ACCOUNTS
of chemical research

Molecular Imaging with Theranostic Nanoparticles

JESSE V. JOKERST[†] AND SANJIV S. GAMBHIR[*, †, ‡]

[†]*Molecular Imaging Program at Stanford (MIPS), Department of Radiology,
318 Campus Drive, Stanford University, Stanford, California 94305-5427,
United States, and* [‡]*Bioengineering, Materials Science & Engineering, Bio-X,
Stanford University, Stanford, California 94305, United States*

RECEIVED ON APRIL 4, 2011

CONSPECTUS

Nanoparticles (NPs) offer diagnostic and therapeutic capabilities not available with small molecules or microscale tools. As the field of molecular imaging has emerged from the blending of molecular biology with medical imaging, NP imaging is increasingly common for both therapeutic and diagnostic applications. The term theranostic describes technology with concurrent and complementary diagnostic and therapeutic capabilities. Although NPs have been FDA-approved for clinical use as transport vehicles for nearly 15 years, full translation of their theranostic potential is incomplete. However, NPs have shown remarkable success in the areas of drug delivery and magnetic resonance imaging. Emerging applications include image-guided resection, optical/photoacoustic imaging in vivo, contrast-enhanced ultrasound, and thermoablative therapy.

Diagnosis with NPs in molecular imaging involves the correlation of the signal with a phenotype. The location and intensity of NP signals emanating from a living subject indicate the disease area's size, stage, and biochemical signature. Therapy with NPs uses the image for resection or delivery of a small molecule or RNA therapeutic. Ablation of the affected area is also possible via heat or radioactivity.

The ideal theranostic NP includes several features: (1) it selectively and rapidly accumulates in diseased tissue; (2) it reports biochemical and morphological characteristics of the area; (3) it delivers an effective therapeutic; and (4) it is safe and biodegrades with nontoxic byproducts. Such a system contains a central imaging core surrounded by small molecule therapeutics. The system targets via ligands such as IgG and is protected from immune scavengers by a cloak of protective polymer. Although no NP has achieved all of the above criteria, many NPs possess one or more of these features. While the most clinically translatable NPs have been used in the field of magnetic resonance imaging, other types in development are quickly becoming more biocompatible through methods that modify their toxicity and biodistribution profiles. In this Account, we describe diagnostic imaging and therapeutic uses of NPs. We propose and offer examples of five primary types of nanoparticles with concurrent diagnostic and therapeutic uses.
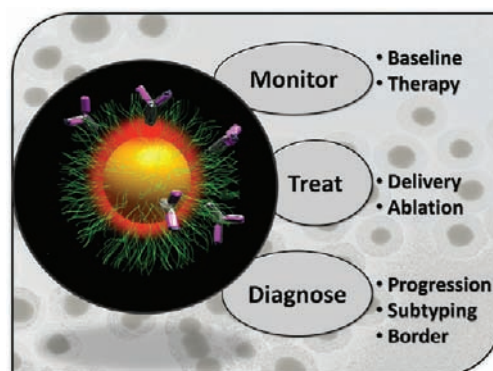
Introduction

Molecular imaging (MI) monitors and measures biological processes in living subjects via spectral data. Traditional modalities such as X-ray, computed tomography (CT), and magnetic resonance imaging (MRI) produce an image of *anatomy*. MI modalities such as positron emission tomography (PET), single photon emission CT (SPECT), optical techniques, and contrast-enhanced CT or MRI produce an image with details on *function*. Molecular imaging monitors and measures biological processes similar to a biopsy but is done noninvasively, in real time, and with potential for sequential, longitudinal monitoring. Applications include early detection of disease, staging of disease, evaluating the response to therapy, and studying biological processes in living subjects.[1,2]

Nanoparticles (NPs) are an emerging instrument in the tool kit of MI because of their intense and stable output, large payload delivery, multimodal signaling capacity,

TABLE 1. NP Applications to Diagnosis and Therapy[a]

Technique	Limitation	NP Solution	NP Type(s)
CT	Sensitivity	Contrast	Gold, Silver, Iodine NPs
Optical	Signal Penetration Poor multiplexing	Intense Signal Fingerprint Spectra	Quantum Dots Raman nanoparticles
MRI	Anatomic Technique	Contrast	Iron Oxide, Gd^{3+}/Silica
PET/SPECT	Spatial Resolution	Multimodal	Radiolabeled NP
Ultrasound	Anatomic Technique	Contrast	Silica, Nanobubble
Resection	Tumor Location	Border Delineation	MRI and/or Fluorescent
Radiation Therapy	Site-specific Delivery	Radio-NPs	Sir-Spheres (microparticles)
Chemotherapy	Delivery/Stability/Toxicity	Drug carriers	Liposome, Micelles
Ablation	Low Efficiency	NP Enhancer	Nanoshell, Nanorod, CuS NPs
RNAi	Delivery	siRNA carrier	Liposome, Polymeric NPs
In vitro Diagnostics	Detection Limit Multiplexing	Intense Signal Spectral Resolution	Quantum Dots Raman/Magnetic NPs

[a]Mechanisms of diagnosis via imaging (pink) and treatment of disease (blue) are aided by nanoparticles. NPs are also critical to next-generation in vitro diagnostics (IVD; brown).

strong target binding via multiple ligands, and tunable biodistribution profiles (Table 1). These synthetic materials (with dimensions from one to hundreds of nanometers) have a long history in drug delivery and are increasingly popular in imaging because of the unique way in which they interact with light, sound, and electromagnetic fields.[3,4] Nanoparticles are particularly intriguing when used for combination applications that are both therapeutic and diagnostic. Such systems have recently been described as *theranostic*.[5] Theranostic nanomedicine is an emerging field that uses nanometer-scale materials to glean diagnostic insight for well-informed treatment. The fundamental advantage of theranostic nanomedicine is the use of patient-specific test results to tailor a treatment regimen producing improved outcomes, reduced costs, and fewer side effects.

While the definition of thernostics continues to evolve, we suggest the following: A *diagnostic* improves the *knowledge* of a disease state. Diagnostics may be performed in vivo or ex vivo and offer information about a disease's metabolic/biochemical state, genotype, size, location(s), morphology, chemical composition, rate of change, and so forth. A *therapeutic* improves the *outcome* of a disease state. Small molecules, proteins, RNA interference, gamma ablation, and surgical explanation are all examples of therapeutics. In the following Account, we recap the state-of-the-art in theranostic NPs and define such systems as synthetic, nanometer-scale materials that simultaneously improve the knowledge and outcome of aberrant biology. Although we focus on cancer biology and in vivo applications, theranostic nanoparticles also have applications in diabetes and in regenerative medicine.[6] Complementary to imaging, theranostic in vitro diagnostic (IVD) approaches also utilize
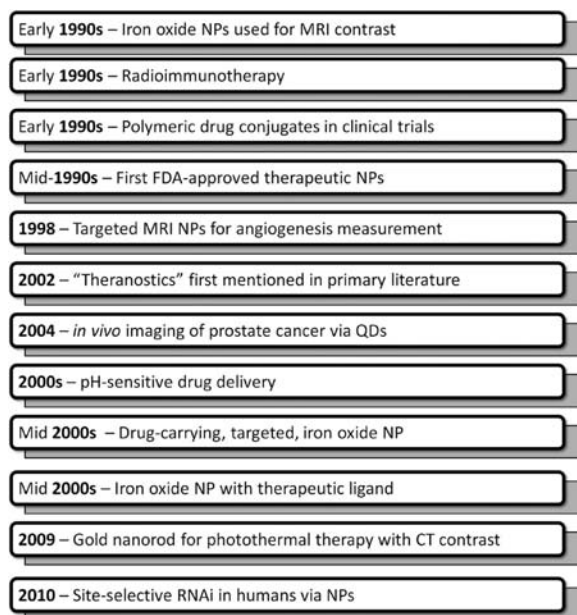

FIGURE 1. Evolution of theranostic NPs. Initial NPs were active in either a therapeutic (delivery) or a diagnostic (imaging) mode. As homing capabilities and multimodal approaches advanced, systems capable of simultaneous therapy and molecular imaging (theranostic) were realized.

nanoparticles and use an ex vivo test result to guide treatment. Helpful discussions of IVD theranostics exist elsewhere.[7]

Nanoparticles

Limitations of some MI techniques include poor multiplexing capabilities, poor spatial resolution, low sensitivity, and poor signal penetration through tissues.[2] For these reasons, MI researchers have increasingly turned to NPs because of their large payloads, high signal intensity/stability, avidity, and the capacity for multiple, simultaneous applications due

NP Type	Description	Therapeutic Role	Diagnostic Role	Examples
I	NP with endogenous contrast	• Targeting ligand occupies cell pathway site • Image-guided resection	• Imaging with site specificity • Border delineation • Phenotyping	• Iron Oxide NP • QD
II	NP carrying imaging agent	• Image-guided resection	• Border delineation • Typing with site specificity	• Radio-labeled silica • Fluorescent silica
III	NP carrying therapeutic agent	• Therapeutic release • Radio-ablation	• Typing with site specificity	• pH-responsive liposomes • RNAi NPs
IV	Labeled NP carrying therapeutic agent	• Therapeutic release	• Imaging with site specificity and fluorphore	• Fluorescent liposomes • See Table 2
V	NP responsive to external stimulus	• Photothermal therapy • Selective drug release	• Thermal imaging • Photoacoustic imaging • CT	• Gold nanorod • Magnetic NPs

FIGURE 2. Types of NPs. The therapeutic (indicated by red circles) and diagnostic (imaging agent indicated by green flash) roles of nanoparticles (gray scaffold) combine in different fashions. Types I and II are self-contained NPs, while types III and IV involve release of an agent. These NPs may target the disease area nonspecifically or specifically via homing ligands (indicated by purple wedges). Type V NPs may include various elements of the following four types; however, they are only activated in the presence of an external stimulus.

to their unique size and the high surface area to volume ratio.[8] NPs are bigger than proteins, yet smaller than cells, and thus behave differently in vivo than other therapies and imaging agents. While both therapeutic nanostructures and imaging NPs have a long history, they have only recently begun to coalesce into the theranostic NPs detailed below (Figure 1). There are currently more than 35 FDA-approved NPs, with a larger number in preclinical studies for both imaging and therapy.[3,9–13] Most FDA-approved NPs are used as mechanisms of drug delivery with the exception of MRI contrast agents.

Diagnostic Capabilities

The diagnostic role of theranostic NPs reports the presence (location(s)) of disease, the status of disease (subtype), or a disease's response to treatment (Figure 2). Through either passive or active targeting, increased binding at the site of interest is achieved. The location and intensity of NP signal after systemic (intravenous) injection correlates to cell surface receptors or molecular phenotype and measures the tumor's size, border, stage, and so forth. NP signaling utilizes radionuclides or fluorophores, or the NP itself may have an intrinsic property for contrast (e.g., iron oxide for MRI.) NPs are often coated with ligands that target angiogenesis markers. For example, the RGD peptide binds to $\alpha_v\beta_3$ integrin and vascular epidermal growth factor (VEGF) binds to the VEGF receptor (VEGF-R). Both identify angiogeneic tumor. Once bound, the NP can help guide resection or monitor response to therapy. Alternatively, passive targeting can be used for anatomic imaging rather than MI.

Diagnostic NPs include superparamagnetic iron oxide (SPIO) and ultrasmall SPIO (USPIO) for MRI contrast and targeted SPIO NPs, which allow MI via MRI.[14] Gold NPs are used for CT and radiograph contrast and MI via ligands.[15] Silica nanoparticles have applications in MRI as gadolinium containers or to protect inner imaging cores.[16] Optical reporters include quantum dots (QDs), fluorophore-doped silica NPs, and fluorophore-doped polymeric NPs.[17] Carbon nanotubes and gold nanorods produce photoacoustic contrast.[18] Surface-enhanced Raman scattering (SERS) NPs are used for multiplexed approaches.[19] Some NPs are multimodal; that is, they report signal through more than one method (e.g., fluorescence and MRI) and are useful when the two modalities have complementary spatial resolution, sensitivity, or depth penetration.[20]

Therapeutic Capabilities

The therapeutic role of the NP can take several forms as well (Figure 2). It may be a material *delivered or released* to the diseased area. Traditionally, this has been small molecule chemotherapeutics such as doxorubicin, paclitaxel, and so

forth. Next-generation systems deliver or miRNA for RNA interference with gene expression.[13,21−23] Mirkin's group has demonstrated that gold NPs not only facilitate cellular delivery of oligonucelotides but also stabilize them from nucleases.[24] The release may be the ablative effect of radionuclides loaded into NPs for destruction of the tumor, causing DNA damage and retarding cell growth.[25] Finally, the release may be in the form of heat or vibrational energy that disrupts the structure of the cells and shrinks the tumor volume. Second, the therapeutic role may be to *guide surgery* for tumor resection. NPs that contain an imaging agent can thus be both diagnostic (determine tumor type, location, and borders) and therapeutic (use that image to guide tumor removal). Intraoperative imaging is visualization of diseased areas exposed during surgery and is especially important as the location of the tumor may change after presurgical imaging and during resection. Finally, the therapeutic role may be in *disrupting a cellular or metabolic pathway*. This approach utilizes a ligand to target the NP and disrupt cell regulation. An example of this is Herceptin-labeled NPs, which occupy the Her-2 cell surface receptors.[26]

In delivery applications, the NP carrier stabilizes the payload (up to 10^5 copies per nanoparticle),[27] allowing a metered release of drug, reducing toxicity and side effects. Hydrophobic drugs are protected by the NP interior.[28] Most therapeutic drug-carrying NPs are in the form of liposomes or lipid-based complexes, as well as polymeric micelles or biodegradable polymer/drug composites.[29] The most common substrate is a blend of poly(lactic-*co*-glycolic acid) (PLGA) and polyethylene glycol (PEG), (PLGA-PEG). Metallic NPs used in tandem with infrared heating are thermoablative NPs; nanoshells and nanorods are the most common examples.[30] Sirspheres are the trademarked name of yttrium-90 loaded nanoparticles used to treat liver cancers. These particles are injected into the hepatic artery and accumulate in the tumor where they ablate the tumor in vivo.[25]

Nanoparticle Types

In Figure 2, five common approaches to designing theranostic NPs are detailed. All have one or more of the above therapeutic and diagnostic roles. While there are obviously many variations on this theme and this list is not exclusive, many NPs do fall into one of these categories. Type I and II NPs are used in tandem with surgery for resection/removal of the tumor/lesion. They either have an intrinsic (type I) signaling nature such as MRI or are loaded with a reporter (type II) like a fluorophore. The diagnostic role is due to the

site-specific accumulation of the NP, caused by the NP's size/ shape, sensitivity to local environment, or targeting ligand. Thus, imaging is used to radically treat the tumor and the location of the NP causes both the diagnosis (tumor location/border/type) and therapy (surgical resection).

Type I NPs include dextran-stabilized iron oxide (MRI), gold nanoshells (photoacoustic imaging), nanobubbles (US), and PEG-coated quantum dots.[31] Type II NPs include dye-loaded silica, radiolabeled silica, and multimodal NPs. Resection using type I or II NPs is one of the best applications of multimodal NPs because the advantages of each modality can be used at different points in the procedure. The mapping of a tumor's sentinel lymph nodes is a key application of these NPs. Limitations to type I and II NPs include nonspecific accumulation, inadequate circulation times/biodistribution, poor biodegradation profiles, and toxicity.[32]

Type III and IV NPs are less invasive (no surgical intervention) and more sophisticated as they carry an agent to the site of interest. Site-specific accumulation allows molecular imaging of the tumor or specific delivery of therapeutic. Drug-carrying liposomes, micelles, and other NPs are type III. Examples include Doxil which is PEGylated, liposomal doxorubicin and Abraxane which is a NP carrier of paclitaxel.[9] More than one type of therapeutic can be loaded per NP. The diagnostic mechanism of type III NPs is their specific accumulation in diseased area, which is an in vivo evaluation of tissue state. Type IV NPs have the additional diagnostic capacity for imaging via a loaded or intrinsic reporter with simultaneous release of a treatment mechanism to the site of interest. One challenge is balancing the limited NP exterior or volume, especially for surface-functionalized NPs. A high amount of therapeutics may reduce the number of bound ligands and vice versa. Still, simultaneous imaging and delivery are illustrated in Table 2 and demonstrate the broad range of deliverable products and targeting ligands.[33,34]

Type V NPs are responsive to external stimulus; NPs with magnetic or thermoablative capacities are of this class. In thermoablative therapy, nanoshells and nanorods are effective because they have absorption peaks in the near-infrared (IR; IR light passes more easily through tissue) and are effective at converting light into heat energy. Bhatia and co-workers reported nanorod accumulation in tumor followed by laser treatment with heating to nearly 70 °C in vivo (Figure 3).[35]

Targeting Ligands

Theranostic NPs demonstrate specific accumulation at the site of interest, which can occur through either active or

TABLE 2. Examples of Type III–V Thernostic NPs[a]

	NP Type	NP Size (nm)	Therapeutic Agent	Diagnostic Agent	Disease State	Target	Reference
Pre-clinical	Iron Oxide	10	Anti-EGFR IgG	Iron Oxide	Brain CA	EGFR	Mao et al. [31]
	Silica	100 – 200	Paclitaxel	Iron Oxide	Many	Folic Acid	Zink et al. [34]
	Liposome	100 – 200	Paclitaxel	pH-responsive membrane	Ovarian CA/ Many Types	EPR	Langer et al. [53]
	Gold Nanorod	10 x 40	Heat	Thermal/CT	Many	EPR	Bhatia et al. [35]
	QD	30	Doxorubicin	QD	Prostate CA	PSMA	Farokhzad et al. [45]
Clinical Trials	Cyclodextrin	70	RNAi	Transferrin	Melanoma	Transferrin Receptor	Calando Pharma. (NCT00689065)
	Gold Nanoshell (Aurolase)	150	Nanoshell (Photothermal)	Nanoshell (MR and optical)	Head and Neck CA	EPR	NanoSpectra (NCT00848042)
	Silica	3-10	cRGD	^{124}I	Melanoma	$\alpha_v\beta_3$ Integrin	MSKCC (NCT01266096)
	Iron Oxide	120 – 180	Injected Cell	Iron Oxide (Endorem)	Healthy Volunteers	None	Univ. of Edinburgh (NCT00972946)
	Gold	27	Tumor Necrosis Factor	Gold NP Size	Solid Tumors	EPR	NCI/Cytimmune (NCT00356980)

[a]Both active and passively targeted NPs are currently in clinical and pre-clinical work. For clinically approved NPs, see Zweck.[3] (EGFR = epidermal growth factor receptor; PSMA = prostate specific membrane antigen; MSKCC = Memorial Sloan Kettering Cancer Center).

passive targeting. Passive targeting takes advantage of the enhanced extravasation, permeation, and retention (EPR) effect in which NPs escape from a tumor's leaky vasculature and accumulate nonspecifically in the lesion. Passive targeting is dependent on the size, shape, and charge of the NP. A key challenge is reducing nonspecific binding. NPs larger than 100 nm, with surface charges (zeta potential $\pm > -20$ mV), or with solid cores are often rapidly (<15 min) cleared from circulation by the liver and spleen before accumulating at the tumor. Drug-containing liposomes are a classic example of NPs (Type III) that target nonspecifically.[9]

Alternatively, actively targeted NPs anneal a recognition element to the NP to bind to cell surface markers for even greater accumulation in the diseased tissue. While passive targeting can increase accumulation, only NPs with ligands that specifically bind to a receptor indicative of a metabolic process are true MI NPs. Ligand types include antibodies, small peptides or molecules, lectins, aptamers, engineered proteins, and protein fragments. Commonly targeted tissue biomarkers include vascular markers and markers of angiogenesis. Folate receptor is overexpressed in many cancer types, and folate-labeled NPs are common.[36] Some NPs are therapeutic by using the monoclonal antibody to occupy a cell surface marker involved in signal transduction preventing tumor growth. In general, expression levels $2-10$ times higher at target than the nontargeted area is sufficient.[37]

A key advantage of using NPs versus molecular scaffolds is the multiple copies of ligand that can be loaded onto the NP. Small NPs such as QDs may have tens of ligands, while larger liposomal NPs may have thousands. This multivalent mode of attachment between the NP and disease area is known as avidity. In addition to the *number* of ligands per NP, the many different *types* of ligands attached to a NP is very advantageous. Because cancer cells often rapidly develop resistance to one type of ligand, combination approaches that target/inhibit through more than one mechanism can be especially useful.[38]

Magnetic NPs. Particles enhancing MRI signal are one of the most established Type I NPs, and iron oxide (stabilized with dextran, PEG, oleic or pluoronic acid) is routine and clinically approved under many brand names including Resovist, Feridex, Ferumoxtran-10, or Gastromark. The main benefits of these NPs are the depth insensitive nature of MRI detection, low toxicity, and hours of circulation time. SPIO and USPIO decrease T1 and T2 relaxation times in a dose-dependent manner. They are used as contrast for lymphography and angiography, bone marrow contrast, or as a perfusion agent of the brain and kidney. Iron oxide particles can be targeted through the addition of a therapeutic ligand such as Herceptin (tratuszamab).[26] There are approximately 20 current clinical trials of SPIO or USPIO.[39] Approved clinical applications of SPIO include imaging of liver metastasis.
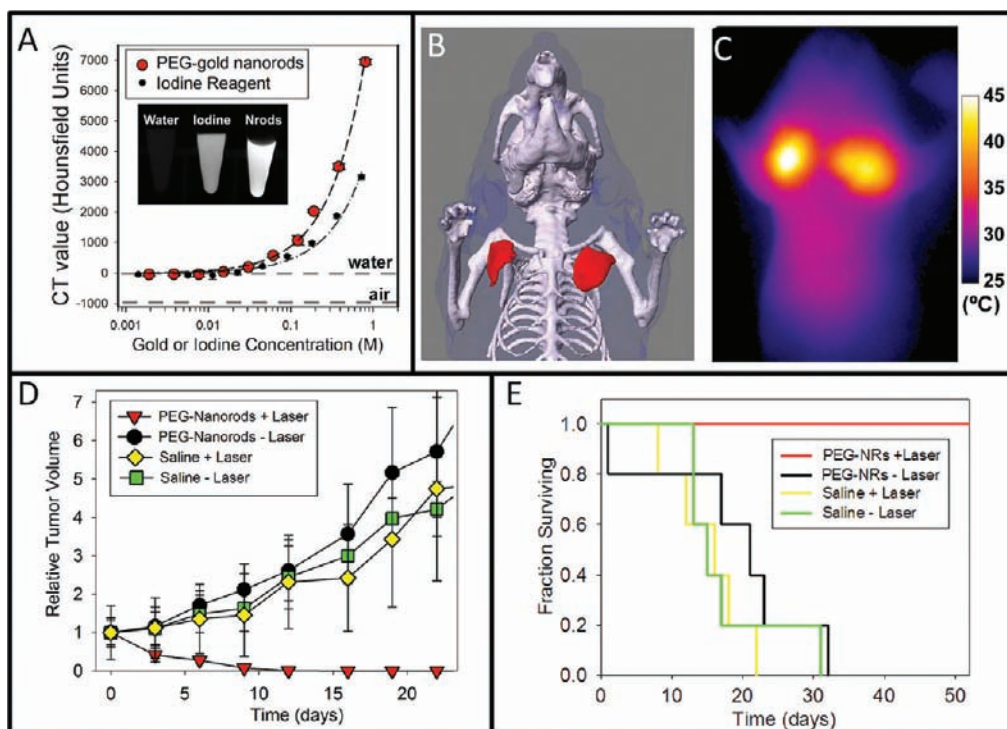
FIGURE 3. Gold nanorods. Nanorods have both imaging and therapeutic capabilities as illustrated in a murine model of breast cancer. (A) Gold absorbs X-rays during CT more strongly than iodine. (B) Nanorods can be used to create a CT map of the tumor. When irradiated with NIR light, the nanorods increase in temperature, which can be mapped via thermal imaging (C). The heating causes tumor death and shrinkage of the tumor (D), resulting in increased survival of treated animals (E). Adapted and reprinted by permission from the American Association for Cancer Research: ref 35.
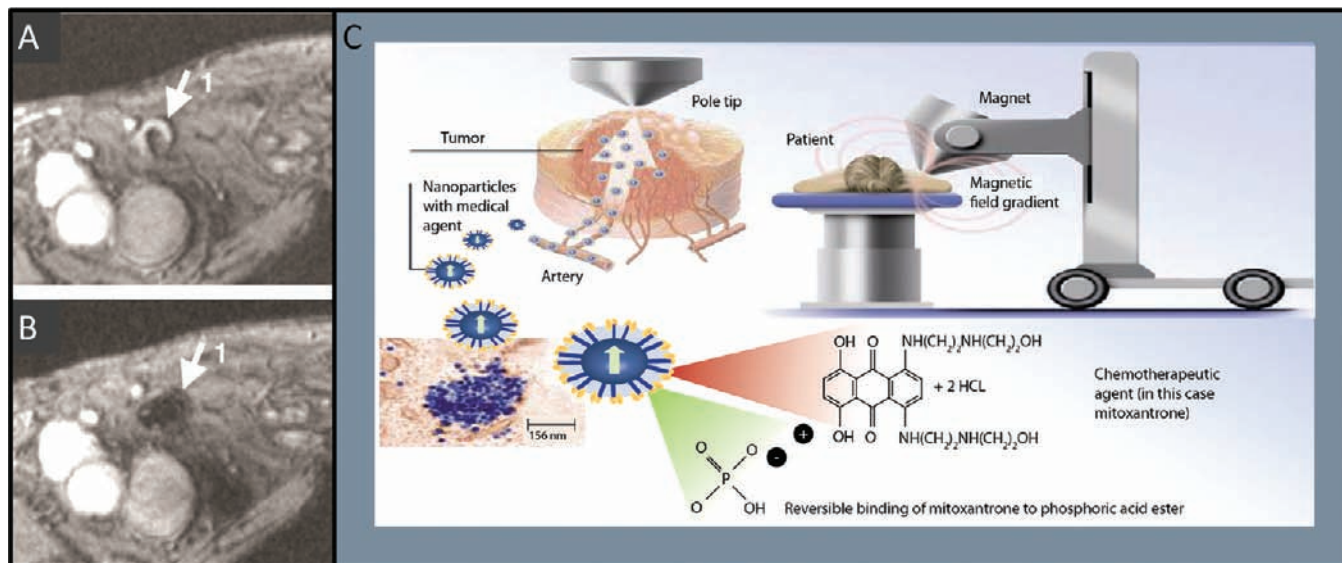

FIGURE 4. Tracking injected cells via magnetic NPs. Human lymph nodes before (A) and after (B) intranodal injection of iron oxide-labeled cells. Cells could be tracked for 2 days after injection as they traveled through lymphatic system. (C) Magnetic NPs can also be loaded with small molecule therapeutics and immobilized at the disease site via external magnetic field to increase dose. Adapted and reprinted with permission from refs 41 and 42. Copyright 2005 and 2009 Future Science Group and Nature Publishing Group.

Theranostic applications of SPIO include labeling cells with iron oxide NPs to be tracked in vivo with potential applications in stem cell monitoring (Figure 4).[40] Figdor and co-workers have shown that cells labeled with magnetic nanoparticles can be monitored and tracked in human patients.[41] Loading of SPIO with NPs was done by coculturing immature cells with 200 mg/mL SPIO. Patients were imaged with gradient echo transversal MRI before both before and

after injection of 7.5×10^6 cells in 200 uL into a lymph node. Sequential imaging showed migration of cells to sentinel lymph nodes. The limit of detection was 1.5×10^5 cells.

Magnetic NPs have also been used to deliver drugs to a diseased area.[42] After systemic injection, a high field magnet is positioned over the tumor to increase NP accumulation by immobilizing circulating particles for cargo release. Plank's group has used SPIO to deliver gene therapy to Wistar rat gut.[43] Labhasetwar's group has shown loading and delivery of paclitaxel and doxorubicin with iron oxide NPs and concurrent MRI.[44]

Multimodal MRI NPs often use optical methods. MRI is a depth insensitive technique and can be used for deep tissue imaging, yet suffers from poor sensitivity and cannot be used in real time. Fluorescence imaging is highly sensitive (fM or pM detection limits), yet has poor depth penetration. Thus, NPs dually functionalized employ the MRI modality for tumor staging and localization and the fluorescent modality intraoperatively to find tumor margins and ensure complete removal of the lesion These particles have been validated in a green fluorescent protein (GFP)-tagged 9L rat gliosarcoma model.[20] Twenty-four hours after 15 mg/kg i.v. injection, imaging with Cy5.5 fluorophore-labeled cross-linked iron oxide via MRI and fluorescence clearly showed tumor margins.

Quantum Dots. QD NPs have broad applications both in vivo and ex vivo.[45,46] Their theranostic applications as Type II NPs have been to identify disease via targeting ligands. Gambhir et al. used PEGylated QDs with the RGD peptide to image $\alpha_v\beta_3$ integrins of a U87 glioma model in vivo and observed tumor to background ratios of 4.42 ± 1.88 at 6 h versus 0.84 ± 0.21 for untargeted QDs. Bhatia et al. have illustrated homing of QDs in cell culture via the nucelolin-binding F3 peptide. These same QDs were also coated with siRNA to inhibit GFP. The authors demonstrated delivery of this siRNA to the nucleus via 50 nM QD incubation and subsequent inhibition by 80% of GFP gene expression.[33] This work has yet to be translated in vivo. Bawendi and colleagues have used QDs in porcine models (Figure 5A). Near IR QDs (200 pmol) allowed imaging of the sentinel lymph nodes in swine in less than 5 min.[47,48]

QD toxicity is the primary barrier to clinical use, and two different approaches are under development to reduce this concern. Encapsulation of the CdSe core with a silica coat prevents leaching of the heavy metal and decreased liver uptake from 57.2 to 16.2%ID/g and splenic uptake from 46.1 to 3.7%ID/g.[49] This decreases leaching of the heavy metal core, but it increases the size of the NP such that renal clearance is modulated. The second approach removes heavy
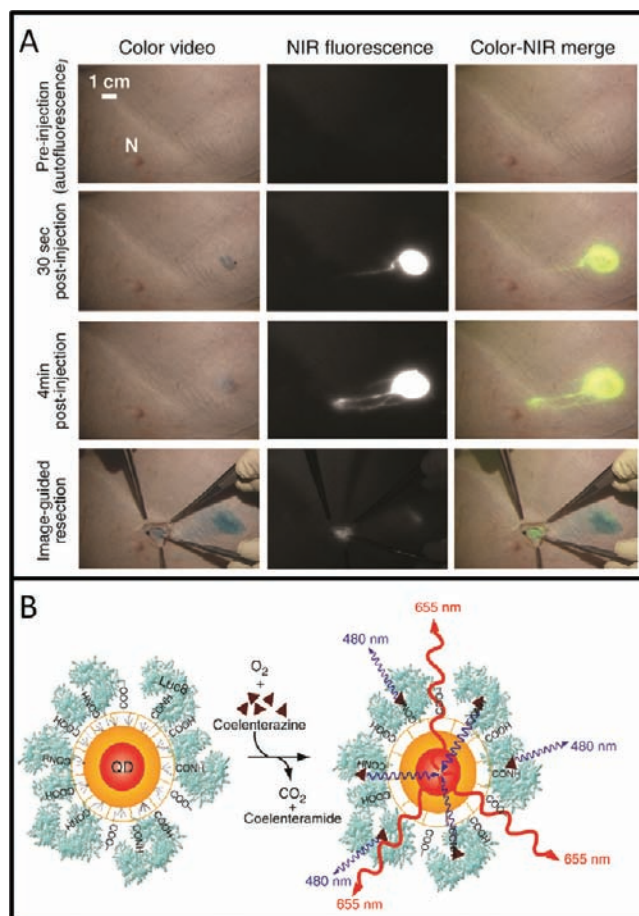

FIGURE 5. Quantum dots. QDs deployed to theranostic applications include the mapping of sentinel lymph nodes (A). A total of 400 pmol of QDs was injected 1 cm deep into living swine tissue. In less than 5 min, the QDs have begun to accumulate in the nearest lymph node for image-guided resection. Surgery is continued until all fluorescence (tumor) is removed. (B) Self-illuminating QDs containing a bioluminescent protein and a QD core use coelenterazine substrate to generate signal. Adapted and reprinted with permission from refs 48 and 51. Copyright 2004, 2006 Nature Publishing Group.

metals, that is, Cd, for ZnSe or ZnS QDs.[50] Self-illuminating QDs combine a bioluminescent protein with a QD core remove the need for an excitation source and improve signal-to-noise because of the low background (Figure 5B).[51]

Activatable TNPs. Activatable or "smart" NPs respond to a change in local environment to instigate the therapeutic/diagnostic mechanism. The key advantage is site-selective therapy for a reduction in side effects; challenges include effective delivery and leaky carriers. The most common triggers for activatable NPs are pH, proteases, and light. First, hypoxia in the tumor microenvironment results in lactic acid production and hence acidic conditions. The diagnostic modality of these NPs is selectivity for tumor environment to release the therapy payload. Liposomes between
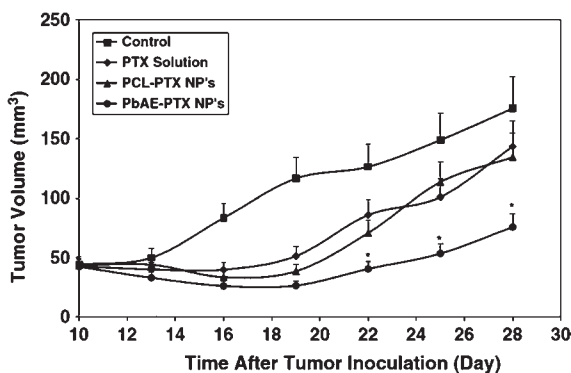
FIGURE 6. pH-sentivive NPs. NPs that selectively release paclitaxel in the presence of acidic tumor environment (PbAE-PTX) show statistically (*$p < 0.05$) greater reduction in tumor volume than control NPs or free paclitaxel. Adapted and reprinted with permission from ref 52. Copyright 2007 Springer.

100 and 200 nm were constructed from pH-sensitive poly-(ethylene oxide)-modified poly(beta-amino ester) carrying paclitaxel and used to treat a murine model of ovarian cancer (Figure 6).[52] At 1 h after injection, there was a 3.0-fold greater accumulation of drug in tumor treated with smart liposome versus non-pH-responsive carrier.

A second class uses the proteases upregulated by tumor for a cleavage event. The family of matrix metalloproteases (MMPs) is commonly used. Bhatia and co-workers demonstrated that, upon cleavage of protective PEG chains by protease, SPIO NPs aggregate at the site of interest.[53] A final class of NPs uses light for activation. One example incorporates the chlorine *meso*-tetraphenylporpholactol into PLGA nanoparticles, which is quenched in NP formulation but regains fluorescence in the presence of cellular lipids, producing singlet oxygen for photodynamic therapy. These NPs were injected in vivo and irradiated with 650 nm light at 191 mW/cm^2, causing a significant reduction in tumor volumes.[54]

Challenges

Some fundamental challenges hamper NP deployment to the clinic. The first among these are delivery obstacles, especially uptake by the reticuloendothelial system (RES) in which NPs are rapidly shuttled out of the circulation to the liver, spleen, and bone marrow. Coating the NP with polyethylene glycol can reduce recognition of the NP by the RES and increase circulation times 2−10 times.[55] Nanoparticles are often limited to vascular applications because their size prevents easy extravasation. NPs below 100 nm are often used to increase extravasation (for solid particles),[54] although extravasation of 400 nm liposomes is reported.[56] NP toxicity concerns often arise because of this RES accumulation.

Aggregation can lead to a loss of function or NP entrapment in the liver, lungs, or elsewhere due to capillary occlusion.[57] Toxicity can also result from the composition of the NP itself, for example, Cd in QDs.

The dose of NPs required for a therapeutic effect may be markedly higher than that required for a diagnostic effect. For example, a drug may need to be present at mg/kg of body weight where a radioactive tracer agent needs much less than 1 μg/kg. A final problem specific to theranostic NPs is circulation time. Imaging requires the area of interest to have higher signal than surrounding tissue for contrast. Thus, most imaging agents are designed to clear from the blood quickly (e.g, in a few minutes to hours). A therapeutic strategy needs to have NPs with longer circulation times for adequate exposure to the tumor and for drug release. A final challenge is oral bioavailability. Current NP design requires i.v. injection; transitioning to a less invasive approach is key.

Theranostic Nanoparticles in the Clinic

Although most NPs are used in preclinical models, some have entered human use (Table 2). The first class are liposomes or micelles for drug delivery. While their diagnostic content is limited to tumor accumulation, nearly 20 are currently commercially available and offer reduced systemic cytotoxicy and drug stability.[3] For example, Abraxane as a NP formulation of paclitaxel demonstrated significantly higher response rates versus free paclitaxel (33% vs 19%, respectively; $P = 0.001$) and longer time to progression (23.0 vs 16.9 weeks.) The side effect of neutropenia was lower for Abraxane versus paclitaxel ($P < 0.001$) despite a 49% higher paclitaxel dose.[58] Ongoing work creates liposomes with active targeting and longer circulation times.[59]

Gold nanoshells under the brand name Aurolase evolved from work by Halas and West.[30] Work in canine models used 1.25×10^9 nanoshells/g body weight. Patients with neck and throat cancers are injected with 120 nm gold nanoshells. These circulate and accumulate in the tumor by EPR. After immobilization of the NPs, laser irradiation at 808 nm causes a temperature increase of $\sim$20 degrees which has been shown to ablate tumor in a wide variety of small animal studies.[30,60] Advantages to photoablative treatment include the ability to customize the treatment with the location and duration of the light pulse. One limitation is that deeper tissue may not receive the same thermal dose as superficial tissue and that location of the tumor is needed prior to the initiation of treatment.

Recent work by Davis and colleagues described a cyclodextrin NP stabilized with PEG and adamantane and targeted to melanoma cells via human transferrin protein.[13] When injected into human melanoma patients, site-selective tumor accumulation was monitored by a Cy3 tag on the siRNA and ribonucleotide reductase-M2 subunit (RRM2) mRNA decreased by 40−70% and protein transcript decreased by ~30% versus predose tissue.[21] Finally, work in the Gambhir and Contag laboratories has shown that SERS NPs can label markers of colon cancer via either affibodies or small peptides.[19] Work is underway to deploy this imaging modality to molecular imaging guided colonosocopy.

Theranostic nanomedicine beyond imaging includes IVD in which an in vivo treatment decision is based on an ex vivo test result. The Mirkin group has developed a bio-barcode assay with excellent sensitivity via gold NPs.[61] Others utilize QDs and magnetic particles. Here, "companion diagnostics" stratify a disease, for example, breast cancer, into subtypes via measurement of a biomarker, for example, circulating Her-2.[7] In general, IVD tests have a shorter time course to gain approval for use in humans than in vivo imaging/diagnostics.[7]

Future Directions

Observing the progression of theranostic NPs in the past decades shows a trend toward less invasive approaches. We suggest NP invasiveness may be staged in the following categories. The current design of NPs can be divided into the following categories of invasiveness.

- Stage A NP: The NP is injected for diagnostic imaging with surgery for therapy.
- Stage B NP: NP is injected and gives information for necessary oral therapy.
- Stage C NP: NP is injected and identifies disease state and selectively treats affected area.
- Stage D NP: NP is constantly circulating and activates theranostics when disease begins.

Stage D NPs have yet to be reported. These systems could be considered synthetic cells and are often described as "nanobots" in the popular scientific press.

Work with theranostic NPs will continue to solve the above challenges. Alternatives to metallic or liposome NPs will allow for tailored circulation times unique to application. Time-sensitive coatings that shed over time could be used for custom circulation profiles. Especially intriguing are the use of bacteria, viruses, or other naturally occurring NP scaffolds.[62] These materials are similar to naturally occurring systems and thus may penetrate the cell membrane much more efficiently than synthetic nanoparticles. Biodegradable nanoparticles are an important nanoparticle type and overcome many of the toxicity and accumulation concerns of other NPs.[63]

BIOGRAPHICAL INFORMATION

Jesse V. Jokerst was born in Missouri and earned his Ph.D. in Analytical Chemistry under the supervision of John T. McDevitt (currently Rice University) at The University of Texas at Austin in 2009. His Ph.D. work used microfluidics and nanoscience to create tools for saliva-based diagnostics and HIV monitoring. He is currently a NIH R25T postdoctoral scholar in the laboratories of Sanjiv Sam Gambhir in the Stanford University School of Medicine. Dr. Jokerst's current research interests are Raman imaging, cell tracking via ultrasound, multimodal nanoparticles, and nanocharacterization.

Sanjiv Sam Gambhir, M.D., Ph.D. is the Chair of Radiology and Virginia & D.K. Ludwig Professor of Radiology, Bioengineering (by courtesy), and Materials Science & Engineering (by courtesy) at Stanford University. He also heads up the new Canary Center at Stanford for Cancer Early Detection. He received his M.D./Ph.D. from the UCLA Medical Scientist Training Program. He has over 375 publications in the field and over 30 patents pending or granted. He is also coeditor of the leading textbook in Nuclear Medicine and a recent comprehensive book on Molecular Imaging.

An internationally recognized researcher in molecular imaging with over $75 million of National Institutes of Health funding as the Principal Investigator, Dr. Gambhir's lab has focused on interrogating fundamental molecular events in living subjects. He has developed and clinically translated several multimodality molecular imaging strategies including imaging of gene and cell therapies. He has also developed strategies for monitoring fundamental cellular events such as protein−protein interactions and protein phosphorylation in living subjects. Much of his work has bridged scientific disciplines including applied physics, chemistry, cell/molecular biology, life sciences, engineering, and biomathematics. He holds several FDA eIND/IND's and has clinically translated PET imaging agents.

Dr. Gambhir serves as an advisor to several major imaging and pharmaceutical companies and has also cofounded several imaging startups. He serves on numerous academic advisory boards for universities around the world and is also a member of the Board of Scientific Advisors of the National Cancer Institute. He was also elected as one of the youngest members of the Institute of Medicine (IOM) of the U.S. National Academies in 2008.

REFERENCES

1 Massoud, T. F.; Gambhir, S. S. Molecular imaging in living subjects: seeing fundamental biological processes in a new light. *Genes Dev.* **2003**, *17* (5), 545–580.

2 Weissleder, R.; Ross, B. D.; Rehemtulla, A.; Gambhir, S. S. *Molecular Imaging: Principles and Practice*; People's Medical Publishing House: Shelton, CT, 2010.

3 Wagner, V.; Dullaart, A.; Bock, A.; Zweck, A. The emerging nanomedicine landscape. *Nat. Biotechnol.* **2006**, *24* (10), 1211–1218.

4 Kim, B. Y.; Rutka, J. T.; Chan, W. C. Nanomedicine. *N. Engl. J. Med.* **2010**, *363* (25), 2434–2443.

5 Sumer, B.; Gao, J. Theranostic nanomedicine for cancer. *Nanomedicine* **2008**, *3* (2), 137–140.

6 Zhang, L.; Gu, F.; Chan, J.; Wang, A.; Langer, R.; Farokhzad, O. Nanoparticles in medicine: therapeutic applications and developments. *Clin. Pharmacol. Ther.* **2007**, *83* (5), 761–769.

7 Papadopoulos, N.; Kinzler, K. W.; Vogelstein, B. The role of companion diagnostics in the development and use of mutation-targeted cancer therapies. *Nat. Biotechnol.* **2006**, *24* (8), 985–995.

8 Jain, P. K.; Huang, X.; El-Sayed, I. H.; El-Sayed, M. A. Noble metals on the nanoscale: optical and photothermal properties and some applications in imaging, sensing, biology, and medicine. *Acc. Chem. Res.* **2008**, *41* (12), 1578–1586.

9 O'Brien, M. E.; Wigler, N.; Inbar, M.; Rosso, R.; Grischke, E.; Santoro, A.; Catane, R.; Kieback, D. G.; Tomczak, P.; Ackland, S. P.; Orlandi, F.; Mellars, L.; Alland, L.; Tendler, C. Reduced cardiotoxicity and comparable efficacy in a phase III trial of pegylated liposomal doxorubicin HCl (CAELYX/Doxil) versus conventional doxorubicin for first-line treatment of metastatic breast cancer. *Ann Oncol.* **2004**, *15* (3), 440–449.

10 Scranton, R.; Cincotta, A. Bromocriptine--unique formulation of a dopamine agonist for the treatment of type 2 diabetes. *Expert Opin. Pharmacother.* **2010**, *11* (2), 269–729.

11 Lim, W. T.; Tan, E. H.; Toh, C. K.; Hee, S. W.; Leong, S. S.; Ang, P. C.; Wong, N. S.; Chowbay, B. Phase I pharmacokinetic study of a weekly liposomal paclitaxel formulation (Genexol-PM) in patients with solid tumors. *Ann. Oncol.* **2009**, *21* (2), 382–388.

12 Winter, P. M.; Morawski, A. M.; Caruthers, S. D.; Fuhrhop, R. W.; Zhang, H.; Williams, T. A.; Allen, J. S.; Lacy, E. K.; Robertson, J. D.; Lanza, G. M.; Wickline, S. A. Molecular imaging of angiogenesis in early-stage atherosclerosis with alpha(v)beta3-integrin-targeted nanoparticles. *Circulation* **2003**, *108* (18), 2270–2274.

13 Davis, M. E.; Zuckerman, J. E.; Choi, C. H. J.; Seligson, D.; Tolcher, A.; Alabi, C. A.; Yen, Y.; Heidel, J. D.; Ribas, A. Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. *Nature* **2010**, *464* (7291), 1067–1070.

14 Artemov, D.; Mori, N.; Okollie, B.; Bhujwalla, Z. MR molecular imaging of the Her 2/neu receptor in breast cancer cells using targeted iron oxide nanoparticles. *Magn. Reson. Med.* **2003**, *49* (3), 403–408.

15 Geso, M. Gold nanoparticles: a new X-ray contrast agent. *Br. J. Radiol.* **2007**, *80* (949), 64–65; author reply 65..

16 Mulvaney, S. P.; Musick, M. D.; Keating, C. D.; Natan, M. J. Glass-Coated, Analyte-Tagged Nanoparticles: A New Tagging System Based on Detection with Surface-Enhanced Raman Scattering. *Langmuir* **2003**, *19* (11), 4784–4790.

17 Cai, W.; Shin, D.-W.; Chen, K.; Gheysens, O.; Cao, Q.; Wang, S. X.; Gambhir, S. S.; Chen, X. Peptide-labeled near-infrared quantum dots for imaging tumor vasculature in living subjects. *Nano Lett.* **2006**, *6* (4), 669–676.

18 De la Zerda, A.; Zavaleta, C.; Keren, S.; Vaithilingam, S.; Bodapati, S.; Liu, Z.; Levi, J.; Smith Bryan, R.; Ma, T.-J.; Oralkan, O.; Cheng, Z.; Chen, X.; Dai, H.; Khuri-Yakub Butrus, T.; Gambhir Sanjiv, S. Carbon nanotubes as photoacoustic molecular imaging agents in living mice. *Nat. Nanotechnol.* **2008**, *3* (9), 557–562.

19 Jokerst, J. V.; Miao, Z.; Zavaleta, C.; Cheng, Z.; Gambhir, S. S. Affibody Functionalized Gold–Silica Nanoparticles for Raman Molecular Imaging of the Epidermal Growth Factor Receptor. *Small* **2011**, *7* (5), 625–633.

20 Kircher, M. F.; Mahmood, U.; King, R. S.; Weissleder, R.; Josephson, L. A multimodal nanoparticle for preoperative magnetic resonance imaging and intraoperative optical brain tumor delineation. *Cancer Res.* **2003**, *63* (23), 8122–8125.

21 Davis, M. E.; Zuckerman, J. E.; Choi, C. H.; Seligson, D.; Tolcher, A.; Alabi, C. A.; Yen, Y.; Heidel, J. D.; Ribas, A. Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. *Nature* **1067**, *464* (7291), 1067–1070.

22 Malek, A.; Merkel, O.; Fink, L.; Czubayko, F.; Kissel, T.; Aigner, A. In vivo pharmacokinetics, tissue distribution and underlying mechanisms of various PEI(-PEG)/siRNA complexes. *Toxicol. Appl. Pharmacol.* **2009**, *236* (1), 97–108.

23 Jacobson, G. B.; Gonzalez-Gonzalez, E.; Spitler, R.; Shinde, R.; Leake, D.; Kaspar, R. L.; Contag, C. H.; Zare, R. N. Biodegradable nanoparticles with sustained release of functional siRNA in skin. *J. Pharm. Sci.* **2010**, *99* (10), 4261–4266.

24 Seferos, D. S.; Prigodich, A. E.; Giljohann, D. A.; Patel, P. C.; Mirkin, C. A. Polyvalent DNA Nanoparticle Conjugates Stabilize Nucleic Acids. *Nano Lett.* **2009**, *9* (1), 308–311.

25 Gray, B.; Van Hazel, G.; Hope, M.; Burton, M.; Moroz, P.; Anderson, J.; Gebski, V. Randomised trial of SIR-Spheres (R) plus chemotherapy vs. chemotherapy alone for treating patients with liver metastases from primary large bowel cancer. *Ann. Oncol.* **2001**, *12* (12), 1711–1720.

26 Lee, J.; Huh, Y.; Jun, Y.; Seo, J.; Jang, J.; Song, H.; Kim, S.; Cho, E.; Yoon, H.; Suh, J. Artificially engineered magnetic nanoparticles for ultra-sensitive molecular imaging. *Nat. Med.* **2006**, *13* (1), 95–99.

27 Noble, C.; Krauze, M.; Drummond, D.; Yamashita, Y.; Saito, R.; Berger, M.; Kirpotin, D.; Bankiewicz, K.; Park, J. Novel nanoliposomal CPT-11 infused by convection-enhanced delivery in intracranial tumors: pharmacology and efficacy. *Cancer Res.* **2006**, *66* (5), 2801.

28 Kwon, G. S. Polymeric micelles for delivery of poorly water-soluble compounds. *Crit. Rev. Ther. Drug Carrier Syst.* **2003**, *20* (5), 357–403.

29 Davis, M. Nanoparticle therapeutics: an emerging treatment modality for cancer. *Nat. Rev. Drug Discovery* **2008**, *7* (9), 771–782.

30 Hirsch, L.; Stafford, R.; Bankson, J.; Sershen, S.; Rivera, B.; Price, R.; Hazle, J.; Halas, N.; West, J. Nanoshell-mediated near-infrared thermal therapy of tumors under magnetic resonance guidance. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100* (23), 13549.

31 Hadjipanayis, C. G.; Machaidze, R.; Kaluzova, M.; Wang, L.; Schuette, A. J.; Chen, H.; Wu, X.; Mao, H. EGFRvIII antibody-conjugated iron oxide nanoparticles for magnetic resonance imaging-guided convection-enhanced delivery and targeted therapy of glioblastoma. *Cancer Res.* **2010**, *70* (15), 6303–6312.

32 Choi Hak, S.; Liu, W.; Misra, P.; Tanaka, E.; Zimmer John, P.; Itty Ipe, B.; Bawendi Moungi, G.; Frangioni John, V. Renal clearance of quantum dots. *Nat. Biotechnol.* **2007**, *25* (10), 1165–1170.

33 Derfus, A. A.; Chen, A. A.; Min, D.-H.; Ruoslahti, E.; Bhatia, S. N. Targeted quantum dot conjugates for siRNA delivery. *Bioconjugate Chem.* **2007**, *18* (5), 1391–1396.

34 Liong, M.; Lu, J.; Kovochich, M.; Xia, T.; Ruehm, S. G.; Nel, A. E.; Tamanoi, F.; Zink, J. I. Multifunctional inorganic nanoparticles for imaging, targeting, and drug delivery. *ACS Nano* **2008**, *2* (5), 889–896.

35 von Maltzahn, G.; Park, J. H.; Agrawal, A.; Bandaru, N. K.; Das, S. K.; Sailor, M. J.; Bhatia, S. N. Computationally guided photothermal tumor therapy using long-circulating gold nanorod antennas. *Cancer Res.* **2009**, *69* (9), 3892–3900.

36 Sonvico, F.; Mornet, S.; Vasseur, S.; Dubernet, C.; Jaillard, D.; Degrouard, J.; Hoebeke, J.; Duguet, E.; Colombo, P.; Couvreur, P. Folate-conjugated iron oxide nanoparticles for solid tumor targeting as potential specific magnetic hyperthermia mediators: synthesis, physico-chemical characterization, and in vitro experiments. *Bioconjugate Chem.* **2005**, *16* (5), 1181–1188.

37 Phelps, M. E. *PET: Physics, Instrumentation, and Scanners*; Springer: New York, NY, 2006.

38 Schiffelers, R. M.; Ansari, A.; Xu, J.; Zhou, Q.; Tang, Q.; Storm, G.; Molema, G.; Lu, P. Y.; Scaria, P. V.; Woodle, M. C. Cancer siRNA therapy by tumor selective delivery with ligand-targeted sterically stabilized nanoparticle. *Nucleic Acids Res.* **2004**, *32* (19), e149.

39 NIH, www.clinicaltrials.gov.

40 Arbab, A. S.; Bashaw, L. A.; Miller, B. R.; Jordan, E. K.; Lewis, B. K.; Kalish, H.; Frank, J. A. Characterization of biophysical and metabolic properties of cells labeled with super-paramagnetic iron oxide nanoparticles and transfection agent for cellular MR imaging 1. *Radiology* **2003**, *229* (3), 838–846.

41 de Vries, I. J. M.; Lesterhuis, W. J.; Barentsz, J. O.; Verdijk, P.; van Krieken, J. H.; Boerman, O. C.; Oyen, W. J. G.; Bonenkamp, J. J.; Boezeman, J. B.; Adema, G. J. Magnetic resonance tracking of dendritic cells in melanoma patients for monitoring of cellular therapy. *Nat. Biotechnol.* **2005**, *23* (11), 1407–1413.

42 Barakat, N. S. Magnetically modulated nanosystems: a unique drug-delivery platform. *Nanomedicine* **2009**, *4* (7), 799–812.

43 Scherer, F.; Anton, M.; Schillinger, U.; Henke, J.; Bergemann, C.; Kruger, A.; Gansbacher, B.; Plank, C. Magnetofection: enhancing and targeting gene delivery by magnetic force in vitro and in vivo. *Gene Ther.* **2002**, *9* (2), 102–109.

44 Jain, T. K.; Foy, S. P.; Erokwu, B.; Dimitrijevic, S.; Flask, C. A.; Labhasetwar, V. Magnetic resonance imaging of multifunctional pluronic stabilized iron-oxide nanoparticles in tumor-bearing mice. *Biomaterials* **2009**, *30* (35), 6748–6756.

45 Michalet, X.; Pinaud, F. F.; Bentolila, L. A.; Tsay, J. M.; Doose, S.; Li, J. J.; Sundaresan, G.; Wu, A. M.; Gambhir, S. S.; Weiss, S. Quantum Dots for Live Cells, in Vivo Imaging, and Diagnostics. *Science* **2005**, *307* (5709), 538–544.

46 Bagalkot, V.; Zhang, L.; Levy-Nissenbaum, E.; Jon, S.; Kantoff, P. W.; Langer, R.; Farokhzad, O. C. Quantum dot-aptamer conjugates for synchronous cancer imaging, therapy, and sensing of drug delivery based on bi-fluorescence resonance energy transfer. *Nano Lett.* **2007**, *7* (10), 3065–3070.

47 Soltesz, E.; Kim, S.; Laurence, R.; DeGrand, A.; Parungo, C.; Dor, D.; Cohn, L.; Bawendi, M.; Frangioni, J.; Mihaljevic, T. Intraoperative sentinel lymph node mapping of the lung using near-infrared fluorescent quantum dots. *Ann. Thorac. Surg.* **2005**, *79* (1), 269–277.

48 Kim, S.; Lim, Y. T.; Soltesz, E. G.; De Grand, A. M.; Lee, J.; Nakayama, A.; Parker, J. A.; Mihaljevic, T.; Laurence, R. G.; Dor, D. M.; Cohn, L. H.; Bawendi, M. G.; Frangioni, J. V. Near-infrared fluorescent type II quantum dots for sentinel lymph node mapping. *Nat. Biotechnol.* **2004**, *22* (1), 93–97.

49 Ma, N.; Marshall, A. F.; Gambhir, S. S.; Rao, J. Facile synthesis, silanization, and biodistribution of biocompatible quantum dots. *Small* **1520**, *6* (14), 1520–1528.

50 Gao, J.; Chen, K.; Xie, R.; Xie, J.; Lee, S.; Cheng, Z.; Peng, X.; Chen, X. Ultrasmall near-infrared non-cadmium quantum dots for in vivo tumor imaging. *Small* **2010**, *6* (2), 256–261.

51 So, M.-K.; Xu, C.; Loening, A. M.; Gambhir, S. S.; Rao, J. Self-illuminating quantum dot conjugates for in vivo imaging. *Nat. Biotechnol.* **2006**, *24* (3), 339–343.

52 Devalapally, H.; Shenoy, D.; Little, S.; Langer, R.; Amiji, M. 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 Chemother. Pharmacol.* **2007**, *59* (4), 477–484.

53 Harris, T. J.; von Maltzahn, G.; Derfus, A. M.; Ruoslahti, E.; Bhatia, S. N. Proteolytic actuation of nanoparticle self-assembly. *Angew. Chem., Int. Ed.* **2006**, *45* (19), 3161–3165.

54 McCarthy, J. R.; Perez, J. M.; Brückner, C.; Weissleder, R. Polymeric nanoparticle preparation that eradicates tumors. *Nano Lett.* **2005**, *5* (12), 2552–2556.

55 Jokerst, J. V.; Lobovkina, T.; Zare, R. N.; Gambhir, S. S. Nanoparticle PEGylation for imaging and therapy. *Nanomedicine* **2011**, *6* (4), 715–728.

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

57 Knop, K.; Hoogenboom, R.; Fischer, D.; Schubert, U. S. Poly(ethylene glycol) in drug delivery: pros and cons as well as potential alternatives. *Angew.* **XXXX**, *49* (36), 6288–6308.

58 Gradishar, W. J.; Tjulandin, S.; Davidson, N.; Shaw, H.; Desai, N.; Bhar, P.; Hawkins, M.; O'Shaughnessy, J. Phase III trial of nanoparticle albumin-bound paclitaxel compared with polyethylated castor oil-based paclitaxel in women with breast cancer. *J. Clin. Oncol.* **2005**, *23* (31), 7794.

59 Park, J. W. Liposome-based drug delivery in breast cancer treatment. *Breast Cancer Res.* **2002**, *4* (3), 95–99.

60 Schwartz, J. A.; Shetty, A. M.; Price, R. E.; Stafford, R. J.; Wang, J. C.; Uthamanthil, R. K.; Pham, K.; McNichols, R. J.; Coleman, C. L.; Payne, J. D. Feasibility study of particle-assisted laser ablation of brain tumors in orthotopic canine model. *Cancer Res.* **2009**, *69* (4), 1659.

61 Nam, J. M.; Stoeva, S. I.; Mirkin, C. A. Bio-bar-code-based DNA detection with PCR-like sensitivity. *J. Am. Chem. Soc.* **2004**, *126* (19), 5932–5933.

62 Akin, D.; Sturgis, J.; Ragheb, K.; Sherman, D.; Burkholder, K.; Robinson, J. P.; Bhunia, A. K.; Mohammed, S.; Bashir, R. Bacteria-mediated delivery of nanoparticles and cargo into cells. *Nat. Nanotechnol.* **2007**, *2* (7), 441–449.

63 Panyam, J.; Labhasetwar, V. Biodegradable nanoparticles for drug and gene delivery to cells and tissue. *Adv. Drug Delivery Rev.* **2003**, *55* (3), 329–347.