Multifunctional Polymeric Nanosystems for Tumor-Targeted Delivery

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1 Introduction

Cancer is the second leading cause of morbidity and mortality in the United States, with occurrences portraying an upward trend for the future. In 2007, approximately 10 million cases of cancer will occur globally, with a total of around 1.5 million new cancer cases and over 560,000 deaths expected in the United States (U.S. National Institute of Health, 2006). Strikingly, remarkable advances in diagnosis and therapy of cancer have been made over the past few decades resulting from significant advances in fundamental cancer biology. What lacks in this case is clinical translation of these advances into effective therapies. A major hurdle in cancer diagnosis and therapy is the targeted and efficacious delivery of agents to the tumor site, while avoiding adverse damage resulting from systemic administration. While systemic drug delivery already hinges largely on physicochemical properties of the drug, such as size, diffusivity, and plasma protein binding affinity, tumors possess a dense, heterogeneous vasculature and an outward net convective flow that act as hurdles to efficient drug deposition at the target site (Jang et al., 2003). Nanocarrier-mediated delivery has emerged as a successful strategy to enhance delivery of therapeutics and imaging agents to tumors, thereby increasing the potential for diagnosis at an earlier stage or for therapeutic success (or both). Based on the initial observation by Maeda and Matsumura that tumors possess a fenestrated vasculature, with pores on average ranging between 200 and 800 nm, and a lack of lymphatic drainage, together termed the enhanced permeability and retention (EPR) effect, it was found that colloidal carriers in the nanometer size range could target tumors passively, by specific extravasation through these fenestrations, and are retained at the site for prolonged time because of lack of lymphatic drainage (Matsumura and Meada, 1986). This physiological advantage has been used successfully to enhance delivery of diagnostic and therapeutic agents, leading to the U.S. Food and Drug Administration (FDA) approval of nanoparticle formulations such as Feridex® for diagnostic applications and Doxil® and Abraxane® for cancer therapy (U.S. Food and Drug Administration, 2006).

The most basic and simple nanoparticle platform for tumor drug delivery is generally lipid- or polymer-based (Fig. 1). Liposomes are the simplest form of a

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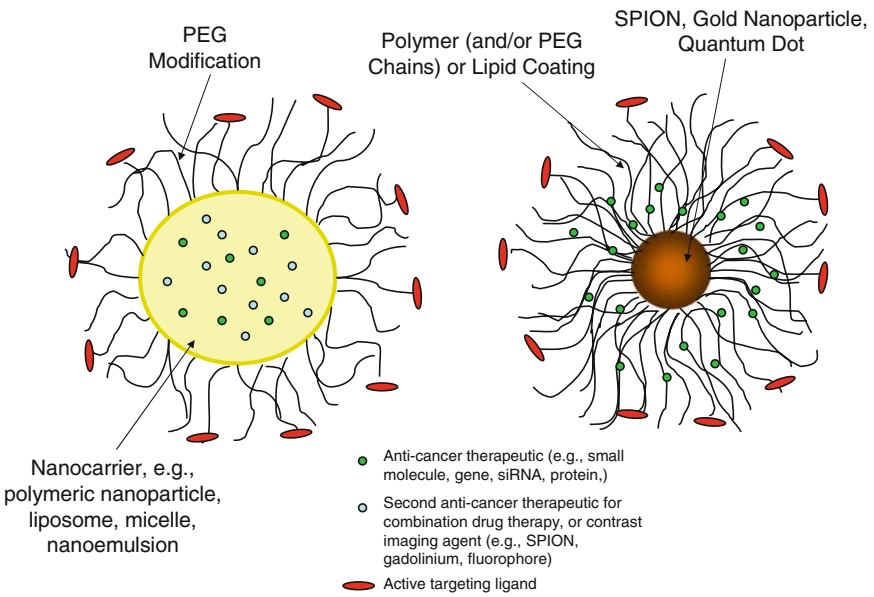

Fig. 1 Typical multifunctional nanoparticle platforms for tumor-targeted therapy

nanoparticle, and became the first system to receive FDA approval for tumor-targeted applications. Constructed from phospholipids as spherical vesicles, they take on the form of aqueous capsules bound by a lipid bilayer, mimicking the plasma membrane of mammalian cells in composition, thereby allowing for great biocompatibility and versatility. Doxil®, a liposomal form of doxorubicin, received FDA approval for the treatment of Kaposi's sarcoma over a decade ago, and is now additionally used against breast cancer and advanced ovarian cancer (U.S. Food and Drug Administration, 2007). Similarly, DaunoXome®, a formulation of daunorubicin, followed suit for the treatment of Kaposi's sarcoma, and a myriad of other liposomal formulations are undergoing preclincal and clinical evaluations as tumor-targeted drug delivery vehicles. Alternatively, micelles have broken through as potential nanocarriers for oncologic applications as well. Micelles are colloidal carriers that spontaneously form through thermodynamically favored aggregation of amphiphiles at or above the critical micellar concentration (CMC). Often such amphiphiles are lipids (lysophopholipids), but amphiphilic polymers and even lipid–polymer hybrids are also frequently used. Micelles are attractive nanocarriers for tumor targeting, due to their small (10–100 nm) size and spontaneous assembly, even though stability of micelles in vivo has been a questionable parameter given their spontaneous disintegration at concentrations below the CMC. Although no micellar formulations have thus far been approved for delivery of anticancer drugs, several are in clinical trials in Asia, and many others are proving quite promising as drug delivery vehicles in early preclinical development. As an example, encapsulation

of paclitaxel into a block-copolymer micelle, composed of monomethoxy poly(ethylene oxide)-*block*-poly(lactide), not only increased the maximum tolerated dose (MTD) threefold, but mice bearing subcutaneous MX-1 breast tumors experienced complete tumor regression by day 24 after treatment initiation, while treatment with Taxol®, a clinically used paclitaxel formulation in Cremophore EL®:ethanol mixture, resulted merely in a partial tumor regression followed by complete regrowth by day 24 (Kim et al., 2001). Similar results were seen when the treatment was repeated on mice bearing subcutaneous SKOV3 ovarian tumors (Kim et al., 2001).

On the other spectrum, nanoparticles constructed of natural or synthetic polymers are another group of nanoscale drug delivery systems widely employed in cancer treatment, whose successes to date also include an FDA approved formulation, Abraxane® – paclitaxel bound into albumin nanoparticles (U.S. Food and Drug Administration, 2007). Polymeric nanoparticles offer a particular advantage as drug delivery vehicles since a myriad of different polymers exist or can be developed for the formulation of nanoparticles (Table 1). Over the past several years, our group has developed a variety of polymeric nanoparticles for tumor drug delivery, all leading to an enhanced in vivo therapeutic efficacy. Some examples of these

Table 1 Illustrative examples of multifunctional nanoparticle systems used in cancer therapy

Active ingredients	Nanoparticle platform	Malignancy	Reference
Combination Drug Therapy			
Doxorubicin and combretastatin-A4	Poly(D,L-lactic-*co*-glycolic acid) nanoparticle core in liposome	Lewis lung carcinoma and B16/F10 melanoma	Sengupta et al. (2005)
Doxorubicin and cyclosporine-A	Poly(alkylcyanoacrylate) nanoparticles	P388 leukemia	Soma et al. (2000)
Doxorubicin and elacridar	Polymer-modified lipid nanoparticles	MDA-MB-435 breast carcinoma	Wong et al. (2006)
Paclitaxel and C_6-ceramide	PEO-modified poly(epsilon-caprolactone) nanoparticles	SKOV3 ovarian carcinoma	van Vlerken et al. (2007)
Combination Hyperthermia and Drug Therapy			
TNF-α	PEG-modified gold nanoparticles	MC38 colon carcinoma	Paciotti et al. (2005)
Bleomycin	Microgels	Small intestine	Blanchette and Peppas (2005)
Doxorubicin	PEG-modified liposomes	Hepatic carcinoma	Goldberg et al. (2002)
Combination Imaging and Drug Therapy			
Doxorubicin	Iron oxide nanoparticles inside PEG-poly (L-lactide) micelles	SLK tumor endothelium	Nasongkla et al. (2006)
Doxorubicin	Dermatan sulfate-modified iron oxide nanoparticles	AT1 prostate carcinoma and MX1 breast carcinoma	Ranney et al. (2005)

(continued)

Table 1 (continued)

Active ingredients	Nanoparticle platform	Malignancy	Reference
Methotrexate	Iron oxide nanoparticles	MCF7 breast carcinoma and HeLa cervical carcinoma	Kohler et al. (2005)
Daunorubicin	3-Mercaptopropionic acid – modified gold nanoparticles	K562 leukemia	Li et al. (2007)
Sialyl-Tn and Lewis-y antigens	Carbohydrate-coated gold nanoparticles	n/a	Ojeda et al. (2007)
TNF-α	PEG-modified gold nanoparticles	MC38 colon carcinoma	Paciotti et al. (2004)
Combination Ultrasound and Drug Therapy			
5-Flurouracil	Perfluorocarbon	C32 melanoma	Larina et al. (2005)

PEG poly(ethylene glycol); *PEO* poly(ethylene oxide); *TNF-α* tumor necrosis factor-α

include the delivery of tamoxifen in poly(ethylene oxide)-modified poly(caprolactone) (PEO-PCL) nanoparticles to MDA-MB-231 breast cancer (Shenoy and Amiji, 2005), the delivery of paclitaxel in PEO-modified PCL and PEO-modified poly(β-amino ester) nanoparticles to SKOV3 ovarian cancer (Devalapally et al., 2006), and even the delivery of a gene therapeutic encoding for sFlt-1 or VEGF-R1 to MDA-MB-435 breast cancer from gelatin nanoparticles (Kommareddy and Amiji, 2007). This versatility of polymer platforms allows for fine tuning of the drug delivery formulation to meet specific advantages. For example, the composition of polymeric matrix can be chosen to match the chemical properties of the encapsulated drug(s) to match loading efficiency and release behavior. Or the composition can be tuned to provide precise drug capture or release in response to environmental triggers. Alternatively, the composition can even be optimized to allow for inclusion of multifunctional properties, such as a combination of therapeutics, targeting, and/or imaging modalities, all within one nanoparticle platform.

A versatile function that is applicable to nearly all nanocarrier platforms is the inclusion of active targeting ligands. While the nanoparticle platform enhances targeting of therapeutics or imaging agents through passive means of the EPR effect, active targeting of these nanoparticles to tumor tissue and cellular surface components uniquely present on target cells can aide in the nanoparticle's ability to locate the target cell type in the tumor mass, or can even enhance internalization of these nanoparticles into their target cells. A wide variety of tumor targeting ligands have been successfully used for active targeting of nanoparticles. Depending on the tumor or cell type, surface proteins overexpress or uniquely express, such as the HER2 receptor, prostate-specific membrane antigen, the folate receptor, the thiamine transporter, integrins, and a myriad of other surface factors that can serve as specific targets to active targeting approaches through inclusion of small molecule

ligands such as folate (Kim et al., 2005; Sun et al., 2006) and thiamine (Oyewumi et al., 2003), sugar residues such as galactose (Jeon et al., 2005), peptides such as argenine-glycine-aspartic acid (RGD) (Schiffelers et al., 2004), proteins such as transferrin (Bellocq et al., 2003) lectins (Gao et al., 2006), as well as antibodies and antibody fragments (Hayes et al., 2006; Elbayoumi and Torchilin, 2006). However, more recent high-throughput construction and validation have led to the use of aptamers (Farokhzad et al., 2006a) and sequences identified by phage display (Nielsen et al., 2002; Simberg et al., 2007) as alternative active targeting ligands, thereby greatly widening the pool of targeting constructs to direct nanoparticles more specifically. Regardless of the targeting moiety, the principle outcome is essentially the same, mainly improved tumor-cell recognition, improved intracellular penetration, and reduced recognition at nonspecific sites.

Nanoparticle platforms are of great use in tumor targeting for enhanced delivery of anticancer therapeutics, spanning the range from small molecule drugs through biotherapeutics such as genes and peptides or proteins. However, the same principle has been widely applied to cancer detection, where passive or active tumor targeting of fluorescent probes or contrast imaging agents can help increase sensitivity of tumor detection or even metastatic behavior to advance diagnostics to improve patient prognosis from the other spectrum. Current nanoparticle research and development is moving towards multifunctionalization of these nanoparticle platforms for cancer treatment, whereby all the applicable uses of nanoparticles are essentially merged together. These advances lead to therapeutic systems that, from a single dose, administers combination drug therapies, combination therapies of chemotherapeutic drugs with physical stressors (such as thermal therapies, radiation, and photodynamic therapies), or even combines therapeutics with imaging agents for envisioning a "real-time" therapeutic approach. Not only does nanotechnology make these advances possible, but many such successful multifunctional nanoparticle strategies are already in circulation. This chapter describes the most recent approaches in use that employ multifunctional nanoparticle strategies to enhance overall cancer therapy (Fig. 2).

2 Multifunctional Nanocarriers to Overcome Biological Barriers

2.1 Nanocarriers for Oral Absorption

The oral route is one of the most attractive methods of drug administration in the body because of opportunities for self-administration and associated high patient compliance. The oral route is also amenable for administration of different formulations, including solid, semi-solid, and liquid dosage forms. For certain drugs (including majority of anticancer therapeutics), their oral route bioavailability is relatively much lower to provide meaningful therapeutic outcomes. This is partly due to the

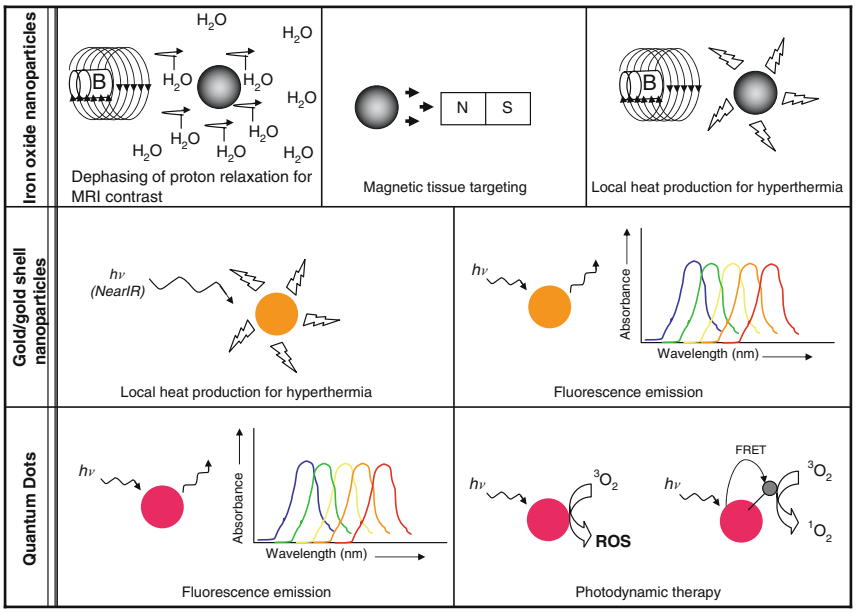

Fig. 2 Opportunity for multiple applications of metallic and semi-conductor nanoparticles in cancer imaging and therapy

presence of large number of multidrug transporters, such as multidrug resistance protein (MRP), *p*-glycoprotein (*p*-gp), and the multispecific organic anion transporter (MOAT) on the enterocyte membrane of the gastrointestinal (GI) tract (Taylor, 2002; Thomas and Coley, 2003). These transporters recognize the therapeutic agent as a substrate and actively effluxes the molecule out of the cells. Different types of strategies have been used to enhance oral bioavailability of drugs, including co-administration of *p*-gp transporter inhibitors and formulation in different nanocarrier delivery systems. Co-administration of a *p*-gp inhibitor with the active therapeutic agent can decrease the efflux of the agent by preferential binding with the *p*-gp pump on the cell membrane (Sadeque et al., 2000; Savolainen et al., 2002). However, this strategy has generally shown higher toxicity in vivo, mostly from the high doses of the *p*-gp inhibitor that are needed, and the additional undesirable pharmacokinetic interactions between the therapeutic of interest and *p*-gp inhibitor.

Another challenge in oral administration is the presence of high concentrations of metabolizing enzymes in the GI lumen. Besides proteases and nucleases, which can degrade protein and nucleic acid therapeutics, respectively, the GI lumen also expresses cytochrome P-450 metabolizing enzyme systems. Premature drug metabolism at the GI lumen before the active molecule can be absorbed into the systemic circulation significantly limits the bioavailability at the active site. Prodrugs have been designed to improve the stability of therapeutic agents in the GI tract by promoting the conversion to active moiety after absorption in the systemic circulation

or, more preferably, at the disease target (Somogyi et al., 1998). In cancer therapy, the prodrug approach can have significant benefit in limiting the toxicity of the agent, if the drug can be selectively activated at the tumor site.

Spray-dried poly(D,L-lactide-*co*-glycolide) (PLGA) nanoparticles have been investigated for oral delivery of amifostine (Pamujula et al., 2004), an organic thiophosphate prodrug that is metabolized by tissue alkaline phosphatase into active thiol metabolite. When administered orally to mice, the amifostine-encapsulated PLGA nanoparticles promoted absorption and the drug was present in blood and other highly-perfused tissues within 30 min of administration. Other polymeric nanoparticles, especially after surface modification to enhance muco- or bioadhesion can be used to enhance the residence time in the GI tract. For instance, tocopheryl poly(ethylene glycol) (PEG) 1,000 succinate (vitamin E-TPGS) modified biodegradable PLGA nanoparticles were proposed for oral administration of paclitaxel. In vitro studies in Caco-2 cells showed a 1.4-fold higher cellular uptake of the TPGS-modified PLGA nanoparticles relative to aqueous solution control.

2.2 Enhancement of Transport Across Other Biological Barriers

Another limitation for drug delivery, especially for systemic brain tumor therapy, is the poor transport across the blood–brain barrier (BBB). The BBB selectively restricts drug transport into the brain because of very tight endothelial cell junctions in the capillary as well as expression of efflux transporters (e.g., *p*-gp) and drug-metabolizing enzymes (Koziara et al., 2004; Ningaraj, 2006). Several studies have shown that poly(alkylcyanoacrylate) nanoparticles promote the delivery of several chemotherapeutic drugs, including doxorubicin, across the BBB when the nanoparticle surfaces are coated with polysorbate (e.g., Tween® 20, 40, 60, and 80) and certain types of poloxamers (e.g., Pluronic® F68). In one example, the therapeutic benefit of doxorubicin administration in sterically stabilized poly(alkylcyanoacrylate) nanoparticulate system was examined in rats bearing intracranial glioblastoma. The investigators proposed that the enhancement in cerebral delivery could probably be due to preferential nanoparticle endocytosis by the low-density lipoprotein receptors on brain capillary endothelial cells after systemic administration. Following cellular internalization, the drug would be able to diffuse out of the nanoparticle matrix and be transported into the brain tissue by transcytosis. The therapeutic potential of this formulation in vivo was studied in rat model with established intracranial 101/8 glioblastoma. Systemic administration of doxorubicin in the polysorbate-modified poly(alkylcyanoacrylate) nanoparticles enabled significantly greater fraction of the animals to survive than did the administration of doxorubicin in solution. Additional opportunities for brain delivery of polymeric nanoparticles can be realized with delivery of combination chemotherapeutic agent and *p*-gp efflux transporter inhibitor. Co-encapsulation of these agents can provide an opportunity to enhance brain delivery of chemotherapeutic agent.

2.3 Enhancement of Drug Availability and Residence at the Tumor Site

For systemic therapy, passive and active targeting strategies are used. Passive targeting relies on the properties of the delivery system and the disease pathology in order to preferentially accumulate the drug at the site of interest and avoid nonspecific distribution. For instance, PEG- or PEO-modified nanocarrier systems can preferentially accumulate in the vicinity of the tumor mass upon intravenous administration based on the hyperpermeability of the newly-formed blood vessels by a process known as EPR effect. Maeda et al. (Maeda, 2001; Jun-Fang et al., 2006) first described the EPR effect in murine solid tumor models and this phenomenon has been confirmed by others. When polymer–drug conjugates are administered, 10–100 fold higher concentrations can be achieved in the tumor (due to EPR effect) than when free drug is administered. Some investigators have also suggested that the EPR effect is present in inflammatory areas and in myocardial infarction. Other approaches for passive targeting involve use of specific stimuli-sensitive delivery system that can release the encapsulated payload only when such a stimuli is present. For instance, the pH around tumor and other hypoxic disease tissues in the body tend to be more acidic (i.e., ~5.5–6.5), relative to physiological pH (i.e., 7.4). We found significant enhancement in drug delivery and accumulation in the tumor mass when pH-sensitive PEO-modified PbAE nanoparticles were used; in contrast drug delivery using non-pH sensitive PEO-PCL nanoparticles in aqueous solution was not as effective (Shenoy et al., 2005). Other approaches for passive targeting involve optimization of nanocarrier size and surface charge modulation. Nanoparticles of <200 nm in diameter and those with positive surface charge are known to preferentially accumulate and reside in the tumor mass for longer duration than do either neutral or negatively charged nanoparticles (van Vlerken and Amiji, 2006). Besides PEG or PEO, other hydrophilic polymers including poly(vinyl alcohol), poly(acryl amide), poly(*N*-vinylpyrrolidone), poly-(*N*-(2-hydroxypropyl)methacrylamide), polysorbate 80 (Tween® 80), and block co-polymers such as poloxomer and poloxamine are also being used to modify the physicochemical properties of the colloidal carriers (Torchilin, 1996; Oupicky et al., 2000; Fenske et al., 2001).

Active targeting to the disease site relies, in addition to PEG modification of nanocarriers to enhance circulation time and achieve passive targeting, coupling of a specific ligand on the surface that will be recognized by the cells present at the disease site. Using solid tumor as an example again, there are several strategies that can be adopted for surface modification of nanocarrier systems for effective targeted delivery to the tumor cells or to endothelial cells of the tumor blood vessels. Since tumor cells are rapidly proliferating, they over-express certain receptors for enhanced uptake of nutrients, including folic acid, vitamins, and sugars. When the surface of nanocarriers is modified with folic acid, they can be targeted to the tumor cells that over-express folate receptors. Tumor and capillary endothelial cells also express specific integrin receptors, such as $\alpha_v\beta_5$ or $\alpha_v\beta_3$ that can bind to RGD

tripeptide sequence. RGD-modification, therefore, has been used to direct nanocarriers to tumor cells and capillary endothelial cells of the angiogenic blood vessels (Cegnar et al., 2005; Chiellini et al., 2006; Gabizon et al., 2006). The phage display method has been used to identify specific peptide sequences that can be used for targeting to tumors and other disease areas in the body. Development of monoclonal antibodies against specific epitopes present only on tumor cells allows for other targeting strategies. Using a monoclonal antibody 2C5 that specifically recognizes antinuclear histones, Torchilin's group (Torchilin, 1994; Torchilin et al., 2001, 2003; Lukyanov et al., 2004; Gupta et al., 2005a) has developed various strategies for active targeted delivery of drugs to the tumor mass using liposomes and micellar delivery systems. Other groups have used transferrin, an iron-binding protein, for surface modification of nanocarriers for delivery to tumors. Recently, Farokhzad et al. (2004, 2006a,b) have elegantly described the use of aptamers, nucleic acid constructs that specifically recognize prostate membrane antigen on prostate cancer cells. The aptamer technology provides an additional strategy for active targeting to tumor cells in the body.

2.4 Enhancement of Intracellular Uptake

Once the nanocarriers are delivered to the specific diseased organ or tissue, they may need to enter the cells of interest and ferry the payload to subcellular organelles. In this case, nonspecific or specific cell penetrating strategies need to be adopted. Nonspecific cell uptake of nanocarriers occurs by endocytotic process, where the membrane envelops the nanocarriers to form a vesicle in the cell called an endosome (Panyam and Labhasetwar, 2003). The endosome then shuttles the content in the cell and can fuse with lyososomes, which are highly acidic organelles rich in degrading enzymes. Endocytosed nanocarriers usually travel in a specific direction and converge at the nuclear membrane. Weissig's group (Weissig, 2005; Weissig et al., 2006) has attempted to direct various nanosized delivery systems to mitochondria using delocalized cationic amphiphiles and other mitochondriotropic vector systems. Specific cellular uptake can occur through receptor-mediated endocytosis, where upon binding of the ligand-modified nanocarrier with the cell-surface receptor leads to internalization of the entire nanocarrier–receptor complex and vesicular transport through the endosomes (Panyam and Labhasetwar, 2003). Following dissociation of the nanocarrier–receptor complex, the receptor can be re-cycled back to the cell membrane. Recently, to enhance cellular uptake, a surge of research effort has been directed towards development of argenine-rich cell penetrating peptides (CPPs) (Torchilin, 2002; Torchilin and Levchenko, 2003; Gupta et al., 2005b; Emerich and Thanos, 2006; Gupta and Torchilin, 2006). Based on the initial work of Dowdy's group (Schwarze and Dowdy, 2000; Becker-Hapak et al., 2001) HIV-1 Tat peptide was identified to promote nonspecific intracellular localization of various molecules upon systemic delivery. This observation has been supported by other groups and a number of cationic peptides have been identified,

including penetratin, to enhance intracellular delivery. The exact mechanism of how Tat and other CPPs enhance cell permeation is still a subject of controversy, but recent data show that it may be through endocytosis as well. Following cellular internalization, stability of the payload in the cytosol and uptake by specific organelle, such as the nucleus, is also essential for nucleic-acid-based therapeutics. For efficient systemic gene therapy using nonviral vectors, nuclear import of plasmid DNA in nondividing cells is considered to be the major limiting factor.

3 Multifunctional Nanocarriers for Combination Drug Therapy

The versatility of nanocarrier platforms opens up possibilities to incorporate combination therapies into a single drug delivery system. Combination drug therapy for cancer was first proposed in a legendary move by Drs. Frei, Freireich, and Holland who revolutionized cancer therapy by implementing combination chemotherapy to treat acute lymphoblastic leukemia (ALL), a malignancy that prior to 1950 was largely incurable (Frei et al., 1958). In this case, it was hypothesized that concurrent use of multiple drugs with differing mechanisms of action would circumvent the development of drug resistance, the likely cause for prior therapeutic failure in ALL. The success of this strategy caused the approach to quickly gain widespread acknowledgement to become a common consideration in current cancer therapy. Furthermore, the idea has been extended beyond combination chemotherapy to combine drugs with entirely distinct pharmacological targets – e.g., combinations of chemotherapeutic agents with angiogenesis inhibitors, protease inhibitors, immunotherapeutics, hormone therapeutics, and modulators of multidrug resistance (MDR) – therapies largely stemming from advances in cancer molecular and cell biology leading to identification of alternate therapeutic targets.

3.1 Combination Antiangiogenic and Cytotoxic Chemotherapy

Angiogenesis is the process of new blood vessel formation (neovascularization), and has been established as the key factor for tumor growth and development past the primary stage (Folkman, 1972). Anti-angiogenic therapy quickly became a popular alternative in cancer therapeutic development versus conventional chemotherapy (Folkman, 1972), leading to FDA approval of the first anti-angiogenesis drug for cancer therapy in 2004. However, since angiogenesis is only implicated in tumor growth and survival beyond the initial avascular tumor core, to date it is standard practice to combine this treatment option with conventional chemotherapy. Such clinically approved regimens include the combination of the angiogenesis inhibitor bevacizumab with standard chemotherapy (ironotecan, 5-fluorouracil, and leucovorin) for metastatic colorectal cancer and with carboplatin and paclitaxel

against non–small-cell lung cancer (Fayette et al., 2005), although an additional variety of such combination therapies also persists in clinical use or clinical trials.

Given the success of nanoparticles in chemotherapeutic drug delivery to tumors, it followed suit that antiangiogenic therapies were delivered in nanoparticles as well, for similar enhancement of tumor-targeting, leading to enhanced therapeutic efficacy, particularly aiding delivery of labile gene therapeutics that have recently found a trend in angiogenesis inhibition. For example, treatment of mice bearing MDA-MD-435 breast tumors with an antiangiogenic gene therapeutic, namely sFlt-1, delivered within long-circulating thiolated gelatin nanoparticles resulted in a nearly sixfold higher transfection efficiency of the gene therapeutic at the tumor-site, a corresponding fourfold decrease in microvessel density in the tumor mass, and a complete tumor growth delay over the course of 25 days (Kommareddy and Amiji, 2007). Similarly, an siRNA therapeutic directed against VEGF-R2 encapsulated within cationic polyplexes bearing an RGD-active targeting moiety also caused a significant inhibition of tumor growth, due to the significant decrease in tumor vascularity (Schiffelers et al., 2004). However, thus far clinical antiangiogenesis therapies are co-administered with chemotherapeutics, leading to an interest in the development of multifunctional nanoparticle formulations for co-administration of the therapeutics. Furthermore, research has alluded to the fact that simultaneous administration of angiogenesis inhibitors and chemotherapeutics may actually cause detrimental effects, where a breakdown of vascularity not only prevents the chemotherapy from accumulating throughout the tumor site, but that it can also lead to tumor hypoxia, which may promote drug resistance and metastasis (Tran et al., 2002). Given this dilemma, it was thought that this form of combination therapy may actually benefit from temporal controlled release, a feat that can well be mediated by using nanoparticles as drug delivery vehicles. On this premise, Sengupta et al. developed a novel multifunctional nanoparticle formulation that, upon localization in the tumor mass, first releases the antiangiogenic drug combretastatin-A4 to shut down tumor vasculature, followed by the sustained release of the cytotoxic agent doxorubicin, already localized within the tumor mass, thereby avoiding the aforementioned problems associated with chemotherapeutic delivery after vascular shutdown (Sengupta et al., 2005). By this mechanism, survival and tumor growth delay of mice bearing either Lewis Lung carcinoma or B16/F10 melanoma models drastically improved when compared with simultaneous nanoparticle administration of the combination therapy lacking temporally controlled release.

3.2 Combination Therapy to Overcome Tumor Drug Resistance

Another treatment target in cancer that greatly benefits from a therapeutic approach that utilizes drug combinations is the treatment of tumors that present with drug resistance, a phenotype whereby the cancer is largely resistant to chemotherapeutic treatment alone. Combination chemotherapy has been extensively used in the clinic to treat cancers that develop resistance, and it is of interest to note that the original

use of combination chemotherapy derived by Frei, Freireich, and Holland was intended to circumvent the establishment of drug resistance in ALL. However, treatment with multiple cytotoxic chemotherapeutic agents lacks in benefit, since these potently toxic drugs can provoke detrimental adverse effects in patients, not to mention the fact that the occurrence of MDR, a cross-resistance to structurally and functionally unrelated classes of anticancer drugs, rules out hope for much of combination chemotherapy (Harris and Hochhauser, 1992). Decades of research into the cellular mechanisms that cause drug resistance to develop have opened up a new avenue of therapeutic targets for combination therapy against drug resistance, most notably aimed at inhibiting drug efflux pumps of the ATP-binding cassette (ABC) family of transporters (most notably *p*-gp/MDR-1), inhibiting drug detoxification mechanisms, and restoring or lowering the apoptotic threshold of MDR cancer cells (Bradley et al., 1988; Harris and Hochhauser, 1992). Initial and some ongoing clinical strategies against MDR used inhibitors of *p*-gp to revert resistance in combination with chemotherapeutic drugs (Gottesman et al., 2002). This principle was quickly combined with the benefits of nanoparticle drug delivery, as demonstrated by Soma et al. who used poly(alkylcyanoacrylate) nanoparticles for co-administration of doxorubicin with the *p*-gp inhibitor cyclosporin A to successfully reverse MDR in monocytic leukemia cell line (p388) (Soma et al., 2000). Similarly, Wong et al. used polymer–lipid nanoparticles to co-administer doxorubicin with GG918 (Elacridar – a third generation *p*-gp inhibitor that has been undergoing testing in clinical trials for the treatment of MDR), to also observe a significantly improved chemosensitization in MDR MDA-MB-435 breast cancer cells (Wong et al., 2006). In the most basic form, intracellular uptake of nanoparticles by endocytotic mechanisms has been explored as a mechanism for chemotherapeutic drugs to bypass drug efflux pumps from the ABC family. Although shown to be a successful approach to chemosensitize MDR cancer types, this benefit of nanoparticles on a cellular level can be used to still deliver a combination therapy against alternate mechanisms of MDR to further improve therapeutic success. We have recently explored this strategy by using PEO-modified PCL nanoparticles to administer a combination therapy of paclitaxel with ceramide, an apoptotic modulator aimed to restore apoptotic signaling in the MDR phenotype. While it was found that the combination therapy significantly improved chemosensitivity in an MDR ovarian cancer model through a restoration of apoptotic activity in response to paclitaxel poisoning, encapsulation of this combination therapy into nanoparticles further enhanced the MDR modulation efficacy on a cellular level, as shown by the multifunctional strategy of simultaneously evading *p*-gp drug efflux as well (van Vlerken et al., 2007).

4 Multifunctional Nanocarriers for Combination Hyperthermia and Drug Therapy

The National Cancer Institute defines hyperthermia as a form of cancer treatment wherein high temperatures of up to 45 °C are applied to the tumor tissue (National Cancer Institute Fact Sheet, 2005). As opposed to thermal ablation, where significantly

higher temperatures (up to 70–80 °C) are used to completely coagulate the tissue for a brief period of time, hyperthermia extends for up to an hour and causes damages to the cellular proteins and organelles, eventually leading to cell death as evident by the tumor shrinkage (National Cancer Institute Fact Sheet, 2005). For general hyperthermia, hot-water baths are commonly used, but local temperature at the tumor site can be raised by 5–8 °C over the physiological temperature using various other thermal techniques such as high radio frequency, ultrasound, infrared, and microwave radiation. High temperatures (43 °C–45 °C) over a fixed period of time (30–60 min) are also used to sensitize tumors to chemotherapy and radiation (National Cancer Institute Fact Sheet, 2005). As such, combination of heat and chemo- or radiotherapy can be used very effectively to augment the therapeutic benefit in cancer leading to better clinical outcomes.

In the context of cancer therapy, hyperthermia has been studied mostly for its increased drug uptake and therapeutic activity enhancement properties. For a long time, hyperthermia has been speculated to preferentially cause changes in tumor metabolism and tumor vasculature by increasing cellular permeability (National Cancer Institute Fact Sheet, 2005). This has been the applied principle in thermal medicine with combination therapy. However, until recently, hyperthermic therapy was not a widely accepted treatment modality due to problems associated in maintaining homogenous temperatures in the target tumor mass and prevention of heat-induced injury to neighboring normal tissue (National Cancer Institute Fact Sheet, 2005). This is particularly challenging in thermotherapy of deep-seeded solid tumors, such as those in the liver, pancreas, prostate, and lung as heat-inducing probes are applied from the exterior, thus making it an invasive and complicated procedure (National Cancer Institute Fact Sheet, 2005). Even to date, clinical hyperthermia is yet to achieve the significance as an adjuvant therapeutic modality. Nevertheless, this treatment option combined with various delivery applications in chemotherapy and radiotherapy can have significant implication in the future.

4.1 Rationale for Combination Thermal Medicine and Drug Therapy

In any cell, hyperthermia triggers the synthesis of heat shock proteins (HSPs), which mediate various cellular defenses, including dynamic protein folding and chaperoning functions throughout the cell, thus inducing thermo-tolerance. Hyperthermic damage to tumor cells is greater when compared to normal cells (van der Zee, 2002), due to various tumor micro-environmental factors, including hypoxia, low pH, and susceptible vasculature, which makes this quite an attractive treatment modality (Ciocca and Calderwood, 2005).

At the molecular level, heat shock factor 1 (HSF-1), in association with heat shock elements (HSEs), mediates the heat shock gene expression (Brade et al., 2000). In the cancerous cell, excessive heat induces production of HSPs leading to cell repair. Cell signaling, apoptosis, and nuclear function involving HSPs have been a prime focus in recent research because of their potential as therapeutic targets.

For example, therapeutic genes that transcribe for cytokines, such as interleukin-2, interleukin-12, and tumor necrosis factor-α (TNF-α), have been successfully targeted in tumor models by using adjuvant hyperthermia (Siddiqui et al., 2007; Visaria et al., 2006). Wild-type HSP-70b promoter was used to control the expression of β-galactosidase reporter gene carried by an adenoviral vector (Brade et al., 2000).

The idea of adjuvant hyperthermia provides some hope as several investigators have recently attempted to address the problems of achieving homogenous therapeutic thermal dose within the tumor interstititum over the necessary period of time. The increased thermal and radio-sensitization brought about by small molecules, which function as radiosensitizers and lower HSF-1 activation, also has shown to cause loss of mitochondrial membrane potential, thus leading to mitochondrial damage (Sekhar et al., 2007).

Systemic chemotherapy has been the most successful mode of cancer therapy for a long time. However, infusing therapeutic doses of cytotoxic drugs into the blood stream and achieving the desired concentration in the tumor without producing toxic effects in the healthy body tissues has been the biggest challenge in cancer chemotherapy. Similarly, gene delivery systems encountered the problem of insufficient uptake, cytotoxicity, and undesirable immunogenic side effects due to the lack of safe tissue- or cell-specific vectors. This problem is being largely addressed by the advent of numerous surface-modified nano-sized drug delivery systems that can escape the reticulo-endothelial system and reach the target tissue with the aid of various target-specific ligands upon systemic administration.

4.2 Select Examples of Nanocarriers for Combination Thermal Medicine and Drug Delivery

Over the last decade, liposomes have been the most studied group of drug delivery systems. Ponce et al. (2007) observed increased uptake of liposomes, loaded with chemotherapeutic drugs when administered in combination with local hyperthermia induced via catheter inserted in the tumor. Further, the drug delivery pattern was observed by magnetic resonance imaging (MRI) to study the antitumor effect of drug loaded liposomes, and reported an increase in the tumor accumulation when administered with hyperthermia (Ponce et al., 2007).

Polymeric nanoparticles are not far behind liposomes or micelles in competing for candidacy of efficient drug delivery systems. Researchers have successfully exploited certain stimuli-responsive polymeric nanocarriers, which undergo thermodynamically reversible lower critical solution temperature (LCST) phase transition, also known as inverted phase transition (Meyer et al., 2001). This means that the polymeric nanocarriers become soluble upon injection in vivo, and then become insoluble only to accumulate at the tumor site due to induction of local hyperthermic state. Poly(*N*-isopropylacrylamide) and certain elastin-like peptides are ideally suited for such thermally-targeted drug delivery in cancer (Meyer et al., 2001). This strategy employs the high loading capacity of the polymeric carriers and the

synergistic effect of macromolecular extravasation by hyperthermia to localize the delivery system at the tumor site (Meyer et al., 2001). Local precipitation of the delivery system in the tumor vasculature due to increase in temperature can also lead to site-specific micro-embolization to prevent oxygen and nutrients diffusing into the tumor mass (Meyer et al., 2001). This is quite an elegant strategy that combines drug delivery with antivascular therapeutic approach to synergistically inhibit tumor growth.

Colloidal gold and microgels are other emerging examples of drug delivery systems that are being used in combination with thermotherapy. A recent study reported that the antitumor efficacy of TNF-α increased significantly upon encapsulation in PEG-coated colloidal gold particles and when administered in combination with thermotherapy (Visaria et al., 2006). In a similar gene-based approach, adjuvant hyperthermia was shown to enhance the anti-angiogenic efficacy of interleukin-12 upon administration with the aid of adenoviral vectors (Siddiqui et al., 2007). Microgels, which are microscopic particles of hydrogels, have gained substantial attention in controlled drug release. Upon cross-linking into mesh systems, various polymers can be used to formulate hydrogels holding large water content (Vinogradov, 2006). Microgels made with polymeric materials that undergo marked volume transitions upon exposure to external stimuli, such as temperature, can be quite useful in chemo-embolization in combination with hyperthermia, especially for liver cancer (Vinogradov, 2006). Certain anticancer drugs such as bleomycin can be encapsulated in the microgels, and upon oral administration, preferential release of the drug at higher pH (of the small intestine) was observed (Blanchette and Peppas, 2005). Further, release of certain encapsulated biomolecules, such as pDNA, can be targeted and controlled by hydrogen or hydrophobic bonding (Vinogradov, 2006).

Radio-frequency (RF) ablation is an image-guided, percutaneous ablative procedure, which applies the principle of tumor necrosis mediated by targeted heat delivery to the tumor mass (Chang, 2003). Electric probes are introduced into the center of the tumor through which high frequency alternating current (up to 550 kHz) is passed to generate heat by agitation of conductive ions, leading to irreversible cellular damage and tumor coagulation (Chang, 2003). Current clinical applications of RF ablation have found importance in the treatment of large lung and liver tumor masses (Chang, 2003).

Recently, RF ablation has become an increasingly popular mode of treatment in malignancies, although tumors larger than 3 cm have shown discouraging outcome (Hines-Peralta et al., 2006). This has prompted several researchers to investigate RFA in conjunction with chemotherapy. Goldberg's group has observed significant increases in tumor accumulation of doxorubicin and antitumor efficacy of the drug encapsulated in PEG-modified liposomes (Doxil®) upon administration in combination with RF ablation. Preliminary results of clinical studies involving patients with hepatic tumors show that high tumor necrosis levels were achieved when RF ablation was used in combination with targeted chemotherapy (Goldberg et al., 2002). Furthermore, favorable results have been reported when the liposomal chemotherapeutic agent was modified to achieve greater tumor coagulation levels.

However, the success of RFA-chemotherapy combination is also influenced by formulation characteristics such as nanoparticle size, the nature, and circulation time of cytotoxic drug delivered there in (Ahmed, 2005). Interestingly, arsenic trioxide (As_2O_3) has been used to enhance RF ablation in solid tumors through its apoptotic activation, antivascular, and thermo-sensitizing properties (Hines-Peralta et al., 2006).

It is critical to evaluate and optimize the current and emerging image-guidance tools applied in tumor ablation with patient-specific temperature maps, also known as isotherms (Wood et al., 2007). Technetium-99 m radiolabeled chemotherapeutic liposomes could be used to monitor drug release, which helps in the calculation of the desired intensity of hyperthermic intervention (Kleiter et al., 2006). X-ray computed tomography may also be used to study the release kinetics of the adjuvant chemotherapeutic agent released in response to thermal trigger at the tumor site and localization in the tumor mass. This is important in the context of physiological changes brought about by the severe tissue damage by RF ablation treatment. Despite the large scope of RF ablation in the treatment of cancer, the size and location of the tumor pose great challenges for successful clinical outcomes as deep-seated solid tumors are often poor targets for RF ablation due to poor visualization, higher probability of incomplete ablation, and potential for cancer relapse (Wood et al., 2007). In deep-seated tumor mass, intense damage to nontarget tissues is also highly possible because of the invasive nature of the treatment modality.

Deep-tissue solid tumors require innovative strategies, which employ minimally invasive, targeted implantation of "thermoseeds" to sensitize the tumor mass to radiotherapy or chemotherapy (or both) (Johannsen et al., 2005). Recently, liposomes or polymeric nanoparticles encapsulating magnetic iron oxide particles have been recruited to induce hyperthermia in tumors and also to serve as MRI contrast agents. In one clinical study, magnetic nanoparticles were evaluated for interstitial thermotherapy where-in iron oxide nanoparticles were suspended in water and administered to 22 patients with pelvic, thoracic, and head-and-neck tumors and exposed to alternating magnetic field to generate local heat (Wust et al., 2006). The results showed good tolerance of magnetic heating of iron oxide nanoparticles by these patients. In a separate study, the same group of investigators evaluated the efficacy of aminosilane-coated iron oxide nanoparticles for thermotherapy of recurrent glioblastoma multiforme and reported that magnetic nanoparticles were safe and efficacious in achieving hyperthermia-mediated tumor control (Maier-Hauff et al., 2007).

5 Multifunctional Nanocarriers for Imaging and Drug Therapy

Perhaps the most common form of nanocarrier multifunctionalization finds itself in the combination of imaging modalities and drug therapy into a single nanoparticle platform. Since the improvement in survival outcome of cancer patients over the last few decades can be largely attributed to improvements in both therapy as well

as diagnostics, the combination of both modalities seems obvious, particularly since the tumor targeting properties of nanoparticles would benefit both therapy and imaging. A concept that is readily attainable through nanoparticles, and would be greatly beneficial to cancer patients, is the idea of "real-time" therapy, a situation whereby a clinician can visually track where in the body the administered dose disperses and how much accumulates at the tumor site, and as a result, can either predict therapeutic outcome, or even go as far as to visually monitor tumor shrinkage over time. Multifunctionalization of nanoparticles through the co-inclusion of therapeutics and imaging contrast agents will allow for such major advances.

Superparamagnetic iron oxide nanoparticles are colloidal suspensions of magnetite (Fe_3O_4) that were approved over a decade ago by the FDA for parental use as a contrast agent in MRI. Originally approved for liver imaging, the superparamagnetic nature of iron oxide nanoparticles enhances contrast of their area of accumulation on a T_2 weighted MRI image, a feat that is advantageous in the tumor detection as well. While MRI in itself is a very useful technique for detection of solid tumors, by providing clear anatomical detail and soft tissue contrast, in the past MRI has been quite insensitive for smaller events in cancer imaging, such as the detection of lymph node metastasis and therapeutic efficacy of cancer treatment. Iron oxide nanoparticles were successful in the detection of 90.5% lymph node metastasis in patients with prostate cancer as opposed to 35.4% detection using conventional MRI, a 2.5-fold greater increase in diagnostic sensitivity (Harisinghani et al., 2003). In a more advanced use of contrast imaging, iron oxide nanoparticles have been shown to image cellular events in vivo. Zhao et al. (2001) targeted iron oxide nanoparticles to anionic phospholipids present on the surface of apoptotic cells by incorporating the C2-domain of synaptotagmin I onto the surface of the nanoparticles, allowing for a real-time visualization of apoptotic activity as an indicator of chemotherapeutic efficacy. Magnetite nanoparticles formulated with PLGA have been successful in combining delivery of chemotherapeutic drugs to the tumor, while retaining enough magnetic strength for imaging contrast enhancement, a potential use for real-time tracking of therapeutic efficacy. This potential has also been demonstrated by Reichardt et al. (2005) who used iron oxide nanoparticles as a tumor contrast enhancement in MRI to visualize the tumor therapeutic response of MV522 colon carcinoma xenografts to a VEGF receptor tyrosine kinase inhibitor over time. From this study, they were able to show a statistically significant decrease in relative vascular volume fraction in real-time over the duration of treatment, as measured by sequential MRI of the tumors using these iron oxide nanoparticles as a tumor-imaging enhancer. Similarly, Nasongkla et al. (2006) developed multifunctional polymeric micelles loaded with doxorubicin and superparamagnetic nanoparticles in the core, and surface modified by inclusion of cyclic RGD for active tumor targeting. Self-assembling dermatan sulfate based nanoparticles formulated as a superparamagnetic nanoparticle with inclusion of the chemotherapeutic drug doxorubicin, is another example of a multifunctional nanoparticle for tumor imaging and treatment (Ranney et al., 2005). Not only have these nanoparticles been shown successful in imaging AT1 tumors in vivo by MRI, surprisingly, therapeutic efficacy against MX-1 breast tumor xenografts increased significantly

when doxorubicin was delivered encapsulated in these nanoparticles, versus treatment with free doxorubicin, as indicated by the drastic tumor growth delay in 60% of mice and complete tumor regression in 40% of mice treated with the nanoparticle formulation, as opposed to the lack of tumor regression and shorter tumor growth delay in mice treated with doxorubicin alone (Ranney et al., 2005). An alternative approach to a similar multifunctional nanoparticle by Kohler et al. (2005) multifunctionalized iron oxide nanoparticles by binding methotrexate to the surface to produce a targeting construct to folate receptors; however, once internalized by the cancer cell, lysosomal pH cleaved methotrexate from the surface, allowing it to further serve as a chemotherapeutic for cancer eradication, thereby producing a multifunctional system that allows for simultaneous tumor therapy and real-time imaging of drug delivery.

Another MRI contrast agent applicable in nanotechnology is gadolinium. Gadolinium-157 is a stable (nonradioactive) nuclide that is frequently used as a contrast agent in MRI diagnostics, to enhance contrast in T1 weighted images (Aime et al., 2004), for example, in MRI in vivo models of lymph node metastasis (Kobayashi et al., 2006). However, an additional benefit of gadolinium nanoparticles is that upon irradiation with thermal neutrons gadolinium-157 produces cytotoxic γ-ray radiation (Barth and Soloway, 1994), enabling gadolinium for the additional use in neutron capture therapy (NCT) of cancer. Thus, the combined therapeutic and imaging properties of gadolinium make it an excellent candidate for multifunctional cancer treatment. Using gadolinium nanoparticles as such as therapeutic modality, tumor growth was significantly suppressed and survival time increased through NCT in mice bearing a radio-resistant melanoma (Tokomitsu et al., 2000). Delivery of gadolinium through gadopentatic acid (Gd-DTPA) allows for association of gadolinium into polymeric nanoparticles, a principle proven by Tokomitsu et al. (Tokumitsu et al., 2000; Shikata et al., 2002) who utilized this concept to associate gadolinium into chitosan nanoparticles for NCT. Although, prior use of Gd-DTPA as an MRI contrast agent and use of chitosan nanoparticles in delivery of chemotherapeutics such as paclitaxel and doxorubicin to tumors (Nsereko and Amiji, 2002) seem evident, the dual use of these gadolinium-containing chitosan nanoparticles in imaging and therapy is yet to be investigated. Thus far, multifunctionalization of gadolinium nanoparticles has been improved through conjugation of folic acid or thiamine to the surface of gadolinium-containing nanoparticles (through distearoylphosphatidylethanolamine (DSPE) and a PEG spacer), greatly enhancing cell uptake of gadolinium to cancer cells expressing receptors for folate and thiamine respectively in vitro and in vivo, thereby potentially improving localization and tumor eradication by NCT (Oyewumi and Mumper, 2002; Oyewumi et al., 2003, 2004). Already, gadolinium nanoparticles present multifunctional properties in their ability to image and ablate the tumor in one system. However, further multifunctionalization of these vectors by conjugation with tumor-specific targeting ligands and incorporation of a drug load remains to be examined.

As another imaging modality, gold nanoparticles and gold nanoshells (silica core nanoparticles surrounded by a layer of gold coating) are favorable to be used

as contrast agents in optical coherence tomography (OCT), since variations in their size and shape allows for precise tuning of their resonance wavelength between near-ultraviolet and mid-infrared (Oldenburg et al., 1999). For example, a gold nanoshell with a 20-nm shell on a 60-nm silica core will resonate at around 700–750 nm, while a nanoshell with a 5-nm shell on the same 60-nm core will resonate at around 1,000–1,050 nm (Loo et al., 2004). In this manner, multifunctionalized gold nanoparticles have been used for tumor imaging and drug delivery. For example, delivery of daunorubicin from gold nanoparticles was shown to cause 15–20% greater inhibition of cell growth of multidrug resistant K562 leukemia cells and over administration of free drug, while retaining imaging capabilities of these cells through fluorescence detection (Li et al., 2007). Similarly, gold nanoparticles have been conjugated to a carbohydrate coating to incorporate glycogen antigens to develop a multifunctional anticancer vaccine (Ojeda et al., 2007). The disperse range permissible to these nanoparticles spans the near-infrared (NIR), and since NIR light experiences maximal tissue penetration with minimal en route absorption, they become beneficial for use in thermal ablation, a property that, like gadolinium nanoparticles, gives these platforms an inherent multifunctional capability in cancer imaging and therapy, aside from the additional inclusion of anticancer drugs. Such combined imaging and therapeutic use of these gold nanoshells has been proven in several cancer models, both in vitro and in vivo (Hirsch et al., 2003; Loo et al., 2004, 2005). It has been shown that thiolated PEG easily assembles onto the nanoshell surface providing a linker for surface incorporation of active tumor targeting moieties or even biotherapeutic agents. Paciotti et al. (2004) have used colloidal gold particles in this manner to successfully deliver TNF-α as an anticancer therapeutic to an MC-38 colon carcinoma in vivo. Mukherjee et al. (2005) reported the inhibition of angiogenesis by gold nanoparticles, through direct binding of the particles to heparin-binding growth factors (VPF or VEGF and FGF specifically), a property that is very useful in halting tumor proliferation. Hainfeld et al. (2004) have shown that gold nanoparticles can help to localize radiotherapy to prolong one-year survival rates of mice bearing EMT-6 mammary carcinomas (86% survival with gold nanoparticles versus 20% survival with x-rays alone). Although the latter three examples used colloidal gold nanoparticles rather than the silicon–gold nanoshells, which bear the combined use of imaging and thermal ablation, future research may allow for the development of a gold nanoshell or a particle that ties together all these uses.

Finally, a more recent nanoparticle platform that emerged for cancer diagnostics, and has further allowed for the multifunctional modality of imaging and therapy is the semiconductor nanocrystal, otherwise known as the quantum dot. Quantum dots are semiconductor-based nanoparticles that function as fluorescent probes for imaging purposes (Gao et al., 2005). Similar to gold nanoshells, quantum dots are favorable imaging agents, that is their absorption properties can be tuned from visible to infrared wavelengths, they emit highly intense signals, and they are chemically, photochemically, and thermally stable (Chan et al., 2002). Quantum dots have the unique property that, from a single excitation wavelength, emission photons can span any wavelength between blue and infrared depending

on the nanocrystal size and composition (Voura et al., 2004). Therefore a number of quantum dots, each actively targeted to a different tumor marker, can be visualized simultaneously, a useful property in real-time cancer imaging. This function has been particularly useful in the tracking of metastatic tumors (Voura et al., 2004). Quantum dots, miniscule in size (2–8 nm in diameter), are easily bioconjugated with peptides, antibodies, and small-molecule drugs through polymer linkers without loss of their fluorescence or tumor localization properties (Gao et al., 2005). Typically, high quality quantum dots are prepared in the organic solvent mixture tri-*n*-octyl phosphine/tri-*n*-octylphosphine oxide (TOP/TOPO) at high temperatures, which caps the quantum dots with a monolayer of the nonpolar solvent. This capping allows for surface adhesion of amphiphilic polymers (such as PEG and poly(ethylene oxide)-containing block copolymers), which not only facilitate solubility and bioavailability of the nanoparticles, but provide a linker for bioconjuation of peptides, antibodies, oligonucleotides, or small molecule drugs, thereby multifunctionalizing the quantum dot for tumor targeting, tumor imaging, and potential drug delivery. A few examples of such incorporation to quantum dots in this manner include antibodies against HER2 (Wu et al., 2003), prostate specific membrane antigen (Gao et al., 2004), HSPs (Medintz et al., 2005), and *p*-gp (Sukhanova et al., 2004). Although from this step forward it seems inherent that drugs can be loaded into the bulk of the polymer coating or grafted onto the surface (successful multifunctionalization of this degree), while retaining the imaging, biocompatibility, and bioavailability properties remains to be proven. Nevertheless, through a recent discovery it appears that quantum dots, such as gold and gadolinium nanoparticles, may possess an inherent therapeutic capability, thereby maintaining the combined tumor imaging and therapy functions that makes these nanoparticles multifunctional. It appears that quantum dots can act as photosensitizers in photodynamic therapy (PDT) (Bakalova et al., 2004). PDT utilizes light, oxygen, and a photosensitizer to selectively destroy target tissue by generating reactive oxygen species, which promotes apoptosis of the target cells. In this, Samia (2006) have shown that cadmium selenide quantum dots can generate the singlet oxygen species that take part in PDT, although generation is at a lower rate than conventional photosensitizers. However, with this nanoparticle tumor therapy system the promise exists for a multifunctional imaging and therapeutic approach, with great benefit to cancer treatment.

6 Other Examples of Multifunctional Nanosystems

6.1 Combination Drug Delivery and Ultrasound

Perfluorocarbon emulsion nanoparticles are under investigation as ultrasound contrast agents and ultrasonically enhanced drug delivery vehicles. With a mean diameter on the order of hundreds of nanometers, approximately 10-fold smaller

than commercially available microbubble contrast agents, targeting of, and extravasation through tumor endothelium may be superior to microbubbles (Kong et al., 2000, 2001). Compared with microbubbles, liquid-filled nanodroplets are more stable under pressure and mechanical stress and are capable of carrying a larger drug payload, although they are also less echogenic. Both microbubbles and liquid-filled nanoparticles can be encapsulated by a molecularly targeted lipid shell.

Lanza and coworkers (Lanza et al., 2002; Lanza and Wickline, 2003; Wickline and Lanza, 2003) have described the use of perfluorocarbon emulsion nanoparticles as ultrasound contrast agents and have developed theoretical models for estimating acoustic reflectivity of different perfluorocarbon nanoparticle formulations (Marsh et al., 1998, 2002a&b; Hall et al., 2000, 2001). Nanoparticles have low acoustic reflectivity in solution; however, their echogenicity increases when they are deposited in a layer, resulting in a targeted contrast agent that is detectable only when adherent at the target site (Lanza and Wickline, 2003). Perfluorocarbon nanoparticles can also serve as MRI contrast agents when gadolinium is incorporated into their lipid shell useful for multimodality imaging studies (Anderson et al., 2000; Winter et al., 2003a,b; Lanza et al., 2004; Morawski et al., 2004; Cyrus et al., 2005; Schmieder et al., 2005). In addition to their application as ultrasound contrast agents, perfluorocarbon nanoparticles have also been used as therapeutic delivery vehicles for doxorubicin, paclitaxel, and other therapeutic agents (Lanza and Wickline, 2001; Wickline and Lanza, 2003; Larina et al., 2005). Crowder et al. (2005) have shown that ultrasound enhances trans-membrane delivery of fluorescent dye from nanoparticles to C32 melanoma cells. Ultrasonic molecular imaging is unique, that is, the optimal application of these agents depends not only on the surface chemistry but also on the applied ultrasound field, which can increase receptor–ligand binding and membrane fusion (Dayton et al., 1999; Zhao et al., 2004; Rychak et al., 2005). Dayton et al. (1999) and Rychak et al. (2005) have previously demonstrated that acoustic radiation force produced by ultrasound can enhance the efficiency of targeted imaging with microbubble-based agents by deflecting targeted particles to the endothelium and facilitating bond formation. Lum et al. (2006) and Shortencarier et al. (2004) have demonstrated that physically localizing drug delivery vehicles with acoustic radiation force can enhance localized drug delivery. Recently, Crowder et al. (2005) have observed acoustically enhanced dye delivery from perfluorocarbon nanoparticles and postulated that acoustic radiation force is partially responsible for this effect.

Using microbubbles as a carrier particle and attaching nanoparticles containing a higher payload of drug allows the biodistribution of such a carrier particle to be controlled by insonation, using ultrasound pulse schemes that are designed to deflect the vehicle to a target vessel wall and then to rupture the larger lipid carrier. When a traveling ultrasonic wave is absorbed by a particle, the momentum associated with the wave produces a net primary ultrasound radiation force (USRF), whereby the radiating sound wave is transferred to the particle. While incompressible objects do experience USRF, compressible objects such as gas bubbles experience far larger forces and are displaced by low-amplitude ultrasound waves (Aaron et al., 2006). Avidinated neutravidin-coated fluorescent nanobeads bound to the biotinylated

lipid shells of preformed microbubbles that specifically targets using USRF and biotin–avidin interactions is demonstrated (Aaron et al., 2006). Targeting of nanobeads was molecularly specific and dependent on, in order of importance, vehicle concentration, wall shear stress, nanobead size, and insonation time. This method of delivery is shown to enable targeted deposition of nanoparticles in shear flow and can be modified to carry therapeutic agents for controlled release in targeted delivery applications.

6.2 Combination Drug Delivery and PDT

Photodynamic therapy (PDT), the activation of a tumor-localized photosensitizer by light, is generally applied as a single modality for the treatment of a variety of solid tumors. Its dominant mechanism of action is the local generation of cytotoxic singlet oxygen, which causes the destruction of tumor cells and damage of the tumor microvasculature (Henderson and Gollnick, 2003). It has been applied to both treatment of superficial tumors (such as cutaneous basal-cell carcinoma and head and neck tumors) and to deeper tumors accessible by endoscopies (including esophageal and lung cancers) (Hopper, 2000). PDT with photofrin has been approved by FDA for the treatment of Barrett's esophagus and endobronchial and esophageal carcinomas, and perhaps the most successful approval of PDT is with verteporfin for injection (Visudyne®) to treat age-related macular degeneration (AMD) (Dolmans et al., 2003).

In general, a photosensitizer is confined within the tumor vasculature initially after injection and PDT that employs a short drug-light interval largely damages tumor vasculature (Veenhuizen et al., 1997). This mechanism is mainly responsible for some of the more successful clinical implementations of PDT today, including AMD treatment with verterpofin (Brown and Mellish, 2001) and prostate cancer treatment with Pd-bacteriopheophorbide TOOKAD (Chen et al., 2002a; Koudinova et al., 2003).

While many studies have explored ways to maximize the therapeutic effect of PDT (Gudgin Dickson et al., 2002), recent efforts are more focused on utilizing targeting strategies that are directed at the tumor vasculature. However, it should be realized that neither vascular targeting nor cellular targeting PDT regime alone is perfect for tumor cell killing. Solely vascular targeting may be a good approach for purely vascular diseases such as AMD (Schmidt-Erfurth et al., 1994), yet it may not be enough for tumors because peripheral tumor vessels are shown to be somewhat resistant to both vascular-targeting agents (Pedley et al., 2001) and PDT-induced vascular effects (Uehara et al., 1998; Chen et al., 2002b; Koudinova et al., 2003).

Despite the extensive central tumor necrosis induced by vascular targeting PDT, tumor vessels or cells can re-grow from the peripheral rim after treatment. The major problem for cellular-targeting PDT is that it suffers from complex issues such as heterogeneity of tumor microenvironment and inhomogeneous photosensitizer distribution. Additionally, tissue hypoxia has been identified as a major obstacle to direct targeting tumor cells by PDT (Dougherty et al., 1998).

Inadequate photosensitizer delivery due to heterogeneous tumor perfusion, vascular permeability, and tumor interstitial pressure can also affect the effectiveness of cellular targeting PDT. Combination of tumor vascular and cellular targeting approaches can be a way to overcome the problem associated with each individual targeting strategy and to achieve maximal opportunity of tumor eradication (Wachsberger et al., 2003). Also, most photosensitizers are hydrophobic and difficult to prepare in an injectable form.

Nanocarriers can provide solution to all the above problems by not only providing a stable dispersion of these drugs into aqueous systems, but also upon systemic administration, these carriers are preferentially taken up by tumor tissues by virtue of the "enhanced permeability and retention effect", which is the property of such tissues to engulf and retain circulating macromolecules and particles owing to their "leaky" vasculature. The carriers include oil dispersions (micelles), liposomes, low-density lipoproteins, polymeric micelles, and hydrophilic drug–polymer complexes. In one study (Qing et al., 2006), Profrin II nanoparticles-PDT results in inhibition of Lovo colon carcinoma growth in post-PDT earlier period in vivo, and were shown to prolong the survival time of nude mice bearing xenografts significantly, whereas Profrin II-PDT could not inhibit the growth of colon tumor completely. In another study (Reddy et al., 2006), multifunctional polymeric nanoparticle consisting of a surface-localized tumor vasculature targeting F3 peptide and encapsulated PDT and imaging agents were shown to specifically bound to the surface of MDA-435 cells in vitro and were internalized conferring photosensitivity to the cells. Treatment of glioma-bearing rats with targeted nanoparticles followed by PDT showed a significant improvement in survival rate when compared with animals who received PDT after administration of nontargeted nanoparticles or systemic photofrin.

Zinc(II) phthalocyanine (ZnPc), a second generation loaded PLGA nanoparticle, was shown to maintain its photo-physical behavior after encapsulation (Ricci-Junior and Marchetti, 2006). Photosensitizer release from nanoparticles was sustained with a moderate burst effect of 15% for 3 days. The photocytotoxicity of ZnPc loaded PLGA Np was evaluated on P388-D1 cells that were incubated with ZnPc loaded Np (5 $\propto$M) by 6 h and exposed to red light (675 nm) for 120 s, and light dose of 30 J/cm^2. After 24 h of incubation, the cellular viability was determined, obtaining 61% of cellular death. From the physical–chemical, photophysical, and photobiological measurements performed it was concluded that ZnPc loaded PLGA nanoparticles is a promising drug delivery system for PDT. In another study, Prasad's group (Cinteza et al., 2006) described the ceramic-based nanoparticles capable of selectively delivering photosensitizers to tumor cells and damaging them in vitro. These studies establish the role of nanocarriers in PDT.

7 Conclusions

Over the last decade, a wide range of nanocarrier systems, such as liposomes, polymeric nanoparticles, nanoemulsions, micelles, and hydrogels have shown tremendous progress in pharmaceutical applications. These engineered multifunctional

nanocarrier systems have successfully evolved to possess some very useful properties such as prolonged circulation in blood, target specificity, and increased cell penetration of the therapeutic drugs and molecules. Prompted by the clinical success of some nanocarriers, most drug delivery research has focused on integrating the various beneficial properties of the nanovectors to make the treatment strategies more direct, specific, stable, less invasive, and in some cases to tackle the problem of MDR.

Current research is also focused on understanding and taking advantage of the features of tumor microenvironment such as pH and temperature changes. Developing nanocarriers that employ various beneficial properties require the assembly of a number of chemical moieties on a single nanoparticle. However, immediate challenges in the formulation of such nanovector system include characteristics such as size, surface charge, cytotoxicity, immunogenicity, cell membrane, and organelle barriers to name a few.

Nanovectors, in their simplest form, could enable deliver a combination of drugs or genes (or both) to take advantage of synergistic or bystander properties of the biomolecules. Currently, various nanocarrier systems undergo surface modification, by synthetic polymers such as PEG and targeting ligands such as peptides, antibodies, or sugar moieties, in order to escape the physiological attack by the reticulo-endothelial sytem in the body and target the disease site. In certain cases, the surface modification could enable the nanovector to pass through the blood–brain barrier. The protective PEG coat of the nanocarriers may inhibit the release of the encapsulated drug, thus encouraging the development of drug delivery systems that could be pH- and temperature-responsive, especially in conditions such as inflammation, infarction, and cancer.

Hyperthermia has been emerging as an important adjuvant mode alongside of chemotherapy, radiotherapy, and surgery. Increased local or whole body temperatures brought about by radiofrequency ablation, ultrasonic waves, or by the administration of magnetic nanoparticles that act in alternating magnetic fields are some of the tested strategies in the clinic. Nanocarriers that enable contrasting agents to transmit a signal drug accumulated site provide for diagnostic and imaging techniques. Gold nanoparticles, quantum dots, liposomes, and micelles are among the successful nanovectors. When formulated in combination, these drug delivery systems could enable imaging and controlled release of drugs or therapeutic molecules in a spatiotemporal pattern. Such multifaceted, versatile nanocarriers and drug delivery systems promise a substantial increase in the efficacy of diagnostic and therapeutic applications in pharmaceutical sciences.

References

Aaron, F. H. L., A. B. Mark, A. D. Paul, et al. (2006). “Ultrasound radiation force enables targeted deposition of model drug carriers loaded on microbubbles.” *J Contr Release*. 111: 128–34.

Ahmed, M., A. N. Lukyanov, V. Torchilin, et al. (2005). Combined radiofrequency ablation and adjuvant liposomal chemotherapy: effect of chemotherapeutic agent, nanoparticle size, and circulation time. *Journal of vascular and interventional radiology*. 16(10): 1365–71.

Aime, S., A. Barge, C. Cabella, et al. (2004). "Targeting cells with MR imaging probes based on paramagnetic Gd(III) chelates." *Curr Pharmaceut Biotechnol*. 5: 509–18.

Alyautdin, R. N., E. B. Tezikov, P. Ramge, et al. (1998). "Significant entry of tubocurarine into the brain of rats by adsorption to polysorbate 80-coated polybutylcyanoacrylate nanoparticles: an in situ brain perfusion study." *J Microencapsul*. 15(1): 67–74.

Alyaudtin, R. N., A. Reichel, R. Lobenberg, et al. (2001). "Interaction of poly(butylcyanoacrylate) nanoparticles with the blood–brain barrier in vivo and in vitro." *J Drug Target*. 9(3): 209–21.

Amiji, M. (2006). "Polymeric delivery - Engineered nanosystems for targeted delivery of drugs and genes". *Future Drug Delivery*. http://www.touchbriefings.com/pdf/1859/amiji.pdf (Accessed August 09, 2006).

Anderson, S. A., R. K. Rader, W. F. Westlin, et al. (2000). "Magnetic resonance contrast enhancement of neovasculature with $\alpha_v\beta_3$-targeted nanoparticles." *Magn Reson Med*. 44(3): 433–9.

Bakalova, R., H. Ohba, Z. Zhelev, et al. (2004). "Quantum dots as photosensitizers?" *Nat Biotech*. 22(11): 1360–1.

Bargoni, A., R. Cavalli, G. P. Zara, et al. (2001). "Transmucosal transport of tobramycin incorporated in solid lipid nanoparticles (SLN) after duodenal administration to rats. Part II—tissue distribution." *Pharmacol Res*. 43(5): 497–502.

Barth, R. F. and A. H. Soloway (1994). "Boron neutron capture therapy of primary and metastatic brain tumors." *Mol Chem Neuropathol*. 21: 139–54.

Becker-Hapak, M., S. S. McAllister and S. F. Dowdy (2001). "TAT-mediated protein transduction into mammalian cells." *Methods*. 24(3): 247–56.

Bellocq, N. C., S. H. Pun, G. S. Jensen, et al. (2003). "Transferrin-containing, cyclodextrin polymer-based particles for tumor-targeted gene delivery." *Bioconjugate Chem*. 14(6): 1122–32.

Bidwell, G. L., III, I. Fokt, W. Priebe, et al. (2007). "Development of elastin-like polypeptide for thermally targeted delivery of doxorubicin." *Biochem Pharmacol*. 73(5): 620–31.

Blanchette, J and N. A. Peppas (2005). Oral chemotherapeutic delivery: design and cellular response. *Ann Biomed Eng*., 33(2):142–9.

Brade, A. M., D. Ngo, P. Szmitco, et al. (2000). Heat-directed gene targeting of adenoviral vectors to tumor cells. *Cancer Gene Ther*. 7(12):1566–74.

Bradley, G., P. F. Juranka and V. Ling (1988). Mechanism of multidrug resitance. *Biochem Biophys Acta.* 948: 87–128.

Brown, S. B. and K. J. Mellish (2001). "Verteporfin: a milestone in opthalmology and photodynamic therapy." *Expert Opin Pharmacother*. 2(2): 351–61.

Calvo, P., B. Gouritin, H. Chacun, et al. (2001a). "Long-circulating PEGylated polycyanoacrylate nanoparticles as new drug carrier for brain delivery." *Pharm Res*. 18(8): 1157–66.

Calvo, P., B. Gouritin, I. Brigger, et al. (2001b). "PEGylated polycyanoacrylate nanoparticles as vector for drug delivery in prion diseases." *J Neurosci Methods*. 111(2): 151–5.

Calvo, P., B. Gouritin, H. Villarroya, et al. (2002). "Quantification and localization of PEGylated polycyanoacrylate nanoparticles in brain and spinal cord during experimental allergic encephalomyelitis in the rat." *Eur J Neurosci*. 15(8): 1317–26.

Cegnar, M., J. Kristl and J. Kos (2005). Nanoscale polymer carriers to deliver chemotherapeutic agents to tumours. *Expert Opinion Biologicy and Therapeutics*. 5(12): 1557–69.

Chan, W. C. W., D. J. Maxwell, X. Gao, et al. (2002). "Luminescent quantum dots for multiplexed biological detection and imaging." *Curr Opin Biotechnol*. 13(1): 40–6.

Chang, I. (2003). Finite element analysis of hepatic radiofrequency ablation probes using temperature-dependent electrical conductivity. *BioMedical Engineering Online*, 2: 12

Chen, B., Y. Xu, T. Roskams, et al. (2001). "Efficacy of antitumoral photodynamic therapy with hypericin: relationship between biodistribution and photodynamic effects in the RIF-1 mouse tumor model." *Int J Cancer*. 93(2): 275–82.

Chen, Q., Z. Huang, D. Luck, et al. (2002a). "Preclinical studies in normal canine prostate of a novel palladium-bacteriopheophorbide (WST09) photosensitizer for photodynamic therapy of prostate cancers." *Photochem Photobiol*. 76(4): 438–45.

Chen, B., T. Roskams and P. A. de Witte (2002b). "Enhancing the antitumoral effect of hypericin-mediated photodynamic therapy by hyperthermia." *Lasers Surg Med*. 31(3): 158–63.

Chen, J., F. Saeki, B. J. Wiley, et al. (2005). "Gold nanocages: bioconjugation and their potential use as optical imaging contrast agents." *Nano Lett*. 5(3): 473–7.

Chiellini, E. E., F. Chiellini and R. Solaro (2006). Bioerodible polymeric nanoparticles for targeted delivery of proteic drugs. *Journal of Nanoscience and Nanotechnology*. 6(9–10): 3040–7.

Cinteza, L. O., T. Y. Ohulchanskyy, Y. Sahoo, et al. (2006). "Diacyllipid micelle-based nanocarrier for magnetically guided delivery of drugs in photodynamic therapy." *Mol Pharm*. 3(4): 415–23.

Ciocca, D. R. and Calderwood, S. K. (2005). Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications. *Cell Stress & Chaperones*., Summer, 10(2): 86–103.

Cole, S. P., G. Bhardwaj, J. H. Gerlach, et al. (1992). "Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line." *Science*. 258(5088): 1650–4.

Crowder, K. C., M. S. Hughes, J. N. Marsh, et al. (2005). "Sonic activation of molecularly-targeted nanoparticles accelerates transmembrane lipid delivery to cancer cells through contact-mediated mechanisms: implications for enhanced local drug delivery." *Ultrasound Med Biol*. 31(12): 1693–700.

Cyrus, T., P. M. Winter, S. D. Caruthers, et al. (2005). "Magnetic resonance nanoparticles for cardiovascular molecular imaging and therapy." *Expert Rev Cardiovasc Ther*. 3(4): 705–15.

Dayton, P., A. Klibanov, G. Brandenburger, et al. (1999). "Acoustic radiation force in vivo: a mechanism to assist targeting of microbubbles." *Ultrasound Med Biol*. 25(8): 1195–201.

Devalapally, H., D. Shenoy, S. Little, et al. (2007). Poly(ethylene oxide)-modified poly(beta-amino ester) nanoparticles as a pH-sensitive system for tumor-targeted delivery of hydrophobic drugs: part 3. Therapeutic efficacy and safety studies in ovarian cancer xenograft model. *Cancer chemotherapy and pharmacology*. 59(4): 477–84.

Dolmans, D. E., D. Fukumura and R. K. Jain (2003). "Photodynamic therapy for cancer." *Nat Rev Cancer*. 3(5): 380–7.

Dougherty, T. J., C. J. Gomer, B. W. Henderson, et al. (1998). "Photodynamic therapy." *J Natl Cancer Inst*. 90(12): 889–905.

Elbayoumi T. A. and V. P. Torchilin (2006). "Enhanced accumulation of long-circulating liposomes modified with the nucleosome-specific monoclonal antibody 2C5 in various tumours in mice: γ-imaging studies." *Eur J Nucl Med Mol Imag*. 33(10): 1196–1205.

Emerich, D. F. and C. G. Thanos (2006). "The pinpoint promise of nanoparticle-based drug delivery and molecular diagnosis." *Biomol Eng*. 23(4): 171–84.

Fang, J., T. Sawa, H. Maeda (2003). Factors and mechanism of "EPR" effect and the enhanced antitumor effects of macromolecular drugs including SMANCS. *Advances in experimental medicine and biology*. 519: 29–49.

Farokhzad, O. C., S. Jon, A. Khademhosseini, et al. (2004). Nanoparticle-aptamer bioconjugates: a new approach for targeting prostate cancer cells. *Cancer Research*. 64(21): 7668–72.

Farokhzad, O. C., J. Cheng, B. A. Teply, et al. (2006a). "Targeted nanoparticle–aptamer bioconjugates for cancer chemotherapy in vivo." *PNAS*. 103(16): 6315–20.

Farokhzad O. C., J. M. Karp and R. Langer (2006b). Nanoparticle-aptamer bioconjugates for cancer targeting. *Expert opinion on drug delivery*. 3(3): 311–24.

Fayette, J., J.-C. Soria and J.-P. Armand (2005). "Use of angiogenesis inhibitors in tumour treatment." *Eur J Canc*. 41(8): 1109–16.

Fellner, S., B. Bauer, D. S. Miller, et al. (2002). "Transport of paclitaxel (Taxol) across the blood–brain barrier in vitro and in vivo." *J Clin Invest*. 110(9): 1309–18.

Fenart, L., A. Casanova, B. Dehouck, et al. (1999). "Evaluation of effect of charge and lipid coating on ability of 60-nm nanoparticles to cross an in vitro model of the blood–brain barrier." *J Pharmacol Exp Ther*. 291(3): 1017–22.

Fenske, D. B., I. MacLachlan and P. R. Cullis (2001). "Long-circulating vectors for the systemic delivery of genes." *Curr Opin Mol Ther*. 3(2): 153–8.

Folkman, J. (1972). "Anti-angiogenesis: new concept for therapy of solid tumors." *Ann Surg*. 175(3): 409–16.

Frei, E., III, J. F. Holland, M. A. Schneiderman, et al. (1958). "A comparative study of two regimens of combination chemotherapy in acute leukemia." *Blood*. 13(12): 1126–48.
Gabizon, A. A., H. Shmeeda, S. Zalipsky (2006). Pros and cons of the liposome platform in cancer drug targeting. *Journal of liposome research*. 16(3): 175–83.
Gao, X., Y. Cui, R. M. Levenson, et al. (2004). "In vivo cancer targeting and imaging with semiconductor quantum dots." *Nat Biotechnol*. 22(8): 969–76.
Gao, X., L. Yang, J. A. Petros, et al. (2005). "In vivo molecular and cellular imaging with quantum dots." *Curr Opin Biotechnol*. 16(1): 63–72.
Gao, X., W. Tao, W. Lu, et al. (2006). "Lectin-conjugated PEG-PLA nanoparticles: preparation and brain delivery after intranasal administration." *Biomaterials*. 27(18): 3482–90.
Genentech Biotechnology. Avastin®, Bevacizumab. Product Information Guide. http://www.avastin.com/avastin/index.jsp?hl=en&lr=&q=Avastin (Accessed September 28, 2006).
Genentech Biotechnology. Herceptin®, Transtuzumab. Product Information Guide. http://www.herceptin.com/herceptin/patient/index.jsp (Accessed September 28, 2006).
Gomez-Lopera, S. A., R. C. Plaza and A. V. Delgado (2001). "Synthesis and characterization of spherical magnetite/biodegradable polymer composite particles." *J Colloid Interface Sci*. 240(1): 40–7.
Gottesman, M. M., Fojo, T. and Bates, S. E. (2002). "Multidrug resistance in cancer: role of ATP-dependent transporters." *Nat Rev Cancer*. 2: 48–58.
Gudgin Dickson, E. F., R. L. Goyan and R. H. Pottier (2002). "New directions in photodynamic therapy." *Cell Mol Biol (Noisy-le-grand)*. 48(8): 939–54.
Gulyaev, A. E., S. E. Gelperina, I. N. Skidan, et al. (1999). "Significant transport of doxorubicin into the brain with polysorbate 80-coated nanoparticles." *Pharm Res*. 16(10): 1564–9.
Gupta, B. and V. P. Torchilin (2006). "Transactivating transcriptional activator-mediated drug delivery." *Expert Opin Drug Deliv*. 3(2): 177–90.
Gupta, B., T. S. Levchenko and V. P. Torchilin (2005). "Intracellular delivery of large molecules and small particles by cell-penetrating proteins and peptides." *Adv Drug Deliv Rev*. 57(4): 637–51.
Hainfeld, J. F., D. N. Slatkin and H. M. Smilowitz (2004). "The use of gold nanoparticles to enhance radiotherapy in mice." *Phys Med Biol*. 49(18): N309–N315.
Hall, C. S., J. N. Marsh, M. J. Scott, et al. (2000). "Time evolution of enhanced ultrasonic reflection using a fibrin-targeted nanoparticulate contrast agent." *J Acoust Soc Am*. 108(6): 3049–57.
Hall, C. S., J. N. Marsh, M. J. Scott, et al. (2001). "Temperature dependence of ultrasonic enhancement with a site-targeted contrast agent." *J Acoust Soc Am*. 110(3, Pt 1): 1677–84.
Harisinghani, M. G., J. Barentsz, P. F. Hahn, et al. (2003). "Noninvasive detection of clinically occult lymph-node metastases in prostate cancer." *N Engl J Med*. 348(25): 2491–9.
Harris, A. L. and D. Hochhauser (1992). Mechanisms of multidrug resistance in cancer treatment. *Acta Oncol.* 31(2): 205–13.
Hayes, M. E., D. C. Drummond, K. Hong, et al. (2006). "Increased target specificity of anti-HER2 genospheres by modification of surface charge and degree of PEGylation." *Mol Pharm*. 3(6): 726–36.
Helm, C. W., C. R. Toler, R. S. Martin, III, et al. (2007). "Cytoreduction and intraperitoneal heated chemotherapy for the treatment of endometrial carcinoma recurrent within the peritoneal cavity." *Int J Gynecol Cancer*. 17(1): 204–9.
Henderson, B. W. and S. O. Gollnick (2003). Mechanistic principles of photodynamic therapy. Boca Raton, CRC Press.
Hildebrandt, B., P. Wust, O. Ahlers, et al. (2002). "The cellular and molecular basis of hyperthermia." *Crit Rev Oncol Hematol*. 43(1): 33–56.
Hines-Peralta, A., V. Sukhatme, M. Regan, et al. (2006). "Improved tumor destruction with arsenic trioxide and radiofrequency ablation in three animal models." *Radiology*. 240(1): 82–9.
Hirsch, L. R., R. J. Stafford, J. A. Bankson, et al. (2003). "Nanoshell-mediated near-infrared thermal therapy of tumors under magnetic resonance guidance." *PNAS*. 100(23): 13549–54.

Hopper, C. (2000). "Photodynamic therapy: a clinical reality in the treatment of cancer." *Lancet Oncol*. 1: 212–19.

Huwyler, J. and W. M. Pardridge (1998). "Examination of blood–brain barrier transferrin receptor by confocal fluorescent microscopy of unfixed isolated rat brain capillaries." *J Neurochem*. 70(2): 883–6.

Huwyler, J., A. Cerletti, G. Fricker, et al. (2002). "By-passing of *p*-glycoprotein using immunoliposomes." *J Drug Target*. 10(1): 73–9.

Iinuma, S., K. T. Schomacker, G. Wagnieres, et al. (1999). "In vivo fluence rate and fractionation effects on tumor response and photobleaching: photodynamic therapy with two photosensitizers in an orthotopic rat tumor model." *Cancer Res*. 59(24): 6164–70.

Illum, L., L. O. Jacobsen, R. H. Muller, et al. (1987). "Surface characteristics and the interaction of colloidal particles with mouse peritoneal macrophages." *Biomaterials*. 8(2): 113–17.

Isomoto, H., A. Ohtsuru, V. Braiden, et al. (2006). "Heat-directed suicide gene therapy mediated by heat shock protein promoter for gastric cancer." *Oncol Rep*. 15(3): 629–35.

Jain, S., V. Mishra, P. Singh, et al. (2003). "RGD-anchored magnetic liposomes for monocytes/neutrophils-mediated brain targeting." *Int J Pharm*. 261(1–2): 43–55.

Jang, S. H., M. G. Wientjes, D. Lu, et al. (2003). "Drug delivery and transport to solid tumors." *Pharm Res*. 20(9): 1337–50.

Jeon, S. I., J. H. L. Andrade and P. G. de Gennes (1991). "Protein-surface interactions in the presence of polyethylene oxide: Simplified theory." *J Colloid Interface Sci*. 142: 149–58.

Jeong, Y. I., S. J. Seo, I. K. Park, et al. (2005). "Cellular recognition of paclitaxel-loaded polymeric nanoparticles composed of poly(γ-benzyl L-glutamate) and poly(ethylene glycol) diblock copolymer endcapped with galactose moiety." *Int J Pharm*. 296(1–2): 151–61.

Jiang, C., N. Koyabu, Y. Yonemitsu, et al. (2003). "In vivo delivery of glial cell-derived neurotrophic factor across the blood–brain barrier by gene transfer into brain capillary endothelial cells." *Hum Gene Ther*. 14(12): 1181–91.

Johannsen, M., U. Gneveckow, L. Eckelt, et al. (2005). "Clinical hyperthermia of prostate cancer using magnetic nanoparticles: presentation of a new interstitial technique." *Int J Hyperthermia*. 21(7): 637–47.

Kakinuma, K., R. Tanaka, H. Takahashi, et al. (1996). "Drug delivery to the brain using thermosensitive liposome and local hyperthermia." *Int J Hyperthermia*. 12(1): 157–65.

Kawashita, M., K. Sadaoka, T. Kokubo, et al. (2006). "Enzymatic preparation of hollow magnetite microspheres for hyperthermic treatment of cancer." *J Mater Sci Mater Med*. 17(7): 605–10.

Kim, S. H., D. W. Kim, Y. H. Shim, et al. (2001). "In vivo evaluation of polymeric micellar paclitaxel formulation: toxicity and efficacy." *J Contr Release*. 72(1–3): 191–202.

Kim, S. H., J. H. Jeong, K. W. Chun, et al. (2005). "Target-specific cellular uptake of PLGA nanoparticles coated with poly(L-lysine)–poly(ethylene glycol)-folate conjugate." *Langmuir*. 21(19): 8852–7.

Kleiter, M. M., D. Yu, L. A. Mohammadian, et al. (2006). A tracer dose of technetium-99m-labeled liposomes can estimate the effect of hyperthermia on intratumoral doxil extravasation. *Clinical cancer research*. 12(22): 6800–7.

Kobayashi, H., S. Kawamoto, M. Bernardo, et al. (2006). "Delivery of gadolinium-labeled nanoparticles to the sentinel lymph node: Comparison of the sentinel node visualization and estimations of intra-nodal gadolinium concentration by the magnetic resonance imaging." *J Contr Release*. 111(3): 343–51.

Kohler, N., C. Sun, J. Wang, et al. (2005). "Methotrexate-modified superparamagnetic nanoparticles and their intracellular uptake into human cancer cells." *Langmuir*. 21: 8858–64.

Kommareddy, S. and M. Amiji (2007). "Antiangiogenic gene therapy with systemically administered sFlt-1 plasmid DNA in engineered gelatin-based nanovectors." *Cancer Gene Ther*. 14(5): 488–98.

Kong, G., R. D. Braun and M. W. Dewhirst (2000). "Hyperthermia enables tumor-specific nanoparticle delivery: effect of particle size." *Cancer Res*. 60(16): 4440–5.

Kong, G., R. D. Braun and M. W. Dewhirst (2001). "Characterization of the effect of hyperthermia on nanoparticle extravasation from tumor vasculature." *Cancer Res*. 61(7): 3027–32.

Koudinova, N. V., J. H. Pinthus, A. Brandis, et al. (2003). "Photodynamic therapy with Pd-Bacteriopheophorbide (TOOKAD): successful in vivo treatment of human prostatic small cell carcinoma xenografts." *Int J Cancer*. 104(6): 782–9.

Koziara, J. M., P. R. Lockman, D. D. Allen, et al. (2004). "Paclitaxel nanoparticles for the potential treatment of brain tumors." *J Contr Release*. 99(2): 259–69.

Kreuter, J. (1994). "Drug targeting with nanoparticles." *Eur J Drug Metab Pharmacokinet*. 19(3): 253–6.

Kreuter, J. (2001). "Nanoparticulate systems for brain delivery of drugs." *Adv Drug Deliv Rev*. 47(1): 65–81.

Kreuter, J. (2004). "Influence of the surface properties on nanoparticle-mediated transport of drugs to the brain." *J Nanosci Nanotechnol*. 4(5): 484–8.

Kreuter, J., R. N. Alyautdin, D. A. Kharkevich, et al. (1995). "Passage of peptides through the blood–brain barrier with colloidal polymer particles (nanoparticles)." *Brain Res*. 674(1): 171–4.

Kreuter, J., P. Ramge, V. Petrov, et al. (2003). "Direct evidence that polysorbate-80-coated poly(butylcyanoacrylate) nanoparticles deliver drugs to the CNS via specific mechanisms requiring prior binding of drug to the nanoparticles." *Pharm Res*. 20(3): 409–16.

Lammers, T., P. Peschke, R. Kuhnlein, et al. (2007). "Effect of radiotherapy and hyperthermia on the tumor accumulation of HPMA copolymer-based drug delivery systems." *J Contr Release*. 117(3): 333–41.

Lanza, G. M. and S. A. Wickline (2001). "Targeted ultrasonic contrast agents for molecular imaging and therapy." *Prog Cardiovasc Dis*. 44(1): 13–31.

Lanza, G. M. and S. A. Wickline (2003). "Targeted ultrasonic contrast agents for molecular imaging and therapy." *Curr Probl Cardiol*. 28(12): 625–53.

Lanza, G. M., D. R. Abendschein, X. Yu, et al. (2002). "Molecular imaging and targeted drug delivery with a novel, ligand-directed paramagnetic nanoparticle technology." *Acad Radiol*. 9 Suppl 2: S330–1.

Lanza, G. M., P. M. Winter, S. D. Caruthers, et al. (2004). "Magnetic resonance molecular imaging with nanoparticles." *J Nucl Cardiol*. 11(6): 733–43.

Larina, I. V., B. M. Evers, T. V. Ashitkov, et al. (2005). "Enhancement of drug delivery in tumors by using interaction of nanoparticles with ultrasound radiation." *Technol Cancer Res Treat*. 4(2): 217–26.

Li, J., X. Wang, C. Wang, et al. (2007). "The enhancement effect of gold nanoparticles in drug delivery and as biomarkers of drug-resistant cancer cells." *ChemMedChem*. 2(3): 374–8.

Liu, W., M. R. Dreher, D. Y. Furgeson, et al. (2006). "Tumor accumulation, degradation and pharmacokinetics of elastin-like polypeptides in nude mice." *J Contr Release*. 116(2): 170–8.

Lockman, P. R., M. O. Oyewumi, J. M. Koziara, et al. (2003). "Brain uptake of thiamine-coated nanoparticles." *J Contr Release*. 93(3): 271–82.

Loo, C., A. Lin, L. Hirsch, et al. (2004). "Nanoshell-enabled photonics-based imaging and therapy of cancer." *Technol Cancer Res Treat*. 3(1): 33–40.

Loo, C., A. Lowery, N. Halas, et al. (2005). "Immunotargeted nanoshells for integrated cancer imaging and therapy." *Nano Lett*. 5(4): 709–11.

Lukyanov, A. N., T. A. Elbayoumi, A. R. Chakilam and V. P. Torchilin (2004). Tumor-targeted liposomes: doxorubicin-loaded long-circulating liposomes modified with anti-cancer antibody. *J Control Release.* 100(1): 135–44.

Lum, A. F., M. A. Borden, P. A. Dayton, et al. (2006). "Ultrasound radiation force enables targeted deposition of model drug carriers loaded on microbubbles." *J Contr Release*. 111(1–2): 128–34.

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.

Maier-Hauff, K., R. Rothe, R. Scholz, et al. (2007). "Intracranial thermotherapy using magnetic nanoparticles combined with external beam radiotherapy: results of a feasibility study on patients with glioblastoma multiforme." *J Neurooncol*. 81(1): 53–60.

Marsh, J. N., M. S. Hughes, C. S. Hall, et al. (1998). "Frequency and concentration dependence of the backscatter coefficient of the ultrasound contrast agent Albunex (R)." *J Acoust Soc Am*. 104: 1654–66.

Marsh, J. N., C. S. Hall, M. J. Scott, et al. (2002a). "Improvements in the ultrasonic contrast of targeted perfluorocarbon nanoparticles using an acoustic transmission line model." *IEEE Trans Ultrason Ferroelectr Freq Contr*. 49(1): 29–38.

Marsh, J. N., C. S. Hall, S. A. Wickline, et al. (2002b). "Temperature dependence of acoustic impedance for specific fluorocarbon liquids." *J Acoust Soc Am*. 112(6): 2858–62.

Matsumura, Y. and H. Maeda (1986). "A new concept for macromolecular therapeutics in cancer chemotherapy: Mechanism of tumoritropic accumulation of proteins and the antitumor agent SMANCS." *Canc Res*. 46: 6387–92.

Matsuo, H., T. Okamura, J. Chen, et al. (2000). "Efficient introduction of macromolecules and oligonucleotides into brain capillary endothelial cells using HVJ-liposomes." *J Drug Target*. 8(4): 207–16.

Medintz, I. L., H. T. Uyeda, E. R. Goldman, et al. (2005). "Quantum dot bioconjugates for imaging, labelling and sensing." *Nat Mater*. 4(6): 435–46.

Meyer, D. E., B. C. Shin, G. A. Kong, et al. (2001). Drug targeting using thermally responsive polymers and local hyperthermia. *Journal of controlled release*. 74(1-3): 213–24

Morawski, A. M., P. M. Winter, K. C. Crowder, et al. (2004). "Targeted nanoparticles for quantitative imaging of sparse molecular epitopes with MRI." *Magn Reson Med*. 51(3): 480–6.

Morel, S., E. Terreno, E. Ugazio, et al. (1998). "NMR relaxometric investigations of solid lipid nanoparticles (SLN) containing gadolinium(III) complexes." *Eur J Pharm Biopharm*. 45(2): 157–63.

Mukherjee, P., R. Bhattacharya, P. Wang, et al. (2005). "Antiangiogenic properties of gold nanoparticles." *Clin Cancer Res*. 11(9): 3530–4.

Murray, C. B., D. J. Norris and M. G. Bawendi (1993). "Synthesis and characterization of nearly monodisperse CdE (E = sulfur, selenium, tellurium) semiconductor nanocrystallites." *J Am Chem Soc*. 115(19): 8706–15.

Nasongkla, N., E. Bey, J. Ren, et al. (2006). "Multifunctional polymeric micelles as cancer-targeted, MRI-ultrasensitive drug delivery systems." *Nano Lett*. 6(11): 2427–30.

National Cancer Institute (2004). Hyperthermia in cancer treatment: questions and answers (FS 7.3). Accessed on February 27, 2007 from http://www.cancer.gov/cancertopics/factsheet/Therapy/hyperthermia

National Cancer Institute (2005). "Hyperthermia in cancer treatment: questions and answers (FS 7.3)." http://www.cancer.gov/PDF/FactSheet/fs7_3.pdf (Accessed April 3, 2007).

Nielsen, U. B., D. B. Kirpotin, E. M. Pickering, et al. (2002). "Therapeutic efficacy of anti-ErbB2 immunoliposomes targeted by a phage antibody selected for cellular endocytosis." *Biochem Biophys Acta*. 1591(1–3): 109–18.

Ningaraj, N. S. (2006). Drug delivery to brain tumours: challenges and progress. *Expert opinion on drug delivery*. 3(4): 499–509.

Nsereko, S. and M. Amiji (2002). "Localized delivery of paclitaxel in solid tumors from biodegradable chitin microparticle formulations." *Biomaterials*. 23(13): 2723–31.

Ojeda, R., J. L. de Paz, A. G. Barrientos, et al. (2007). "Preparation of multifunctional glyconanoparticles as a platform for potential carbohydrate-based anticancer vaccines." *Carbohydr Res*. 342(3–4): 448–59.

Olbrich, C., A. Gessner, O. Kayser, et al. (2002). "Lipid–drug-conjugate (LDC) nanoparticles as novel carrier system for the hydrophilic antitrypanosomal drug diminazenediaceturate." *J Drug Target*. 10(5): 387–96.

Oldenburg, S. J., J. B. Jackson, S. L. Westcott, et al. (1999). "Infrared extinction properties of gold nanoshells." *Appl Phys Lett*. 75(19): 2897–9.

Olivier, J. C., L. Fenart, R. Chauvet, et al. (1999). "Indirect evidence that drug brain targeting using polysorbate 80-coated polybutylcyanoacrylate nanoparticles is related to toxicity." *Pharm Res*. 16(12): 1836–42.

Oupicky, D., K. A. Howard, C. Konak, et al. (2000). "Steric stabilization of poly-L-lysine/DNA complexes by the covalent attachment of semitelechelic poly(*N*-(2-hydroxypropyl)methacryla mide)." *Bioconjugate Chem*. 11(4): 492–501.

Oyewumi, M. O. and R. J. Mumper (2002). "Engineering tumor-targeted gadolinium hexanedione nanoparticles for potential application in neutron capture therapy." *Bioconjugate Chem*. 13(6): 1328–35.

Oyewumi, M. O., S. Liu, J. A. Moscow, et al. (2003). "Specific association of thiamine-coated gadolinium nanoparticles with human breast cancer cells expressing thiamine transporters." *Bioconjugate Chem*. 14(2): 404–11.

Oyewumi, M. O., R. A. Yokel, M. Jay, et al. (2004). "Comparison of cell uptake, biodistribution and tumor retention of folate-coated and PEG-coated gadolinium nanoparticles in tumor-bearing mice." *J Contr Release*. 95(3): 613–26.

Paciotti, G. F., L. Myer, D. Weinreich, et al. (2004). "Colloidal gold: a novel nanoparticle vector for tumor directed drug delivery." *Drug Deliv*. 11(3): 169–83.

Pamujula, S., R. A. Graves, T. Freeman, et al. (2004). Oral delivery of spray dried PLGA/amifostine nanoparticles. *The Journal of Pharmacy and Pharmacology*. 56(9): 1119–25.

Panyam, J. and V. Labhasetwar (2003). "Biodegradable nanoparticles for drug and gene delivery to cells and tissue." *Adv Drug Deliv Rev*. 55(3): 329–47.

Pedley, R. B., S. A. Hill, G. M. Boxer, et al. (2001). "Eradication of colorectal xenografts by combined radioimmunotherapy and combretastatin a-4 3-O-phosphate." *Cancer Res*. 61(12): 4716–22.

Peira, E., P. Marzola, V. Podio, et al. (2003). "In vitro and in vivo study of solid lipid nanoparticles loaded with superparamagnetic iron oxide." *J Drug Target*. 11(1): 19–24.

Pelz, J. O., J. Doerfer, W. Hohenberger, et al. (2005). "A new survival model for hyperthermic intraperitoneal chemotherapy (HIPEC) in tumor-bearing rats in the treatment of peritoneal carcinomatosis." *BMC Canc*. 5(1): 56.

Ponce, A. M., B. L. Viglianti, D. Yu, et al. (2007). Magnetic resonance imaging of temperature-sensitive liposome release: drug dose painting and antitumor effects. *Journal of the National Cancer Institute*. 99(1): 53–63.

Qing, S. H., L. Y. Li, X. H. Sheng, et al. (2006). "Photosensitizer nanoparticles photodynamic therapy on LOVO human colon cancer xenografts in athymic mice." *Zhonghua Wei Chang Wai Ke Za Zhi*. 9(6): 530–3.

Ramge, P., J. Kreuter and B. Lemmer (1999). "Circadian phase-dependent antinociceptive reaction in mice determined by the hot-plate test and the tail-flick test after intravenous injection of dalargin-loaded nanoparticles." *Chronobiol Int*. 16(6): 767–77.

Ranney, D., P. Antich, E. Dadey, et al. (2005). "Dermatan carriers for neovascular transport targeting, deep tumor penetration and improved therapy." *J Contr Release*. 109(1–3): 222–35.

Reddy, G. R., M. S. Bhojani, P. McConville, et al. (2006). "Vascular targeted nanoparticles for imaging and treatment of brain tumors." *Clin Cancer Res*. 12(22): 6677–86.

Reichardt, W., D. Hu-Lowe, D. Torres, et al. (2005). "Imaging of VEGF receptor kinase inhibitorinduced antiangiogenic effects in Drug-Resistant Human Adenocarcinoma Model." *Neoplasia*. 7: 847–53.

Ricci-Junior, E. and J. M. Marchetti (2006). "Preparation, characterization, photocytotoxicity assay of PLGA nanoparticles containing zinc (II) phthalocyanine for photodynamic therapy use." *J Microencapsul*. 23(5): 523–38.

Rychak, J. J., A. L. Klibanov and J. A. Hossack (2005). "Acoustic radiation force enhances targeted delivery of ultrasound contrast microbubbles: in vitro verification." *IEEE Trans Ultrason Ferroelectr Freq Contr*. 52(3): 421–33.

Sadeque, A. J., C. Wandel, H. He, et al. (2000). "Increased drug delivery to the brain by *p*-glycoprotein inhibition." *Clin Pharmacol Ther*. 68(3): 231–7.

Samia, A. C. S., S. Dayal and C. Burda (2006). Quantum Dot-based Energy Transfer: Perspectives and Potential for Applications in Photodynamic Therapy. *Photochemistry and Photobiology*. 82(3): 617–625.

Sauer, I., I. R. Dunay, K. Weisgraber, et al. (2005). "An apolipoprotein E-derived peptide mediates uptake of sterically stabilized liposomes into brain capillary endothelial cells." *Biochemistry*. 44(6): 2021–9.

Savolainen, J., J. E. Edwards, M. E. Morgan, et al. (2002). "Effects of a *p*-glycoprotein inhibitor on brain and plasma concentrations of anti-human immunodeficiency virus drugs administered in combination in rats." *Drug Metabol Dispos*. 30(5): 479–82.

Schiffelers, R. M., A. Ansari, J. Xu, et al. (2004). "Cancer siRNA therapy by tumor selective delivery with ligand-targeted sterically stabilized nanoparticle." *Nucleic Acids Res*. 32(19): e149.

Schmidt-Erfurth, U., T. Hasan, E. Gragoudas, et al. (1994). "Vascular targeting in photodynamic occlusion of subretinal vessels." *Ophthalmology*. 101(12): 1953–61.

Schmieder, A. H., P. M. Winter, S. D. Caruthers, et al. (2005). "Molecular MR imaging of melanoma angiogenesis with $\alpha_v\beta_3$-targeted paramagnetic nanoparticles." *Magn Reson Med*. 53(3): 621–7.

Schroeder, U. and B. A. Sabel (1996). "Nanoparticles, a drug carrier system to pass the blood–brain barrier, permit central analgesic effects of i.v. dalargin injections." *Brain Res*. 710(1–2): 121–4.

Schroeder, U., P. Sommerfeld and B. A. Sabel (1998). "Efficacy of oral dalargin-loaded nanoparticle delivery across the blood–brain barrier." *Peptides*. 19(4): 777–80.

Schwarze, S. R. and S. F. Dowdy (2000). "In vivo protein transduction: intracellular delivery of biologically active proteins, compounds and DNA." *Trends Pharmacol Sci*. 21(2): 45–8.

Sekhar, K. R., V. N. Sonar, V. Muthusamy, et al. (2007). Novel chemical enhancers of heat shock increase thermal radiosensitization through a mitotic catastrophe pathway. *Cancer research*. 67(2): 695–701.

Sengupta, S., D. Eavarone, I. Capila, et al. (2005). "Temporal targeting of tumour cells and neovasculature with a nanoscale delivery system." *Nature*. 436(7050): 568–72.

Shenoy, D. B. and M. M. Amiji (2005). Poly(ethylene oxide)-modified poly(epsilon-caprolactone) nanoparticles for targeted delivery of tamox ifen in breast cancer. *International journal of pharmaceutics*. 293(1-2):261-70.

Shenoy, D., S. Little, R. Langer and M. Amiji (2005). Poly(ethylene oxide)-modified poly(beta-amino ester) nanoparticles as a pH-sensitive system for tumor-targeted delivery of hydrophobic drugs: part 2. In vivo distribution and tumor localization studies. *Pharmaceutical research*. 22(12): 2107–14.

Shi, N., Y. Zhang, C. Zhu, et al. (2001). "Brain-specific expression of an exogenous gene after i.v. administration." *Proc Natl Acad Sci U S A*. 98(22): 12754–9.

Shikata, F., H. Tokumitsu, H. Ichikawa, et al. (2002). "In vitro cellular accumulation of gadolinium incorporated into chitosan nanoparticles designed for neutron-capture therapy of cancer." *Eur J Pharm Biopharm*. 53(1): 57–63.

Shortencarier, M. J., P. A. Dayton, S. H. Bloch, et al. (2004). "A method for radiation-force localized drug delivery using gas-filled lipospheres." *IEEE Trans Ultrason Ferroelectr Freq Contr*. 51(7): 822–31.

Siddiqui, F., C. Y. Li, S. M. Larue, et al. (2007). "A phase I trial of hyperthermia-induced interleukin-12 gene therapy in spontaneously arising feline soft tissue sarcomas." *Mol Cancer Ther*. 6(1): 380–9.

Simberg, D., T. Duza, J. H. Park, et al. (2007). "Biomimetic amplification of nanoparticle homing to tumors." *PNAS*. 104(3): 932–6.

Soma, E. C., C. Dubernet, D. Bentolila, et al. (2000). "Reversion of multidrug resistance by co-encapsulation of doxorubicin and cyclosporin A in polyalkylcyanoacrylate nanoparticles." *Biomaterials*. 21(1): 1–7.

Somogyi, G., L. Prokai and N. Bodor (1998). "Targeted drug delivery to the brain via phosphonate derivatives II. Anionic chemical delivery system for zidovudine (AZT)." *Int J Pharm*. 166: 27–35.

Stewart, F., P. Baas and W. Star (1998). "What does photodynamic therapy have to offer radiation oncologists (or their cancer patients)?" *Radiother Oncol*. 48(3): 233–48.

Storm G., T. Daemen and D. D. Lasic (1995). "Surface modification of nanoparticles to oppose uptake by the mononuclear phagocyte system." *Adv Drug Deliv Rev*. 17: 31–48.

Sukhanova, A., J. Devy, L. Venteo, et al. (2004). "Biocompatible fluorescent nanocrystals for immunolabeling of membrane proteins and cells." *Anal Biochem*. 324(1): 60–7.

Sun, C., R. Sze and M. Zhang (2006). "Folic acid-PEG conjugated superparamagnetic nanoparticles for targeted cellular uptake and detection by MRI." *J Biomed Mater Res* 78(3): 550–7.

Szymanski-Exner, A., N. T. Stowe, R. S. Lazebnik, et al. (2002). "Noninvasive monitoring of local drug release in a rabbit radiofrequency (RF) ablation model using X-ray computed tomography." *J Contr Release*. 83(3): 415–25.

Taylor, E. M. (2002). "The impact of efflux transporters in the brain on the development of drugs for CNS disorders." *Clin Pharmacokinet*. 41(2): 81–92.

Thomas, H. and H. M. Coley (2003). Overcoming multidrug resistance in cancer: an update on the clinical strategy of inhibiting p-glycoprotein. *Cancer control*. 10(2): 159–65.

Tokes, Z. A., A. K. St Peteri and J. A. Todd (1980). "Availability of liposome content to the nervous system. Liposomes and the blood–brain barrier." *Brain Res*. 188(1): 282–6.

Tokumitsu, H., J. Hiratsuka, Y. Sakurai, T. Kobayashi, H. Ichikawa and Y. Fukumori (2000). "Gadolinium neutron-capture therapy using novel gadopentetic acid-chitosan complex nanoparticles: in vivo growth suppression of experimental melanoma solid tumor." *Canc Lett*. 150(2): 177–82.

Torchilin, V. P. (1996). "How do polymers prolong circulation time of liposomes?" *J Liposome Res*. 6: 99–116.

Torchilin, V. P., T. S. Levchenko, A. N. Lukyanov, et al. (2001). p-Nitrophenylcarbonyl-PEG-PE-liposomes: fast and simple attachment of specific ligands, including monoclonal antibodies, to distal ends of PEG chains via p-nitrophenylcarbonyl groups. *Biochim Biophys Acta.* 1511(2): 397–411.

Torchilin, V. P. (2002). "TAT peptide-modified liposomes for intracellular delivery of drugs and DNA." *Cell Mol Biol Lett*. 7(2): 265–7.

Torchilin, V. P. and T. S. Levchenko (2003). "TAT-liposomes: a novel intracellular drug carrier." *Curr Protein Pept Sci*. 4(2): 133–40.

Tran, J., Z. Master, J. L. Yu, et al. (2002). "A role for survivin in chemoresistance of endothelial cells mediated by VEGF." *PNAS*. 99: 4349–54.

Tusji, A., Ed. (2000). The blood–brain barrier and drug delivery to the CNS. New York, Marcel Dekker.

Uehara, M., T. Inokuchi, K. Sano, et al. (1998). "The anti-tumor effect of photodynamic therapy evaluated by bromodeoxyuridine immunohistochemistry." *Int J Oral Maxillofac Surg*. 27(3): 204–8.

U.S. Food and Drug Administration, "Center for Drug Evaluation and Research." http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm (Accessed April 25, 2006).

U.S. National Institute of Health, "Cancer Statistics" http://www.cancer.gov/statistics/ (Accessed September 15, 2006).

van der Zee, J. (2002). Heating the patient: a promising approach? *Ann Oncol*. 13:1173–84.

van Vlerken, L. E. and M. M. Amiji (2006). Multi-functional polymeric nanoparticles for tumour-targeted drug delivery. *Expert opinion on drug delivery*. 3(2): 205–16.

van Vlerken, L. E., Z. Duan, M. V. Seiden, et al. (2007). "Modulation of intracellular ceramide using polymeric nanoparticles to overcome multidrug resistance in cancer." *Canc Res*. 67(10): 4843–50.

Veenhuizen, R., H. Oppelaar, M. Ruevekamp, et al. (1997). "Does tumour uptake of Foscan determine PDT efficacy?" *Int J Cancer*. 73(2): 236–9.

Vinogradov, S. V. (2006). Colloidal microgels in drug delivery applications. *Current Pharmaceutical Design.* 2006;12(36): 4703–12.

Visaria, R. K., R. J. Griffin, B. W. Williams (2006). Enhancement of tumor thermal therapy using gold nanoparticle-assisted tumor necrosis factor-alpha delivery. *Molecular cancer therapeutics*. 5(4): 1014–20.

Voura, E. B., J. K. Jaiswal, H. Mattoussi, et al. (2004). "Tracking metastatic tumor cell extravasation with quantum dot nanocrystals and fluorescence emission-scanning microscopy." *Nat Med*. 10(9): 993–8.

Wachsberger, P., R. Burd and A. P. Dicker (2003). "Tumor response to ionizing radiation combined with antiangiogenesis or vascular targeting agents: exploring mechanisms of interaction." *Clin Cancer Res*. 9(6): 1957–71.

Wani, M. C., H. L. Taylor, M. E. Wall, et al. (1971). "Plant antitumor agents VI. The isolation and structure of Taxol, a novel antitumor and anitleukemic agent from *Taxus brevifolia*." *J Am Chem Soc*. 18(3): 242–60.

Weinberg, B. D., E. Blanco, S. F. Lempka, et al. (2007). "Combined radiofrequency ablation and doxorubicin-eluting polymer implants for liver cancer treatment." *J Biomed Mater Res A*. 81(1): 205–13.

Weissig, V. 2005. Targeted drug delivery to mammalian mitochondria in living cells. *Expert Opin Drug Deliv.* 2(1): 89–102.

Weissig, V., S. V. Boddapati, S. M. Cheng and G. G. D'Souza (2006). Liposomes and liposome-like vesicles for drug and DNA delivery to mitochondria. *J Liposome Res.* 16(3): 249–64.

Wickline, S. A. and G. M. Lanza (2003). "Nanotechnology for molecular imaging and targeted therapy." *Circulation*. 107(8): 1092–5.

Winter, P. M., S. D. Caruthers, A. Kassner, et al. (2003a). "Molecular imaging of angiogenesis in nascent Vx-2 rabbit tumors using a novel $\alpha_v\beta_3$-targeted nanoparticle and 1.5 tesla magnetic resonance imaging." *Canc Res*. 63(18): 5838–43.

Winter, P. M., A. M. Morawski, S. D. Caruthers, et al. (2003b). "Molecular imaging of angiogenesis in early-stage atherosclerosis with $\alpha_v\beta_3$-integrin-targeted nanoparticles." *Circulation*. 108(18): 2270–4.

Wong, H. L., R. Bendayan, A. M. Rauth, et al. (2006). "Simultaneous delivery of doxorubicin and GG918 (Elacridar) by new polymer-lipid hybrid nanoparticles (PLN) for enhanced treatment of multidrug-resistant breast cancer." *J Contr Release*. 116(3): 275–84.

Wood, B. J., J. K. Locklin, A. Viswanathan, et al. (2007). Technologies for guidance of radiofrequency ablation in the multimodality interventional suite of the future. *Journal of vascular and interventional radiology*. 18(1 Pt 1): 9–24.

Wu, X., H. Liu, J. Liu, et al. (2003). "Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots." *Nat Biotechnol*. 21(1): 41–6.

Wust, P., B. Hildebrandt, G. Sreenivasa, et al. (2002). "Hyperthermia in combined treatment of cancer." *Lancet Oncol*. 3(8): 487–97.

Wust, P., U. Gneveckow, M. Johannsen, et al. (2006). "Magnetic nanoparticles for interstitial thermotherapy—feasibility, tolerance and achieved temperatures." *Int J Hyperther*. 22(8): 673–85.

Yang, S. C., L. F. Lu, Y. Cai, et al. (1999a). "Body distribution in mice of intravenously injected camptothecin solid lipid nanoparticles and targeting effect on brain." *J Contr Release*. 59(3): 299–307.

Yang, S., J. Zhu, Y. Lu, et al. (1999b). "Body distribution of camptothecin solid lipid nanoparticles after oral administration." *Pharmaceut Res*. 16(5): 751–7.

Zara, G. P., R. Cavalli, A. Bargoni, et al. (2002a). "Intravenous administration to rabbits of non-stealth and stealth doxorubicin-loaded solid lipid nanoparticles at increasing concentrations of stealth agent: pharmacokinetics and distribution of doxorubicin in brain and other tissues." *J Drug Target*. 10(4): 327–35.

Zara, G. P., A. Bargoni, R. Cavalli, et al. (2002b). "Pharmacokinetics and tissue distribution of idarubicin-loaded solid lipid nanoparticles after duodenal administration to rats." *J Pharmaceut Sci*. 91(5): 1324–33.

Zhang, X., J. Xie, S. Li, et al. (2003). "The study on brain targeting of the amphotericin B liposomes." *J Drug Target*. 11(2): 117–22.

Zhao, M., D. A. Beauregard, L. Loizou, et al. (2001). "Non-invasive detection of apoptosis using magnetic resonance imaging and a targeted contrast agent." *Natl Med*. 1: 1241–1244.

Zhao, S., M. Borden, S. H. Bloch, et al. (2004). "Radiation-force assisted targeting facilitates ultrasonic molecular imaging." *Mol Imag*. 3(3): 135–48.

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