a nanoscale-based material, the size of which falls into a size range similar to intra- and extracellular biological structures [1]. The principal goal of *nanopharmacy* is to create novel agents with improved therapeutic efficacy utilizing nanoscience. This can be achieved either by using novel therapeutic molecules (small molecules, proteins, peptides, nucleic acids) or by reformulating existing ones (i.e., poorly soluble drugs and antibodies) [2,3]. In most cases, the broader aim is to increase the bioavailable drug concentration at the desired (e.g., target) site, while simultaneously minimizing toxic responses by reducing off-target effects [4,5]. Nanopharmaceuticals have been developed to address conditions for which traditional pharmaceutical treatments are ineffective (e.g., antibacterial resistance), diseases for which therapies are available but should be more adjusted to the patient's needs (cardiovascular diseases, cancer) and diseases for which therapeutic interventions are not available (e.g., stroke and Alzheimer's disease) [6].

Nanoparticles are of immense scientific interest because their properties differ from the bulk material or the isolated atoms and molecules used to fabricate or assemble them. For example, gold nanoparticles have different optical properties from atomic gold, as they are highly efficient in absorbing and scattering light. Gold nanoparticle size also commonly influences the light-induced collective oscillation of electrons on the metal surface, a phenomenon known as surface plasmon resonance. With increased gold nanoparticle size, the surface plasmon resonance wavelength shifts to longer wavelengths. This unique property is only one example that has been exploited in cancer research for the development of multiple imaging, diagnostic, and therapeutic purposes [7]. Nanopharmacy

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requires the interdisciplinary contribution from fields ranging from chemical sciences to biology and from physics to pharmacology. This is not entirely unexpected, since different and new scientific disciplines evolve constantly. Gene therapy is another example of a research field waiting to mature into a clinical discipline, where various disciplines need to converge toward the achievement of the same goal: effective delivery of biologically active nucleic acid sequences for the genetic treatment or alleviation of various pathological conditions.

The design of delivery systems is largely dependent on a variety of factors that are intricately related to each specific therapeutic or diagnostic application. The ability to fine-tune the pharmacological properties (biodistribution, tissue uptake, and pharmacokinetics) of biologically active molecules through their reformulation using nanomaterials is considered of utmost importance. There are various technologies and approaches that allow such fine-tuning and optimization. First, the route of administration affects nanoparticle biodistribution and provides an initial level of targeting. Second, surface functionalization of nanoparticles with specific moieties modifies their pharmacokinetic profile (e.g., PEGylation). Tissue uptake and cellular internalization of nanoparticles can also be controlled by decorating the outer surface of nanoparticles with targeting moieties. This can take place through molecular surface modifications, either in the form of chemically conjugated targeting ligands or proteins adsorbed from the local environment, that have the ability to dramatically alter the nature of the cellular interactions. Lastly, the engineering of nanoparticles designed to release their payload in response to various stimuli, can also provide an alternative method to target specific tissues [3,8]. The potential of nanopharmacy is challenged though from the high expectations linked to new discoveries, rapid nanotechnology advancements, and the need for short-term deliverables. Sensationalism is also another challenge. Components represented as *"nano"* commonly attain either the connotation of a "wonder" technology, full of promises of revolutionizing therapy, or in direct contrast, as an unparalleled threat to safety. Both connotations have very little basis in scientific reality, and do not take into consideration that some of the most stringent requirements for therapeutic efficacy and safety already govern the approval of new nanopharmaceuticals in a process that can lasts for decades. The clinical translation of multicomponent, sophisticated nanodrugs with chemical and biological functionalizations, such as targeting moieties, remains challenging and requires the use of already existing safety, efficacy, and quality assessments tools as well as the development of new testing strategies [6].

In short, nanopharmacy is an emergent, multidisciplinary field with great potential and expectations, however, will require time and persistent investment in order to allow maturation into clinical reality. A plethora of studies at the preclinical and clinical levels are currently carried out for new nanopharmaceuticals, some of which may have different and intriguing mode of action due to complex mechanisms involving the interplay among mechanical, chemical, pharmacological, and immunological components. This chapter aims to offer an

overview of how nanotechnology advances can impact the design and development of novel pharmacological agents and contribute to the emerging field of nanopharmacy.

3.2
First Generation of Nanopharmaceuticals: From Drug Molecules to Nanodrugs

Early efforts in *nanopharmacy* were focused on improving the properties of already existing therapeutic agents, aiming to improve their pharmacokinetics, reduce the adverse effects arising from them, and specifically target them at the site of action. A multitude of materials has been investigated as drug delivery systems, including biological substances, such as proteins and phospholipids, as well as chemical substances, such as polymers, metals, carbon, and silica [9]. This section, based on the example of two clinically used anticancer agents, doxorubicin and paclitaxel, will highlight the major advantages associated with the formulation of therapeutic agents into nanoparticle-based moieties.

Nanoscale delivery systems have been explored for a diverse range of applications, with oncology being the most notable beneficiary to date. One of the major challenges to the treatment of solid tumors is the accumulation of the drug at the target tissue, while avoiding healthy tissue damage. The encapsulation of chemotherapeutic agents inside nanocarriers has been an established strategy to reduce the drug-associated toxicity to normal tissues and to simultaneously increase their accumulation into highly vascularized solid tumors [10,11]. Nanocarriers selectively accumulate into the tumor tissue as a result of the pathophysiological characteristics of the tumor, namely, leaky vasculature and poor lymphatic drainage. This phenomenon is known as enhanced permeability and retention (EPR) effect (Figure 3.1a) [12,13]. Key to the passive accumulation of nanocarriers at the tumor site is their long blood circulation time. Nanoparticles are recognized as foreign bodies and are opsonized by the cells of the reticuloendothelial system (mononuclear phagocyte system, MPS), and thus the availability of the drug at the required site is diminished [14,15]. A major breakthrough was the surface coating of nanoparticles with the hydrophilic polymer polyethylene glycol (PEG), which imparts steric stabilization and reduces the interaction of NPs with serum proteins, resulting in a substantial increase in the circulation time [16,17]. Because of the ability of these nanocarriers to avoid uptake by the MPS cells, they were termed “stealth” drug delivery systems.

Among the nanosized drug delivery systems, liposomes were the first and the most extensively used nanopharmaceuticals for cancer therapy [18]. Liposomes can be described as “spherical phospholipid vesicles consisting of one or more concentric lipid bilayers enclosing an aqueous core” [19]. Their ability to encapsulate hydrophilic molecules in their aqueous inner space [20] as well as hydrophobic molecules in their phospholipid bilayer membranes [21], makes them very attractive as drug delivery systems. They are now considered to be the most clinically established nanotechnology platform, with more than

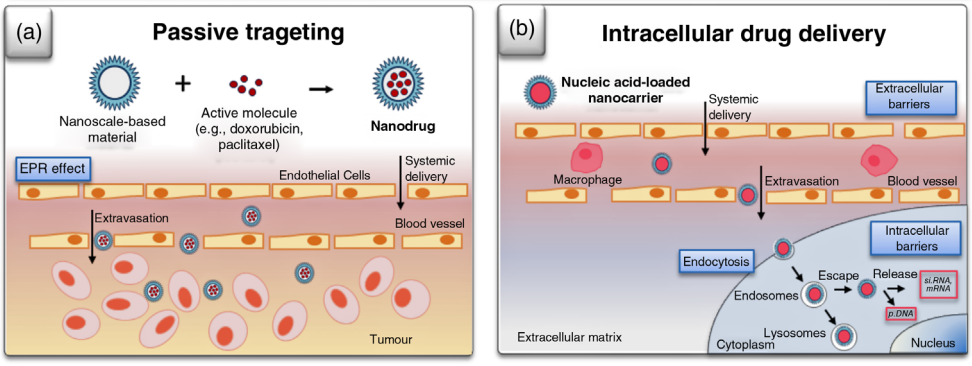

Figure 3.1 From drug molecules to nanodrugs. (a) Passive targeting of nanodrugs-EPR effect. Nanocarriers selectively accumulate into the tumor tissue as a result of the pathophysiological characteristics of the tumor, namely, leaky vasculature and poor lymphatic drainage, phenomenon known as enhanced permeability and retention effect (EPR). (b) Intracellular targeting. After intravenous injection, the vector: nucleic acid complex is distributed to organs via blood circulation and simultaneously undergoes elimination. In addition, cells of the reticuloendothelial system attempt to degrade and eliminate the vector, which is recognized as foreign. After extravasation, the vector is transported across the interstitial space to the target cells, where it has to cross the cell membrane barrier, translocate into the cytoplasm, and release its cargo to its intracellular therapeutic site. To exert its action, DNA has ultimately to reach the nucleus of the target cell, while the action of RNA molecules occurs in the cytoplasm.

12 liposome-based drugs approved for clinical use and many more in various stages of clinical trials [1,18]. Their success can be attributed to their ability to extensively accumulate into regions of enhanced vascular permeability and for their ability to reduce the side effects of encapsulated drugs [22]. The significant reduction in the cardiotoxicity of the anticancer agent doxorubicin (anthracycline antibiotic) after its encapsulation in PEGylated liposomes led to the development of the first nanodrug, Doxil®, approved by the FDA in 1995 for the treatment of AIDS-related Kaposi's sarcoma [23]. PEGylated liposomal doxorubicin shows superiority to free doxorubicin clinical performance owing to (a) prolonged circulation time due to the surface functionalization of liposomes with PEG, (b) stable retention of the drug inside the liposomes while in circulation, (c) increased tumor accumulation attributed to the EPR effect, and (d) decreased cardiac toxicity [23,24].

In addition to the adverse effects, the use of conventional drugs is often hindered by their insolubility in aqueous solutions [25]. The conversion of poor water-soluble drugs into nanodrugs, by their conjugation with macromolecular polymers or proteins, has been a successful strategy to avoid the use of organic solvent-based formulations, often associated with serious and dose-limiting toxicities. An example of a highly hydrophobic drug, successfully formulated into nanoscale delivery systems, is the anticancer agent paclitaxel. Abraxane®, albumin-based, 130-nm-sized nanoparticles conjugated with paclitaxel was licensed

in 2005 for the treatment of metastatic breast cancer and in 2012 as a first-line treatment for advanced nonsmall-cell lung cancer. Albumin-based nanoparticles, similarly to other nanocarriers, passively accumulate at the side of solid tumors, via the EPR effect. In addition, dissociation of albumin nanoparticles into individual drug-bound albumin molecules has been found to facilitate specific albumin-receptor-mediated uptake by the endothelial cell walls of tumor microvessels that further increases the intratumor concentration of paclitaxel [26–28]. Another nanosystem developed for the delivery of paclitaxel is Opaxio®, approved in 2012 for the treatment of glioblastoma. Opaxio®, consists of paclitaxel covalently linked to solid polymer-based nanoparticles, composed of poly(L-glutamic acid). When bound to the polymer, then paclitaxel is inactive, thus, preventing paclitaxel-associated toxicity to healthy tissues. The success of this formulation is based on the enzymatic hydrolysis of the polymer after the accumulation of the nanoparticles inside the solid tumor, which results in the release of active paclitaxel from the polymeric backbone [29,30].

In addition to albumin- and polymer-based solid nanoparticles, micelles provide an alternative for parenteral administration of poorly water-soluble drugs. Micelles are self-assembled spherical nanoparticles with a hydrophobic core and a hydrophilic shell, made of amphiphilic copolymers [31]. An example of micellar formulation product available in the market is Genexol-PM, approved in South Korea for breast cancer and small-cell lung cancer. Genexol-PM consists of 20–50 nm micelles formed by the self-assembly of polyethylene glycol and polylactide polymers, the core of which contains paclitaxel. The co-polymer increases the water solubility of paclitaxel and allows delivery of higher doses than those achievable with paclitaxel alone [32].

In brief, the conversion of therapeutic molecules into nanodrugs requires a comprehensive understanding of both, the nanomaterial design and disease pathophysiology. Nanosized delivery systems offer the opportunity to reformulate conventional active molecules in order to improve their bioavailability, efficacy, and toxicity profile.

3.2.1
Making New Therapies Happen: The Example of Nucleic Acid Therapeutics

Besides improving the efficacy of already established therapeutic molecules, nanotechnology has enabled the development of new therapies. The example of gene therapy is a great case to illustrate the pivotal role of nanotechnology for the clinical translation of novel therapeutic strategies. Gene therapy can be described as the exogenous introduction of nucleic acids (DNA or RNA) into specific host cells, to intentionally modulate gene expression in order to treat or prevent pathological conditions. The treatment of inherited monogenic diseases caused by a single-gene defect by the introduction of a functional copy of the gene represents the prototype of gene therapy and has been referred to as “gene replacement.” Other gene manipulation strategies are now at the preclinical stage of investigation, including gene knockdown by RNA interference (RNAi),

gene addition, and gene editing with enormous therapeutic potential [33]. From the drug delivery point of view, nucleic acids have unfavorable physicochemical characteristics as therapeutic agents and the use of a delivery vector is a necessity for their clinical application [34].

One of the main challenges for the systemic delivery of nucleic acids is their short half-life due to the degradation by serum nucleases. In addition, the high molecular weight and negative charge of unmodified nucleic acids hamper their cellular uptake. The administration of nucleic acids requires the development of safe and efficient delivery vectors with the ability to target nucleic acids to the region of interest provide protection from nuclease degradation, shield recognition by the immune system, and limit excretion through the kidneys. The two main approaches for nucleic acid therapy are based on viral and nonviral delivery vectors, with ~70% of gene therapy clinical trials carried out so far using modified viruses, such as retroviruses, lentiviruses, adenoviruses, and adeno-associated viruses [35,36].

The ability of viruses to insert their genetic information into mammalian host cells has been successfully utilized for gene delivery. Viral nanoparticles used in gene therapy are engineered from viruses by replacing most of their pathogenic genes with a therapeutic gene cassette, while retaining their infectious nature [37]. The world's first gene therapeutics, Gendicine and Oncorine (adenoviral-vector based), were approved in China, for the treatment of neck and head cancer, in 2003 and 2006, respectively. In 2012, the European Medicines Agency recommended for the first time a gene therapy product, Glybera (adeno-associated viral vector based), for the treatment of lipoprotein lipase deficiency. Although, viral vectors have substantially advanced nucleic acid delivery, several limitations are associated their use, including their immunogenicity, mutagenesis after random integration into the host genome, limited nucleic acid capacity, and difficulty of vector production. Synthetic nonviral vector systems have the potential to address many of these limitations, particularly with respect to safety. Size-independent delivery of nucleic acids, simpler quality control, and ease of preparation are some of the advantages that nonviral vectors can offer. However, only few nonviral vectors have progressed into clinical trials, and none of these vectors has been approved by regulatory authorities. This is mainly due to the compromised therapeutic efficiency of nonviral vectors compared to viral vectors [35].

Liposomes were the first nonviral gene delivery systems to reach clinical trials. It was first described, in 1980, that liposomes could entrap and deliver DNA to monkey kidney cells [38]. Few years later, Felgner *et al.* demonstrated that synthetic cationic liposomes could complex DNA and facilitate efficient transfection of various mammalian cell lines [39]. Since then, the development of nonviral vectors is rapidly expanding, with a broad spectrum of nanoconstructs being under preclinical investigation, including polymeric nanoparticles, carbon-based materials [40], and peptides [41]. Among the various synthetic vectors, cationic lipids and polymers are the most studied nonviral delivery vesicles so far with few being currently under clinical investigation [42]. Liposomes, formed from

the cationic lipid DOTAP and cholesterol, have been well characterized as gene delivery systems and have progressed in clinical trials. For example, DOTAP–cholesterol complexed with a plasmid encoding a tumor suppressor protein, FUS1, is currently in phase II for the treatment of nonsmall-cell lung cancer [43]. Polyethylenimine (PEI) is among the most studied polymeric materials for gene delivery. PEI–DNA complexes are currently in stages I and II of clinical trials for local gene therapy of ovarian, pancreatic, and bladder cancers [42,44,45]. To improve its stability and biocompatibility, PEI has been also modified with other polymers. A PEG–PEI–cholesterol lipopolymer, complexed with plasmid DNA encoding the human gene for interleukin 12 (IL-12), is currently under clinical investigation for ovarian and colorectal cancer treatment [46,47].

Despite the significant efforts, nonviral gene therapy is still clinically challenging and requires a better understanding of the fate of nanodrugs after administration. The barriers for effective delivery of nucleic acids by synthetic vectors depend on the target organ and on the route of administration. In general, local administration has fewer barriers compared with systemic delivery. After intravenous injection, the vector–nucleic acid complex is distributed to organs via blood circulation and simultaneously undergoes elimination. In addition, cells of the reticuloendothelial system attempt to degrade and eliminate the vector, which is recognized as foreign. After extravasation, the vector is transported across the interstitial space to the target cells, where it has to cross the cell membrane barrier, translocate into the cytoplasm, and release its cargo to its intracellular therapeutic site. To exert its action, DNA has ultimately to reach the nucleus of the target cell, while the action of RNA molecules occurs in the cytoplasm (Figure 3.1b) [34].

The need to develop vectors for nucleic acid-based therapeutics initiated the idea of organelle-specific drug delivery, as an attractive strategy to increase the therapeutic index of a drug. Intracellular targeting has been recently attempted for other novel therapeutic molecules, including the delivery of proapoptotic compounds to mitochondria, and the delivery of enzymes to defective lysosomes [48–50]. Although in its infancy stages, the intracellular spatial control of drug-containing nanoparticles is expected to sharply increase the therapeutic efficacy of nanodrugs and thus allow their clinical application.

3.2.1.1 Making Nanodrugs Smarter: Multifunctional Nanodrugs

The first generation of nanodrugs described earlier was mainly designed to overcome a single challenge, for example, increase drug stability, prolong plasma circulation half-life, or the need to passively target specific organs taken the advantage of the accompanied pathological changes [1]. Advances in the development of pharmaceutical nanotechnology has led to possibility of engineering multifunctional nanodrugs that can perform more than one task (concurrently or sequentially) [51,52] to efficiently deliver their payload (single or multiple) [53,54] to the target site. For the purpose of this chapter, multifunctional nanodrugs have been classified into three main categories: targeted, responsive, and diagnostic/theranostic nanodrugs.

Targeted Nanodrugs

Actively targeted nanodrugs represent the first group of multifunctional nanodrugs and can be accomplished by the attachment of targeting ligands on their outer surface, such as monoclonal antibodies, antibody fragments, peptides, and aptamers. It has been well established that the best way to prevent shielding of the targeting ligand by the polymeric coating layer (e.g., PEG) is to chemically attach the targeting moiety to the distal end of the polymer chain that is coating these nanodrugs (Figure 3.2a) [55]. An example of such actively targeted nanodrug is PEGylated doxorubicin-loaded liposomes that have human epidermal receptor 2 (HER2) monoclonal antibody attached to their surface [56]. These liposomes were successfully used to target HER2-overexpressing tumor cells in mice [56] and are currently under phase I/II clinical testing for HER2-positive breast cancer patient [57,58].

It is important to clarify that active targeting of long circulating nanodrugs is not necessarily independent from passive targeting and in most of the cases

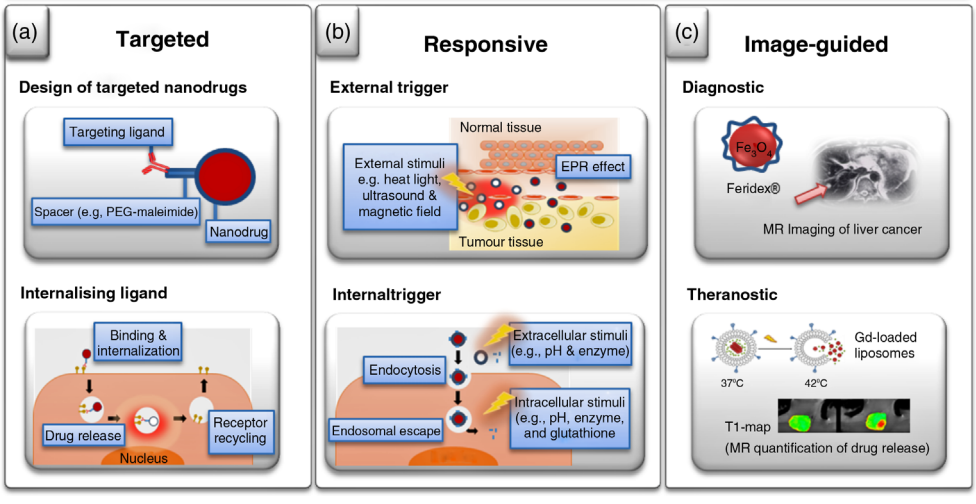

Figure 3.2 Making nanodrugs smarter (a) targeted nanodrugs. The ideal design of targeted nanodrugs is to attach the targeting ligand to the distal end of the polymeric coating layer in order to reserve its binding capacity. After binding with target cells, activation of receptor-mediated uptake process of nanodrug occurs, carrying therapeutic payload into the target cell. (b) Responsive nanodrugs are designed to release their cargo in the vicinity of the target tissue (such as a tumor) in response to external stimuli (such as heat) or internal stimuli (such as a change in pH). (c) Image-guided nanodrugs. The selective uptake of Feridex® nanoparticles by normal hepatic cells compared to tumor cells, allows malignant tissue to be easily distinguished by appearing as a bright spot compared to the surrounding normal tissue. Encapsulation of MR contrast agents that can change signal intensity based on the interaction with surrounding water molecules allows real-time monitoring of drug release. The increase in MR signal after release of encapsulated MR contrast agent and drug from liposomes provides information about spatial and temporal drug release.

these two phenomena are closely connected. This is because the accumulation of targeted nanodrugs to the pathological tissue is mainly dependent of the EPR effect (passive targeting), while the interaction with the target cell will be driven by ligand-mediated interaction (active targeting) [52]. Therefore, any improvement in therapeutic activity is not due to higher accumulation of actively targeted nanodrugs in the diseased site *per se*, but due to the activation of receptor-mediated uptake process (cellular internalization) of nanodrugs carrying therapeutic payload into the target cells [59]. As a result, the premature loss of the therapeutic payload prior to binding and uptake by target cells (drug retention/stability) [60] and the rate of drug release (bioavailability) [61] are very crucial to increase the therapeutic activity compared to nontargeted nanodrugs. In the case of HER2-targeted liposomes, for example, previous studies in mice have shown that their overall tumor accumulation did not increase compared to nontargeted liposome. However, the intratumoral microdistribution and cellular localization of targeted anti-HER2 liposomes were different due to their cellular internalization capacity. Significant fraction of HER2-targeted liposomes was observed within cancer cells, compared to nontargeted liposomes mainly found in stromal cells [56]. There are many factors that affect the efficiency of targeted nanodrugs such as vascular permeability, tissue penetrability, binding site barrier, receptor density, targeting ligands affinity to their target, and interaction with plasma proteins [62]. Therefore, targeted nanodrugs have shown to have the best therapeutic effect when directed toward easily reached target, such as tumor vasculature, micrometastasis, blood cancer [63], and when combined with permeability enhancers [51,63]. The clinical progress of targeted nanodrugs has been much slower compared to nontargeted ones and only few examples have progressed into the clinic such as HER2-targeted liposomal doxorubicin (MM-302) for HER2-positive breast cancer (Phase I/II) [57,58] and Docetaxel-loaded polymeric nanodrugs targeted against prostate-specific membrane antigen (Phase I/II) [64]. The main reason behind that is the substantial increase in the manufacturing cost, such as the production of high-quality antibodies. The therapeutic benefits achieved from targeted nanodrugs have to be considerably higher than what can be achieved from nontargeted counterparts to justify their approval [18].

Responsive Nanodrugs

The second group of multifunctional nanodrugs combine responsive functionality to several stimuli. This can be achieved by the inclusion of specific components that respond (in a "smart," responsive manner) to different types of stimuli that can change their properties or behavior (Figure 3.2b). Smart nanodrugs can be designed to respond to triggers at the cellular levels, such as pH-sensitive nanodrugs to trigger the release of their payload in the late endosome or lysosomes or to facilitate the escape from the lysosome to the cytoplasm. Alternatively, many other formulations were designed to take advantage of the specific microenvironmental changes at the tissue level of certain pathological conditions such as low interstitial pH [65], changes in the level of certain

enzymes (phospholipase A2 [66] and matrix metalloproteinases [67]), or alteration in the level of glutathione (redox-responsive nanodrugs) [68]. Among these pathological changes, neoplastic conditions remained the main focus in addition to other diseases such as inflammation, infection, and ischemia [52]. Stimuli-responsive nanodrugs can also respond to external triggers from outside the body (external stimuli), for example, changes in temperature, light, magnetic field, or ultrasound [69]. Interest in designing stimuli-responsive nanodrugs increased with the realization that more efficient delivery strategies are needed. One example on that is the need to provide a tailored release profile (triggered release) with excellent control on the time, duration, and site of drug release. This is mainly because the early generation of nanodrugs, such as Doxil, has been clinically approved to reduce drug-associated toxicity rather than improving efficacy [70].

The concept of stimuli-responsive nanodrugs was first suggested in late 1970s, and was based on hyperthermia (external trigger) induced-release of methotrexate from thermosensitive liposomes. That early example was demonstrated to be four times more efficient to deliver drug to heated tumor compared to nonheated control tumors [71]. Shortly after, many other examples of smart responsive nanodrugs followed [69,72–74]. In another widely explored type of responsive nanoparticle-based approach, the acidic environment of some tumor interstitial spaces [65] or endosomes has been utilized to design pH-sensitive systems. The inclusion of pH-sensitive components is essential in the design of this type. PEGylated poly(L-histidine) copolymer self-assembles into a stable form of polymeric micelles at neutral pH, but as the pH drops to 6.5 or less, protonation of histidine residues in the poly(L-histidine) block occurs resulting in destablization of the micelle structure and release of the incorporated drug. The generation of high local drug concentration in the tumor interstitium or at the cellular level has proved to be effective when resistance to certain drugs is likely to happen, for example, due to drug efflux [75,76]. Redox-responsive nanodrugs is another type of smart systems that respond to the differences in the redox potential such as the changes in glutathione level. Glutathione level increases significantly by more than 100-fold intracellularly compared to extracellular environment and in tumor tissue compared to healthy ones [68,77]. Disulfide bonding is one of the most widely used chemical strategy in the design of redox-responsive nanodrugs, since it is prone to rapid cleavage by glutathione to attain redox sensitivity [77]. Many examples have been described in this regard by the inclusion of disulfide bonds either in the core [78] or in the shell [79] of micelles and the lipid component of liposomes [74] that lead to rapid structural disassembly and release of encapsulated drugs. Although conceptually promising, in practice only a few examples of responsive nanodrugs have progressed to clinical evaluation. In particular, internal triggers are often seen harder to control because of the variability from one patient to another or even within the same tissue, such as the variability in the degree of pH changes at the tumor site. On the other hand, external triggers could be better controlled and indeed most of the progress that has been made in responsive systems has

been in that area [69]. To that end, we would like to mention two examples of externally triggered nanodrugs, currently in clinical trials. These two examples are similar in concept, but very different in design. The first example is ThermoDox®, a liposomal doxorubicin formulation that releases drug in response to external mild hyperthermia (41–45 °C) [80]. The responsive nature of ThermoDox® is based on the presence of lipids, such as dipalmitoylphosphatidylcholine and lysolipids, with a suitable phase transition temperature that undergo changes from gel-to-liquid phase and stabilize long-lasting pores in the liposomal membrane for the drug to be released, in response to mild increase in temperature [81]. These changes in the liposomal structure lead to ultrafast release of encapsulated drug in the tumor vasculature [82] and showed efficient tumor growth control in several tumor models in mice [83,84] and currently been tested in human patients [85]. Initial data from phase III clinical trial for hepatocellular carcinoma (HCC) with optimum radiofrequency ablative heating have shown 58% improvement in overall survival [86,87]. ThermoDox® is also currently clinically tested with other external heating techniques such as high-intensity focused ultrasound (HIFU) for HCC [88] or with external microwave hyperthermia for recurrent chest wall breast cancer. NanoTherm® is another example of an externally responsive nanodrug that has advanced to clinical testing for magnetic thermal ablation. NanoTherm® consists of aminosilane-coated superparamagnetic iron oxide nanoparticles with 15 nm core diameter. It is important to stress here that the nanomaterial (iron oxide nanopartciles) is playing the key therapeutic role in this example. In this particular case, the nanodrugs is directly introduced into the solid tumor mass and externally activated by external exposure to alternating magnetic field. The changes in the polarity of the applied magnetics force up to hundred thousand times per second caused the iron-oxide nanoparticles to significantly increase their core temperature. The temperature in the tumor can be controlled by changing the duration of exposure to the oscillating magnetic field to achieve intratumoral temperature in the ablative region (>50 °C) that can directly destroy cancer cells. NanoTherm® is currently clinically tested for glioblastoma multiforme [89] and prostate cancer [90].

Diagnostic/Theranostic Nanodrugs

The third group of multifunctional nanodrugs comprises those having additional imaging functionality either for diagnostic purposes only or in combination with therapy (theranostics) (Figure 3.2c). The inclusion of imaging component in the design of nanodrugs enables not only the track of their pharmacokinetics, accumulation in the target organ and off-target (healthy tissues), but also allows the efficacy of the therapy to be monitored noninvasively and in real time [54]. Due to the significant potential this group of nanodrugs holds, many examples of nanodrugs for image-guidance and theranostic purposes have been reported and the progress is still exponential. Although widely different in functionality, the design of these nanodrugs can be divided into two main categories: (a) those based on traditional nanodrugs (such as liposomes and micelles) that can be

functionalized with contrast imaging modalities [53]; and (b) those that are built on a template nanoparticle, such as gold and iron oxide, that have intrinsic imaging properties but can be further modified with a second imaging modality or therapeutic molecule [52,91].

Diagnostic These types of nanodrugs include those designed for purely diagnostic purposes, utilizing the potential diagnostic advantages offered compared to low-molecular-weight contrast agents. These possible benefits can be summarized as (a) flexibility in the chemical composition and size to improve biocompatibility; (b) improved biological stability and reduced clearance rate to prolong the time window for imaging; (c) capability of active targeting via surface modification with specific ligands; and (d) ability to design multifunctional contrast agents by using a combination of more than one contrast agent, for example, for magnetic and optical imaging [92]. Feridex®, a colloidal aqueous dispersion of iron oxide nanoparticles associated with dextran that was approved by the FDA for magnetic resonance (MR) imaging. The size and paramagnetic properties of iron oxide nanoparticles offer unique advantages compared to other MR contrast agents such as gadolinium chelates. Feridex® nanoparticles are between 80 and 150 nm, therefore, their renal clearance is reduced and their uptake is diverted toward the reticuloendothelial system resulting in rapid accumulation in the liver and spleen. Consequently, hepatic imaging was the first application of these nanoparticles for detection of hepatic cancer [93]. In addition, the uptake of iron oxide nanoparticles by activated inflammatory cells such as macrophages and activated microglia makes them excellent contrast agent for imaging inflammatory processes associated with some pathologies such as multiple sclerosis, stroke, and brain tumors [91].

Theranostic One of the key advantages that nanodrugs offer is improved localization of the therapeutic molecules to the target site, particularly after systemic administration. Determination of their pharmacokinetic profile and accumulation into target sites is of significant usefulness [54]. An example of this is the use of radioactive isotope indium-111 to label PEGylated liposomes to monitor their accumulation in the tumor tissue in patients with different cancer types such as breast, head and neck, and Kaposi's sarcoma [94]. This technology has been able to demonstrate in real time that 50% of the PEGylated liposomes were still in systemic circulation 48 h after administration. It also proved that radiolabeled liposomes localized efficiently into the tumor, but the level of accumulation varied considerably from one tumor type to another [94]. In the case of doxorubicin-loaded PEGylated liposomes, a significant percentage of liposomes can be taken up by circulating and tumor-associated macrophages. Since these macrophages are known to play an important role in the clearance mechanism of liposomes, the direct cytotoxic effect of drug-loaded liposomes on the macrophages (following drug release inside the macrophages) can extensively change the pharmacokinetics and distribution of liposomes [95]. The use of theranostic nanodrugs can also guide the patient selection process in clinical practice

(personalized medicine). This will help to justify only those patients that show evidence of high drug accumulation in the desired target site, and good therapeutic response to the initial treatment cycles, should be considered for continuation. Otherwise, replacement or adding alternative therapeutic options should be taken into account [54].

Another important advantage that theranostic nanodrugs offer is the ability to monitor drug release. In order for a nanodrug to be therapeutically effective, the encapsulated drug should be released once accumulated at the pathological site. MR contrast agents such as gadolinium and manganese have been highly useful for this purpose as their signal depends on the interaction with the surrounding water molecules. The MR signal generated can vary significantly when these agents are encapsulated within a nanoparticle (limited interaction with water molecules), compared to that obtained after the release of the contrast agent (free interaction with water molecules). One such example are paramagnetic temperature-responsive liposomes, consisting of Gd(HPDO3A) – a clinically approved MR contrast agent (ProHance®) – coencapsulated with doxorubicin inside the aqueous space of liposomes [96,97]. The expectation is that even more efforts will be invested in developing theranostic nanodrugs, and that these systems and strategies will contribute substantially to realizing the potential of personalized medicine.

3.3 Conclusion

Nanopharmacy as new discipline brings together the chemical, physical, and engineering sciences with pharmaceutical sciences aiming to generate nanoscale systems of diagnostic and therapeutic value. Initial efforts intended to improve the performance of already existing therapeutics. The conversion of therapeutic agents to nanodrugs requires a comprehensive understanding of both nanomaterial design and disease pathophysiology. The encapsulation of chemotherapeutics into nanosized delivery systems has been a successful strategy to obtain high drug accumulation in target disease sites and decreased off-target drug-associated toxicities. In addition, reformulation of poorly soluble drugs into nanosized particles (e.g., Abraxane) has enabled the development of therapies for cancer by improving drug bioavailability.

Besides improving the efficacy of already established therapeutic molecules, the application of nanotechnology in pharmaceutical science has enabled the development of new therapies. Nanomedicine-based gene therapy has opened the avenue to treat disorders by delivering nucleic acids intracellularly. Smart nanodrugs of different chemistry are being developed to enable targeting and design of responsive nanoparticles to microenvironment changes (pH, enzymes) or external stimuli (temperature, light, magnetic fields). Incorporation of the imaging component in the development of nanomedicines is increasing the potential to couple therapy with diagnostic capabilities. Imaging modalities have

opened up an entirely new avenue to design sophisticated nanopharmaceuticals that can simultaneously achieve diagnosis and therapy.

Due to the complexity of their mode of action, the assessment of safety and risk/benefit of new nanopharmaceuticals remains challenging at the present: new tools for nanocharacterization and testing strategies for quality and safety assessments remain to be defined. Undoubtedly, the emergence of nanopharmacy will contribute to solution of unmet needs in the development of new nanoscale technologies, aiming to improve clinical outcomes in a variety of clinical settings. At present, the major field of application is in oncology; however, nanomedicines will most likely have many more possibilities in the treatment of neurology [98] and immune disorders [99].

References

1 Weissig, V., Pettinger, T.K., and Murdock, N. (2014) Nanopharmaceuticals (part 1): products on the market. *Int. J. Nanomedicine*, **9**, 4357–4373.

2 Langer, R. and Weissleder, R. (2015) Nanotechnology. *JAMA*, **313**, 135–136.

3 Cheng, C.J., Tietjen, G.T., Saucier-Sawyer, J.K., and Saltzman, W.M. (2015) A holistic approach to targeting disease with polymeric nanoparticles. *Nat. Rev. Drug Discov.*, **14**, 239–247.

4 Wong, I.Y., Bhatia, S.N., and Toner, M. (2013) Nanotechnology: emerging tools for biology and medicine. *Gene Dev.*, **27**, 2397–2408.

5 Xu, X., Ho, W., Zhang, X., Bertrand, N., and Farokhzad, O. (2015) Cancer nanomedicine: from targeted delivery to combination therapy. *Trends Mol. Med.*, **21**, 223–232.

6 Bremer-Hoffmann, S., Amenta, V., and Rossi, F. (2015) Nanomedicines in the European translational process. *Eur. J. Nanomedicine*, **7**, 135–268.

7 Huang, X. and El-Sayed, M.A. (2010) Gold nanoparticles: optical properties and implementations in cancer diagnosis and photothermal therapy. *J. Adv. Res.*, **1**, 13–28.

8 Kostarelos, K. (2003) Rational design and engineering of delivery systems for therapeutics: biomedical exercises in colloid and surface science. *Adv. Colloid Interface Sci.*, **106**, 147–168.

9 Onoue, S., Yamada, S., and Chan, H.K. (2014) Nanodrugs: pharmacokinetics and safety. *Int. J. Nanomedicine*, **9**, 1025–1037.

10 Peer, D., Karp, J.M., Hong, S., FaroKHzad, O.C., Margalit, R., and Langer, R. (2007) Nanocarriers as an emerging platform for cancer therapy. *Nat. Nanotechnol.*, **2**, 751–760.

11 Davis, M.E., Chen, Z.G., and Shin, D.M. (2008) Nanoparticle therapeutics: an emerging treatment modality for cancer. *Nat. Rev. Drug Discov.*, **7**, 771–782.

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

13 Maeda, H. (2001) The enhanced permeability and retention (EPR) effect in tumor vasculature: the key role of tumor-selective macromolecular drug targeting. *Adv. Enzyme Regul.*, **41**, 189–207.

14 Gregoriadis, G. and Neerunjun, D.E. (1974) Control of the rate of hepatic uptake and catabolism of liposome-entrapped proteins injected into rats. Possible therapeutic applications. *Eur. J. Biochem.*, **47**, 179–185.

15 Iyer, A.K., Khaled, G., Fang, J., and Maeda, H. (2006) Exploiting the enhanced permeability and retention effect for tumor targeting. *Drug Discov. Today*, **11**, 812–818.

16 Allen, T.M. and Hansen, C. (1991) Pharmacokinetics of stealth versus

conventional liposomes: effect of dose. *Biochim. Biophys. Acta*, **1068**, 133–141.
17 Allen, T.M., Hansen, C., Martin, F., Redemann, C., and Yau-Young, A. (1991) Liposomes containing synthetic lipid derivatives of poly(ethylene glycol) show prolonged circulation half-lives *in vivo*. *Biochim. Biophys. Acta*, **1066**, 29–36.
18 Allen, T.M. and Cullis, P.R. (2013) Liposomal drug delivery systems: from concept to clinical applications. *Adv. Drug Deliv. Rev.*, **65**, 36–48.
19 Bangham, A.D. and Horne, R.W. (1964) Negative staining of phospholipids and their structural modification by surface-active agents as observed in electron microscope. *J. Mol. Biol.*, **8** (5), 660–668.
20 Li, X., Hirsh, D.J., Cabral-Lilly, D., Zirkel, A., Gruner, S.M., Janoff, A.S., and Perkins, W.R. (1998) Doxorubicin physical state in solution and inside liposomes loaded via a pH gradient. *Biochim. Biophys. Acta*, **1415**, 23–40.
21 Koudelka, S. and Turanek, J. (2012) Liposomal paclitaxel formulations. *J. Control Release*, **163**, 322–334.
22 Gabizon, A., Catane, R., Uziely, B., Kaufman, B., Safra, T., Cohen, R., Martin, F., Huang, A., and Barenholz, Y. (1994) Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes. *Cancer Res.*, **54**, 987–992.
23 Barenholz, Y. (2012) Doxil® – the first FDA-approved nano-drug: lessons learned. *J. Control Release.*, **160**, 117–134.
24 Gabizon, A., Shmeeda, H., and Barenholz, Y. (2003) Pharmacokinetics of pegylated liposomal doxorubicin: review of animal and human studies. *Clin. Pharmacokinet.*, **42**, 419–436.
25 De Jong, W.H. and Borm, P.J. (2008) Drug delivery and nanoparticles: applications and hazards. *Int. J. Nanomedicine*, **3**, 133–149.
26 Desai, N., Trieu, V., Yao, Z., Louie, L., Ci, S., Yang, A., Tao, C., De, T., Beals, B., Dykes, D., Noker, P., Yao, R., Labao, E., Hawkins, M., and Soon-Shiong, P. (2006) Increased antitumor activity, intratumor paclitaxel concentrations, and endothelial cell transport of cremophor-free, albumin-bound paclitaxel, ABI-007, compared with cremophor-based paclitaxel. *Clin. Cancer Res.*, **12** (4), 1317–1324.
27 Gradishar, W.J. (2006) Albumin-bound paclitaxel: a next-generation taxane. *Expert Opin. Pharmacother.*, **7**, 1041–1053.
28 Green, M.R., Manikhas, G.M., Orlov, S., Afanasyev, B., Makhson, A.M., Bhar, P., and Hawkins, M.J. (2006) Abraxane, a novel Cremophor-free, albumin-bound particle form of paclitaxel for the treatment of advanced non-small-cell lung cancer. *Ann. Oncol.*, **17**, 1263–1268.
29 Li, C., Yu, D.F., Newman, R.A., Cabral, F., Stephens, L.C., Hunter, N., Milas, L., and Wallace, S. (1998) Complete regression of well-established tumors using a novel water-soluble poly(L-glutamic acid) paclitaxel conjugate. *Cancer Res.*, **58**, 2404–2409.
30 Jeyapalan, S., Boxerman, J., Donahue, J., Goldman, M., Kinsella, T., Dipetrillo, T., Evans, D., Elinzano, H., Constantinou, M., Stopa, E., Puthawala, Y., Cielo, D., Santaniello, A., Oyelese, A., Mantripragada, K., Rosati, K., Isdale, D., and Safran, H. (2014) Paclitaxel poliglumex, temozolomide, and radiation for newly diagnosed high-grade glioma: a Brown University Oncology Group Study. *Am. J. Clin. Oncol.*, **37** 444–449.
31 Cho, H., Lai, T.C., Tomoda, K., and Kwon, G.S. (2015) Polymeric micelles for multi-drug delivery in cancer. *AAPS Pharm. Sci. Tech.*, **16**, 10–20.
32 Kim, T.Y., Kim, D.W., Chung, J.Y., Shin, S.G., Kim, S.C., Heo, D.S., Kim, N.K., and Bang, Y.J. (2004) Phase I and pharmacokinetic study of Genexol-PM, a cremophor-free, polymeric micelle-formulated paclitaxel, in patients with advanced malignancies. *Clin. Cancer Res.*, **10**, 3708–3716.
33 Wang, D. and Gao, G. (2014) State-of-the-art human gene therapy: part II. Gene therapy strategies and clinical applications. *Discovery Medicine*, **18**, 151–161.
34 Kostarelos, K. and Miller, A.D. (2005) Synthetic, self-assembly ABCD nanoparticles, a structural paradigm for viable synthetic non-viral vectors. *Chem. Soc. Rev.*, **34**, 970–994.

35 Nguyen, J. and Szoka, F.C. (2012) Nucleic acid delivery: the missing pieces of the puzzle? *Acc. Chem. Res.*, **45**, 1153–1162.

36 Ginn, S.L., Alexander, I.E., Edelstein, M.L., Abedi, M.R., and Wixon, J. (2013) Gene therapy clinical trials worldwide to 2012 – an update. *J. Gene Med.*, **15**, 65–77.

37 Wang, D. and Gao, G. (2014) State-of-the-art human gene therapy: part I. Gene delivery technologies. *Discov. Med.*, **18**, 67–77.

38 Fraley, R., Subramani, S., Berg, P., and Papahadjopoulos, D. (1980) Introduction of liposome-encapsulated SV40 DNA into cells. *J. Biol. Chem.*, **255**, 10431–10435.

39 Felgner, P.L., Gadek, T.R., Holm, M., Roman, R., Chan, H.W., Wenz, M., Northrop, J.P., Ringold, G.M., and Danielsen, M. (1987) Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. *Proc. Natl. Acad. Sci. USA*, **84**, 7413–7417.

40 Podesta, J.E., Al-Jamal, K.T., Herrero, M.A., Tian, B., Ali-Boucetta, H., Hegde, V., Bianco, A., Prato, M., and Kostarelos, K. (2009) Antitumor activity and prolonged survival by carbon-nanotube-mediated therapeutic siRNA silencing in a human lung xenograft model. *Small*, **5**, 1176–1185.

41 Mazza, M., Hadjidemetriou, M., de Lazaro, I., Bussy, C., and Kostarelos, K. (2015) Peptide nanofiber complexes with siRNA for deep brain gene silencing by stereotactic neurosurgery. *ACS Nano*, **9**, 1137–1149.

42 Yin, H., Kanasty, R.L., Eltoukhy, A.A., Vegas, A.J., Dorkin, J.R., and Anderson, D.G. (2014) Non-viral vectors for gene-based therapy. *Nat. Rev. Genet.*, **15**, 541–555.

43 Lu, C., Stewart, D.J., Lee, J.J., Ji, L., Ramesh, R., Jayachandran, G., Nunez, M.I., Wistuba, I.I., Erasmus, J.J., Hicks, M.E., Grimm, E.A., Reuben, J.M., Baladandayuthapani, V., Templeton, N.S., McMannis, J.D., and Roth, J.A. (2012) Phase I clinical trial of systemically administered TUSC2(FUS1)-nanoparticles mediating functional gene transfer in humans. *PLoS One*, **7**, e34833.

44 Gofrit, O.N., Benjamin, S., Halachmi, S., Leibovitch, I., Dotan, Z., Lamm, D.L., Ehrlich, N., Yutkin, V., Ben-Am, M., and Hochberg, A. (2014) DNA based therapy with diphtheria toxin-A BC-819: a phase 2b marker lesion trial in patients with intermediate risk nonmuscle invasive bladder cancer. *J. Urol.*, **191**, 1697–1702.

45 Buscail, L., Bournet, B., Vernejoul, F., Cambois, G., Lulka, H., Hanoun, N., Dufresne, M., Meulle, A., Vignolle-Vidoni, A., Ligat, L., Saint-Laurent, N., Pont, F., Dejean, S., Gayral, M., Martins, F., Torrisani, J., Barbey, O., Gross, F., Guimbaud, R., Otal, P., Lopez, F., Tiraby, G., and Cordelier, P. (2015.) First-in-man phase 1 clinical trial of gene therapy for advanced pancreatic cancer: safety, biodistribution, and preliminary clinical findings. *Mol. Ther.*, **23**, 779–789.

46 Anwer, K., Kelly, F.J., Chu, C., Fewell, J.G., Lewis, D., and Alvarez, R.D. (2013) Phase I trial of a formulated IL-12 plasmid in combination with carboplatin and docetaxel chemotherapy in the treatment of platinum-sensitive recurrent ovarian cancer. *Gynecol. Oncol.*, **131**, 169–173.

47 Alvarez, R.D., Sill, M.W., Davidson, S.A., Muller, C.Y., Bender, D.P., DeBernardo, R.L., Behbakht, K., and Huh, W.K. (2014) A phase II trial of intraperitoneal EGEN-001, an IL-12 plasmid formulated with PEG-PEI-cholesterol lipopolymer in the treatment of persistent or recurrent epithelial ovarian, fallopian tube or primary peritoneal cancer: a gynecologic oncology group study. *Gynecol. Oncol.*, **133**, 433–438.

48 Sakhrani, N.M. and Padh, H. (2013) Organelle targeting: third level of drug targeting. *Drug Des. Devel. Ther.*, **7**, 585–599.

49 Biswas, S. and Torchilin, V.P. (2014) Nanopreparations for organelle-specific delivery in cancer. *Adv. Drug Deliv. Rev.*, **66**, 26–41.

50 Paulo, C.S., Pires das Neves, R., and Ferreira, L.S. (2011) Nanoparticles for intracellular-targeted drug delivery. *Nanotechnology*, **22**, 494002.

51 Al-Ahmady, Z.S., Chaloin, O., and Kostarelos, K. (2014) Monoclonal antibody-targeted, temperature-sensitive liposomes: *in vivo* tumor chemotherapeutics in combination with

mild hyperthermia. *J. Control. Release*, **196**, 332–343.
52 Torchilin, V.P. (2014) Multifunctional, stimuli-sensitive nanoparticulate systems for drug delivery. *Nat. Rev. Drug Discov.*, **13**, 813–827.
53 Lozano, N., Al-Ahmady, Z.S., Beziere, N.S., Ntziachristos, V., and Kostarelos, K. (2015) Monoclonal antibody-targeted PEGylated liposome-ICG encapsulating doxorubicin as a potential theranostic agent. *Int. J. Pharm.*, **482**, 2–10.
54 Lammers, T., Aime, S., Hennink, W.E., Storm, G., and Kiessling, F. (2011) Theranostic nanomedicine. *Acc. Chem. Res.*, **44**, 1029–1038.
55 Moreira, J.N., Ishida, T., Gaspar, R., and Allen, T.M. (2002) Use of the post-insertion technique to insert peptide ligands into pre-formed stealth liposomes with retention of binding activity and cytotoxicity. *Pharm. Res.*, **19**, 265–269.
56 Kirpotin, D.B., Drummond, D.C., Shao, Y., Shalaby, M.R., Hong, K., Nielsen, U.B., Marks, J.D., Benz, C.C., and Park, J.W. (2006) Antibody targeting of long-circulating lipidic nanoparticles does not increase tumor localization but does increase internalization in animal models. *Cancer Res.*, **66**, 6732–6740.
57 ClinicalTrials.gov (2015) Safety and Pharmacokinetic Study of MM-302 in Patients With Advanced Breast Cancer (NCT01304797). Available at https://clinicaltrials.gov/ct2/show/NCT01304797?term=mm302&rank=1.
58 ClinicalTrials.gov (2016) MM-302 Plus Trastuzumab vs. Chemotherapy of Physician's Choice Plus Trastuzumab in HER2-Positive Locally Advanced/Metastatic Breast Cancer Patients (HERMIONE) (NCT02213744). Available at https://clinicaltrials.gov/ct2/show/NCT02213744?term=mm302&rank=2.
59 Sapra, P. and Allen, T.M. (2002) Internalizing antibodies are necessary for improved therapeutic efficacy of antibody-targeted liposomal drugs. *Cancer Res.*, **62**, 7190–7194.
60 Vingerhoeds, M.H., Steerenberg, P.A., Hendriks, J.J., Dekker, L.C., Van Hoesel, Q.G., Crommelin, D.J., and Storm, G. (1996) Immunoliposome-mediated targeting of doxorubicin to human ovarian carcinoma *in vitro* and *in vivo*. *Br. J. Cancer*, **74**, 1023–1029.
61 Sapra, P. and Allen, T.M. (2004) Improved outcome when B-cell lymphoma is treated with combinations of immunoliposomal anticancer drugs targeted to both the CD19 and CD20 epitopes. *Clin. Cancer Res.*, **10**, 2530–2537.
62 Hadjidemetriou, M., Al-Ahmady, Z., Mazza, M., Collins, R.F., Dawson, K., and Kostarelos, K. (2015) *In vivo* biomolecule corona around blood-circulating, clinically used and antibody-targeted lipid bilayer nanoscale vesicles. *ACS Nano*, **9**, 8142–8156.
63 Lammers, T., Kiessling, F., Hennink, W.E., and Storm, G. (2012) Drug targeting to tumors: principles, pitfalls and (pre-) clinical progress. *J. Control. Release*, **161**, 175–187.
64 ClinicalTrials.gov (2016) A Phase 2 Study to Determine the Safety and Efficacy of BIND-014 (Docetaxel Nanoparticles for Injectable Suspension), Administered to Patients With Metastatic Castration-Resistant Prostate Cancer (NCT01812746). Available at https://clinicaltrials.gov/ct2/show/NCT01812746?term=bind-014&rank=1.
65 Wojtkowiak, J.W., Verduzco, D., Schramm, K.J., and Gillies, R.J. (2011) Drug resistance and cellular adaptation to tumor acidic pH microenvironment. *Mol. Pharmaceutics*, **8**, 2032–2038.
66 Andresen, T.L., Jensen, S.S., Kaasgaard, T., and Jorgensen, K. (2005) Triggered activation and release of liposomal prodrugs and drugs in cancer tissue by secretory phospholipase A2. *Curr. Drug Deliv.*, **2**, 353–362.
67 Sarkar, N., Banerjee, J., Hanson, A.J., Elegbede, A.I., Rosendahl, T., Krueger, A.B., Banerjee, A.L., Tobwala, S., Wang, R., Lu, X., Mallik, S., and Srivastava, D.K. (2008) Matrix metalloproteinase-assisted triggered release of liposomal contents. *Bioconjug. Chem.*, **19**, 57–64.
68 Torchilin, V. (2009) Multifunctional and stimuli-sensitive pharmaceutical nanocarriers. *Eur. J. Pharm. Biopharm.*, **71**, 431–444.

69 Mura, S., Nicolas, J., and Couvreur, P. (2013) Stimuli-responsive nanocarriers for drug delivery. *Nat. Mater.*, **12**, 991–1003.

70 O'Brien, M.E., Wigler, N., Inbar, M., Rosso, R., Grischke, E., Santoro, A., Catane, R., Kieback, D.G., Tomczak, P., Ackland, S.P., Orlandi, F., Mellars, L., Alland, L., and Tendler, C. (2004) Reduced cardiotoxicity and comparable efficacy in a phase III trial of pegylated liposomal doxorubicin HCl (CAELYX/Doxil) versus conventional doxorubicin for first-line treatment of metastatic breast cancer. *Ann. Oncol.*, **15**, 440–449.

71 Weinstein, J.N., Magin, R.L., Yatvin, M.B., and Zaharko, D.S. (1979) Liposomes and local hyperthermia: selective delivery of methotrexate to heated tumors. *Supramol. Sci.*, **204**, 188–191.

72 Al-Ahmady, Z.S., Scudamore, C.L., and Kostarelos, K. (2015) Triggered doxorubicin release in solid tumors from thermosensitive liposome-peptide hybrids: critical parameters and therapeutic efficacy. *Int. J. Cancer*, **137**, 731–743.

73 Al-Ahmady, Z.S., Al-Jamal, W.T., Bossche, J.V., Bui, T.T., Drake, A.F., Mason, A.J., and Kostarelos, K. (2012) Lipid-peptide vesicle nanoscale hybrids for triggered drug release by mild hyperthermia *in vitro* and *in vivo*. *ACS Nano*, **6**, 9335–9346.

74 Goldenbogen, B., Brodersen, N., Gramatica, A., Loew, M., Liebscher, J., Herrmann, A., Egger, H., Budde, B., and Arbuzova, A. (2011) Reduction-sensitive liposomes from a multifunctional lipid conjugate and natural phospholipids: reduction and release kinetics and cellular uptake. *Langmuir*, **27**, 10820–10829.

75 Kim, D., Gao, Z.G., Lee, E.S., and Bae, Y.H. (2009) *In vivo* evaluation of doxorubicin-loaded polymeric micelles targeting folate receptors and early endosomal pH in drug-resistant ovarian cancer. *Mol. Pharmaceutics*, **6**, 1353–1362.

76 Kim, D., Lee, E.S., Oh, K.T., Gao, Z.G., and Bae, Y.H. (2008) Doxorubicin-loaded polymeric micelle overcomes multidrug resistance of cancer by double-targeting folate receptor and early endosomal pH. *Small*, **4**, 2043–2050.

77 Saito, G., Swanson, J.A., and Lee, K.D. (2003) Drug delivery strategy utilizing conjugation via reversible disulfide linkages: role and site of cellular reducing activities. *Adv. Drug Deliv. Rev.*, **55**, 199–215.

78 Li, Y., Xiao, K., Luo, J., Xiao, W., Lee, J.S., Gonik, A.M., Kato, J., Dong, T.A., and Lam, K.S. (2011) Well-defined, reversible disulfide cross-linked micelles for on-demand paclitaxel delivery. *Biomaterials*, **32**, 6633–6645.

79 Wen, H.Y., Dong, H.Q., Xie, W.J., Li, Y.Y., Wang, K., Pauletti, G.M., and Shi, D.L. (2011) Rapidly disassembling nanomicelles with disulfide-linked PEG shells for glutathione-mediated intracellular drug delivery. *Chem. Commun.*, **47**, 3550–3552.

80 Landon, C.D., Park, J.Y., Needham, D., and Dewhirst, M.W. (2011) Nanoscale drug delivery and hyperthermia: the materials design and preclinical and clinical testing of low temperature-sensitive liposomes used in combination with mild hyperthermia in the treatment of local cancer. *Open Nanomed. J.*, **3**, 38–64.

81 Needham, D., Park, J.Y., Wright, A.M., and Tong, J. (2013) Materials characterization of the low temperature sensitive liposome (LTSL): effects of the lipid composition (lysolipid and DSPE-PEG2000) on the thermal transition and release of doxorubicin. *Faraday Discuss.*, **161**, 515–534.

82 Manzoor, A.A., Lindner, L.H., Landon, C.D., Park, J.Y., Simnick, A.J., Dreher, M.R., Das, S., Hanna, G., Park, W., Chilkoti, A., Koning, G.A., ten Hagen, T.L., Needham, D., and Dewhirst, M.W. (2012) Overcoming limitations in nanoparticle drug delivery: triggered, intravascular release to improve drug penetration into tumors. *Cancer Res.*, **72**, 5566–5575.

83 Kong, G., Anyarambhatla, G., Petros, W.P., Braun, R.D., Colvin, O.M., Needham, D., and Dewhirst, M.W. (2000) Efficacy of liposomes and hyperthermia in a human tumor xenograft model: importance of triggered drug release. *Cancer Res.*, **60**, 6950–6957.

84 Yarmolenko, P.S., Zhao, Y., Landon, C., Spasojevic, I., Yuan, F., Needham, D., Viglianti, B.L., and Dewhirst, M.W. (2010) Comparative effects of thermosensitive doxorubicin-containing liposomes and

hyperthermia in human and murine tumours. *Int. J. Hyperthermia*, **26**, 485–498.

85 Celsion.com (2015) Optima Study Highlighted at ILCA Symposium. Available at http://celsion.com/docs/OPTIMA_Study_ ILCA_Symposium.

86 Celsion.com (2015) THERMODOX®. Available at http://celsion.com/docs/technology_thermodox.

87 ClinicalTrials.gov (2016) A Study of ThermoDox™ in Combination With Radiofrequency Ablation (RFA) in Primary and Metastatic Tumors of the Liver (NCT00441376). Availabel at https://clinicaltrials.gov/ct2/show/NCT00441376?term=thermodox&rank=3.

88 ClinicalTrials.gov (2016) Targeted Chemotherapy Using Focused Ultrasound for Liver Tumours (TARDOX) (NCT02181075). Available at https://clinicaltrials.gov/ct2/show/NCT02181075?term=thermodox&rank=4.

89 Maier-Hauff, K., Rothe, R., Scholz, R., Gneveckow, U., Wust, P., Thiesen, B., Feussner, A.von, Deimling, A., Waldoefner, N., Felix, R., and Jordan, A. (2007) Intracranial thermotherapy using magnetic nanoparticles combined with external beam radiotherapy: results of a feasibility study on patients with glioblastoma multiforme. *J. Neurooncol.*, **81**, 53–60.

90 Johannsen, M., Thiesen, B., Wust, P., and Jordan, A. (2010) Magnetic nanoparticle hyperthermia for prostate cancer. *Int. J. Hyperthermia*, **26**, 790–795.

91 Wang, Y.X. (2011) Superparamagnetic iron oxide based MRI contrast agents: current status of clinical application. *Quant. Imaging Med. Surg.*, **1**, 35–40.

92 Weissig, V. and Guzman-Villanueva, D. (2015) Nanopharmaceuticals (part 2): products in the pipeline. *Int. J. Nanomedicine*, **10**, 1245–1257.

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

94 Harrington, K.J., Mohammadtaghi, S., Uster, P.S., Glass, D., Peters, A.M., Vile, R.G., and Stewart, J.S. (2001) Effective targeting of solid tumors in patients with locally advanced cancers by radiolabeled pegylated liposomes. *Clin. Cancer Res.*, **7**, 243–254.

95 Dams, E.T., Laverman, P., Oyen, W.J., Storm, G., Scherphof, G.L., van Der Meer, J.W., Corstens, F.H., and Boerman, O.C. (2000) Accelerated blood clearance and altered biodistribution of repeated injections of sterically stabilized liposomes. *J. Pharmacol. Exp. Ther.*, **292**, 1071–1079.

96 de Smet, M., Heijman, E., Langereis, S., Hijnen, N.M., and Grull, H. (2011) Magnetic resonance imaging of high intensity focused ultrasound mediated drug delivery from temperature-sensitive liposomes: an *in vivo* proof-of-concept study. *J. Control. Release*, **150**, 102–110.

97 de Smet, M., Langereis, S., van den Bosch, S., and Grull, H. (2010) Temperature-sensitive liposomes for doxorubicin delivery under MRI guidance. *J. Control. Release*, **143**, 120–127.

98 Gendelman, H.E., Anantharam, V., Bronich, T., Ghaisas, S., Jin, H.J., Kanthasamy, A.G., Liu, X.M., McMillan, J., Mosley, R.L., Narasimhan, B., and Mallapragada, S.K. (2015) Nanoneuromedicines for degenerative, inflammatory, and infectious nervous system diseases. *Nanomed. Nanotechnol.*, **11**, 751–767.

99 Gharagozloo, M., Majewski, S., and Foldvari, M. (2015) Therapeutic applications of nanomedicine in autoimmune diseases: from immunosuppression to tolerance induction. *Nanomedicine*, **11**, 1003–1018.