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Imaging of Nanoparticle Distribution to Assess Treatments That Alter Delivery

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Abstract

Molecular imaging is a vital tool to non-invasively measure nanoparticle delivery to solid tumors. Despite the myriad of nanoparticles studied for cancer, successful applications of nanoparticles in humans is limited by inconsistent and ineffective delivery. Successful nanoparticle delivery in preclinical models is often attributed to enhanced permeability and retention (EPR)—a set of conditions that is heterogeneous and transient in patients. Thus, researchers are evaluating therapeutic strategies to modify nanoparticle delivery, particularly treatments which have demonstrated effects on EPR conditions. Imaging nanoparticle distribution provides a means to measure the effects of therapeutic intervention on nanoparticle delivery to solid tumors. This review focuses on the utility of imaging to measure treatment-induced changes in nanoparticle delivery to tumors and provides preclinical examples studying a broad range of therapeutic interventions.

Keywords

Nanoparticles; Imaging; Theranostics; Chemotherapy

Introduction

As interest in nanoparticles (NP) for the delivery of therapeutic agents to solid tumors grows, methods to monitor or predict their effective delivery are critically needed. Imaging NP can streamline the development and implementation of NP treatments and can serve as tools for personalized medicine. NP platforms for drug delivery are designed to enhance drug deposition in tumor tissues to increase effective therapeutic doses [1]. However, preclinical successes in treating tumors with NP often fail to successfully translate to human trials due to ineffective delivery to tumors in the heterogeneous patient population [2].

By providing non-invasive, quantitative measures of NP localization, imaging can supply invaluable information of NP distribution in tumors. With imaging, the delivery of NP can

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Conflict of Interest

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be assessed in a lesion, predict therapeutic efficacy of NP treatments, and monitor distribution over time or as a response to treatment [3, 4]. Since ineffectual NP delivery in human tumors is a critical barrier for clinical use, researchers are studying therapies which alter the tumor and its microenvironment to improve NP delivery [5]. Imaging NP delivery could identify and monitor strategies for improving NP localization to solid tumors. This review focuses on the utility of imaging to measure treatment-induced changes in nanoparticle delivery to tumors and provides preclinical examples studying a broad range of therapeutic interventions.

“Nanotheranostics” to Visualize Delivery with Non-invasive Imaging

In addition to delivering therapeutic payloads, many NP can be designed or modified for imaging to act as a tracer or contrast agent. Imaging with NP (diagnostic NP; Dx-NP) that mimic the systemic distribution of drug-loaded NP (therapeutic NP; Tx-NP) can assess the tumor-localizing capacity of the NP platform. Dx-NP may or may not contain a therapeutic component and are often administered in lower doses than Tx-NP [6–8]. Unlike traditional diagnostics, Dx-NP are not typically used for tumor staging. Instead, Dx-NP can serve as surrogate measure of NP delivery to predict the success of Tx-NP delivery. Further, Dx-NP do not provide explicit information about therapeutic action of drug payloads. While ineffective delivery of NP platforms to human tumors is a critical clinical barrier, drug efficacy is an independent variable which depends upon tumor sensitivity.

Recently, coupling of targeted therapies with complimentary diagnostic imaging has been termed “theranostics” [9]. From this, the emerging field of “nanotheranostics” provides tools to measure NP delivery which may predict efficacy of NP therapy on an individual basis [10, 11]. Examples of a variety of NP platforms that have been used preclinically to image NP delivery are outlined in Table 1. While imaging with Dx-NP to predict therapeutic response has been the goal of nanotheranostics, the potential utilities of imaging in NP research are myriad. Dx-NP can measure the release of payloads or assess drug availability [27]. Non-invasive scans can be repeated over time to monitor delivery through the course of treatment. Perhaps, the newest and least explored utility for nanotheranostics is in evaluating strategies to improve NP deposition in tumors with therapies that have an impact on enhanced permeability and retention (EPR). Imaging with Dx-NP can allow researchers evaluate how therapies such as radiation, chemotherapy, and anti-vascular agents affect the delivery of NP. Utilizing imaging with NP could streamline NP development, identify the best combination therapies and treatment timelines, and narrow the gap between preclinical studies and clinical application of NP.

Enhanced Permeability and Retention: from Mice to Mankind

NP have long been thought to localize to solid tumors via EPR. Tumors generate aberrant “leaky” blood vessels which, coupled with poor lymphatic drainage, causes large particles to become trapped in tumor interstitial spaces [28]. NP, which are usually 10 to 100 nm, have been shown preclinically to passively accumulate in tumors due to EPR, often regardless of targeting surface moieties [29–31]. Promising Tx-NP formulations often demonstrate successful EPR-associated delivery to pre-clinical tumor models [32, 33]. This can lead to

improved drug efficacy compared to non-NP formulations [34, 35]. However, delivery and penetration of NP in solid tumors has remained a prominent problem in the translation of NP to humans [2, 36]. The physiological conditions which define tumor EPR, and many aspects of NP delivery, are highly variable and dynamic in humans. Researchers have since suggested that EPR is hampered in humans by conditions of high interstitial fluid pressures (IFP), increased pericyte coverage, inconsistent vessel pore sizes, and thicker collagen and extracellular matrix (ECM) layers [37–40].

The task of improving NP accumulation in solid tumors can be viewed from two perspectives: (1) adjustment of the physical parameters of the NP and (2) therapeutic modulation of the tumor and its microenvironment [41]. Studies of the former are already actively utilizing imaging [42, 43]. By adding diagnostic components to the NP platform, researchers can measure differences in systemic distribution of NP during the design, modification, or fine-tuning of NP. This can mean manipulating the size and shape, surface chemistry, targeting moieties, *etc.* [44], and is beyond the scope of this review. Instead, this discussion focuses on the latter and the use of imaging to monitor therapeutic interventions which make tumor tissues more available to NP infiltration. Many currently accessible treatments have the capacity to influence EPR parameters. In attempts to improve NP delivery, multiple groups are utilizing therapies that affect tumor blood flow, vascular permeability, IFP, and ECM components. The goal is to reduce or remodel the physical barriers which hamper macromolecular profusion in tumors. While multiple preclinical studies have begun testing therapeutic alteration of delivery, a select few have incorporated imaging to measure these effects. These studies demonstrate the utility of imaging NP delivery to measure and monitor the effects of therapeutic intervention, as well as streamline preclinical studies searching for promising combination therapies.

Imaging to Measure and Monitor Therapeutically Altered Nanoparticle Delivery to Solid Tumors

Nanotheranostics studies utilize various imaging modalities to measure and monitor differences in NP distribution patterns which result from additional therapies/interventions. A summary of these studies is provided in Table 2. These studies identify tools and techniques for personalization of NP therapies for cancer.

Drugs Which Inhibit Angiogenesis, Disrupt Vessel Structure, and Alter Permeability

EPR is attributed, in part, to aberrant tumor vasculature defined by poorly formed vessels comprised of “leaky” vascular endothelium. Large pores and gaps in the vessel walls allow for extravasation of macromolecules in circulation, including NP [45]. These characteristics are heterogeneous in clinical populations, making them a somewhat difficult target for cancer therapies [46, 47]. Although often lacking widespread impact as monotherapies, drugs which target angiogenesis or vessel integrity have been shown to improve outcomes when combined with chemotherapy [48, 49]. However, the ability to modulate tumor vasculature properties is an attractive concept when facing the problem of inconsistent NP

distribution in tumors. Thus, agents which target a number of vascular properties have been suggested as a means of altering EPR to enhance NP delivery.

Targeting Tumor Angiogenesis

Anti-angiogenic therapies like bevacizumab (Avastin™; Genentech, San Francisco, CA), a vascular endothelial growth factor (VEGF)-targeted antibody, are designed to disrupt tumor blood vessel formation and ultimately starve tumors of nutrients. These drugs have led to modest improvements in clinical outcomes when combined with conventional chemotherapy [48, 50]. While depletion of blood vessels is the intended outcome of anti-angiogenic therapy, there is some evidence that these drugs cause temporary remodeling or “normalization” of blood vessels, which may affect drug delivery for a short time [51, 52]. With the expanding use of anti-angiogenic therapies in the clinic, a complete understanding of their effect on NP delivery will be important as more NP therapies are developed and studied.

A study performed by Wilmes *et al.* measured the effect of blocking VEGF signaling on perfusion of small and large contrast agents with dynamic contrast-enhanced (DCE) magnetic resonance imaging (MRI) in BT474 breast tumor xenografts in mice [26]. The group utilized a novel small molecule inhibitor of VEGF receptor tyrosine kinase, axitinib (AG-013736; Inlyta®; Pfizer, New York, NY) to disrupt tumor vessel properties and growth. Administration of the drug for 3 weeks showed dramatic antitumor action. DCE-MRI images to measure early drug effects were obtained with both macromolecular albumin-(GdDTPA)₃₀ and low molecular weight GdDTPA contrast agents before and after axitinib therapy.

After only 7 days of axitinib administration, the authors noted a marked decrease in tumor perfusion compared to control tumors. Reduced vessel permeability was evident from significant drops in tumor endothelial transfer coefficients (K^{ps}) calculated for both contrast agents. Histology staining for CD31 performed in resected tumor tissues showed a reduced number of microvessels after 7 days of treatment, which complements the imaging data. The measurable decrease in macromolecular albumin-(GdDTPA)₃₀ perfusion into tumor tissues following short-term axitinib therapy suggests that axitinib treatment may not be a viable option for combination with NP therapies. Importantly, imaging with DCE-MRI was able to provide an early assessment of these effects. While the precise influence of anti-angiogenic therapy on NP delivery will depend upon the agents and conditions examined, imaging represents an invaluable tool to streamline these studies.

TGF- β Pathway Disruption

Multiple studies have demonstrated that NP accumulation in solid tumors can be enhanced by treatment with agents which cause tumor vessels to become leaky [53, 54]. A popular target is the transforming growth factor (TGF)- β pathway, since blocking the kinase activity of the TGF- β 1 receptor has been shown to increase tumor vessel leakage [55]. Drugs that inhibit TGF- β 1, also known as activin-like kinase 5 (Alk5), are widely available and relatively well characterized, which simplifies their incorporation into nanotheranostics studies.

Daldrup-Link *et al.* chose to utilize MR imaging to measure the effect of Alk5-inhibitor [3-(pyridine-2-yl)-4-(4-quinonyl)]-1H-pyrazole (LY-364947 Calbiochem, San Diego, CA) on the delivery of NP-based contrast agents in transgenic mouse mammary tumor virus-driven expression of the polyoma middle T oncogene (MMTV-PyMT) adenocarcinoma, as well as an orthotopic glioblastoma model [23]. MRI images were performed with gadofosveset trisodium (Ablavar®), a small molecule contrast agent which binds albumin to form macromolecular complexes in circulation, as well as ferumoxytol (Feraheme™), an iron oxide NP. Images were obtained at baseline, and following 6 days of treatment with LY-364947, i.p. every other day to visualize the effect of Alk5 inhibition on NP delivery.

In tumors subjected to Alk5 inhibition, tumor enhancement increased threefold compared to that of controls in MR images with gadofosveset, primarily in the tumor periphery, and twofold in images with ferumoxytol, throughout tumor tissues. The authors suggest that Alk5 inhibition may be able to improve NP delivery and efficacy, and that this effect can be visualized with NP contrast agents for MR imaging. In this way, image-guided modulation of TGF- β signaling can be used to personalize NP therapies.

Another study, carried out by Minowa *et al.* in mice bearing colon 26 tumors, measured the effect of Alk5 inhibition with A-83-01 on NP delivery by performing DCE-MRI with liposomal GdDTPA [12]. The authors compared baseline scans to scans acquired 24 h after initiating treatment, which consisted of two injections of A-83-01. Compared to baseline scans, treatment resulted in a 3.8-fold increase in the AUC of Gd concentration (Fig. 1). This implies that even short-term Alk5 inhibition can markedly improve liposome delivery to the tumor. Importantly, imaging with a liposomal contrast agent for MRI was able to identify improved liposomal delivery very early into Alk5 inhibition with A-83-01.

Cytotoxic Debulking

In some rapidly dividing tumors, densely packed populations of tumor cells may be a greater barrier to NP delivery than vascular structure. Excessive cellular bulk often leaves little interstitial space through which NP can travel after extravasation from the circulation, reducing drug penetration. Dense tumors can also disrupt lymphatic drainage, leading to high IFP which can prevent NP from collecting in tumors [56]. Thus, it has been proposed that administration of cytotoxic agents prior to NP treatment could prime the tumors for more efficient NP delivery by modestly debulking tumor mass [57].

Cytotoxic debulking has led to improved NP delivery in multiple preclinical models. In colon and GI stromal tumor models in mice, Apo2/TRAIL pre-treatment was found to increase tumor vessel size and widen stromal areas [58]. This led to increased uptake and improved efficacy of a liposomal formulation of gemcitabine. Priming tumors with paclitaxel treatment has been shown to increase latex bead and doxorubicin-filled liposomes to solid tumors, with no evidence of increased accumulation in healthy tissues [59]. Short-term paclitaxel treatment in a model of pancreatic cancer was even found to be useful in priming tumors for delivery of PEGylated cationic-siRNA directed against survivin, a protein known to promote paclitaxel resistance [60]. Interestingly, while paclitaxel priming followed by siRNA delivery managed to reduce surviving levels, NP delivery without

priming had no effect, suggesting that interstitial transport was the primary barrier. Although the evidence for cytotoxic debulking is compelling in preclinical models, a non-invasive imaging method would be very useful for elucidating priming timelines, and the success of debulking techniques in individuals.

Tumor Priming with Cyclophosphamide

Studies performed by Geretti *et al.* in HER-2-positive breast tumor xenografts utilized a Cu-64 labeled, trastuzumab-coated doxorubicin-filled liposome (MM-302, Merrimack Pharmaceuticals Inc., Cambridge, MA) for positron emission tomography (PET) imaging of the effects of cyclophosphamide therapy on liposome delivery (Fig. 2) [13]. Interestingly, tumor priming with a single dose of cyclophosphamide 4 days before liposome administration resulted in significant increases in [⁶⁴Cu]liposome accumulation in breast tumors compared to that of vehicle controls. Tissue analysis revealed that while HER-2 expression, vessel density, and collagen remained unchanged, cyclophosphamide pre-treatment resulted in reduced tumor cell density and interstitial space. The authors also observed reductions in measured IFP in treated tumors. Analysis of CD31 and FITC-lectin in tissues demonstrated that treated tumors exhibited increases in vessel perfusion.

The evidence of enhanced liposome delivery seen in PET was supported by efficacy studies in the same model, which demonstrated increased efficacy of MM-302 treatment following priming with cyclophosphamide. This effect was robust and continued through multiple cycles of MM-302 (each preceded by cyclophosphamide). The authors measured the intratumoral distribution of fluorescently labeled MM-302. Analysis of doxorubicin (Dox) content *via* HPLC of resected tumor tissues demonstrated increased levels of total Dox in tumors primed with cyclophosphamide. Consistent with the previously mentioned measures of perfusion and interstitial space, fluorescence was detected at greater distances from vessels in tumors which received pre-treatment. Impressively, the number of Dox-positive nuclei was 12-fold greater in tumors pre-treated with cyclophosphamide, which is consistent with the observed enhancement in efficacy. Thus, PET was able to visualize enhanced delivery of [⁶⁴Cu]NP following cyclophosphamide pre-treatment, an effect which led to greater efficacy of NP treatment.

Pre-treatment with S-1

Similar to cyclophosphamide, pre-treatment with S-1 (tegafur), an orally available pro-drug of 5-fluorouracil (5-FU), has been suggested as a means to increase NP deposition in solid tumors by reducing tumor bulk and IFP. In mice bearing C26 murine colon tumors, Doi *et al.* administered sub-maximum-tolerated doses of S-1 for 7 days, prior to administering fluorescently labeled PEGylated liposomes [14]. Fluorescence in untreated tumors was often greater in the tumor periphery, whereas tumors treated with S-1 for 1 week showed greater overall liposome uptake, with more homogeneous distribution throughout the tumor. Further analysis of resected tissues indicated apoptotic induction in vascular endothelial cells and surrounding tumor cells in treated tumors.

The authors furthered their study in DLD-1 human colon tumor xenografts and assessed intratumoral accumulation of fluorescent liposomes *in vivo* with NiR imaging at multiple

time points post-injection. Again, liposomal localization to tumor was measurably enhanced in DLD-1 tumors treated with S-1 for 7 days compared to untreated controls.

In a similar study of DLD-1 tumors, Nakamura *et al.* observed enhanced delivery of fluorescently labeled PEGylated siRNA-lipoplexes containing siBcl-2 following 7 days of S-1 pre-treatment [15]. The authors chose to target Bcl-2, as the Bcl-2 pathway had been indicated as a major mechanism of 5-FU and S-1 resistance [61]. At all time points imaged post-injection of the lipoplexes, the authors measured increased uptake in tumors of mice which had received daily S-1 dosing. When measuring therapeutic efficacy, the authors found that combining daily oral tegafur with siBcl-2-lipoplexes administered every other day for 2 weeks resulted in significant reduction in tumor burden. Further, the authors found that Bcl-2 protein expression was suppressed to a greater extent in tumors treated with S-1 as well as the siBcl-2 lipoplexes, compared to tumors treated with the NP alone. The enhanced delivery of the NP measured in the scans after 7 days of S-1 treatment was likely an important factor in the improved efficacy seen in the combination treatment after 2 weeks.

Radiation

Similar to certain types of chemotherapy, radiation therapy (RT) has been shown to induce time-dependent, temporary effects on tumor endothelium and stromal cells which may influence drug delivery [62]. RT can induce cycles of hypoxia followed by rapid reoxygenation and may increase vascular permeability due to transient increases in cytokines.

Preclinical studies have suggested that the effects of RT may improve NP delivery and solid tumor penetration. In osteosarcoma xenografts, single and fractionated doses of RT administered 1 day after injection of liposomal doxorubicin (Caelyx®) lead to increased accumulation of liposomes in tumor tissues [63]. Decreased IFP and increased vascular permeability were seen in mouse mammary adenocarcinomas after a single 15-Gy fraction of RT, resulting in a nearly two-fold increase in accumulation of superparamagnetic iron oxide (SPIO) NP [64]. These experiments, as well as others, have spurred a widespread interest in imaging RT effects on NP delivery.

Enhancing NP Delivery with High-Dose RT

Studies employing multiple imaging modalities have demonstrated that a single, high dose of radiation can dramatically affect tumor vascularity. Kobayashi *et al.* utilized MR imaging with a gadolinium-labeled dendrimer contrast agent (G8-Gd-D) to measure changes in vascular properties in irradiated xenografts of squamous cell carcinoma [22]. Compared to non-irradiated controls, the authors found that tumor irradiation with a single 15-Gy dose was followed by increased enhancement seen in MR scans as early as 7 h post-RT. This effect was still seen at 24 h, and returned to baseline by 36 h post-RT. Although significant enhancement was seen, MR was clearly able to demonstrate that the effects of RT on G8-Gd-D were transient. This emphasizes the importance of timing when attempting to augment NP delivery and the invaluable role of imaging to determine appropriate dosing schedules.

Along with determining changes in enhancement over time, the authors found that the increases in enhancement with G8-Gd-D after a single dose of 15 Gy were not seen in tumors treated with single lower doses of RT. Further, tumors which received fractionated RT also showed no enhancement in G8-Gd-D with MR, including mice treated according to a 5 Gy $\times$ 5 protocol. The authors concluded that, along with scheduling, the specific dose delivered to tumors was critical in augmenting NP delivery. By utilizing MR with a dendrimer NP contrast agent, the authors were able to efficiently measure the increase in NP delivery to tumors following RT, elucidate how long these effects lasted, and determine the optimal RT dose to induce this effect.

In a genetic mouse model of primary sarcomas with mutant *KRAS* and p53, Moding *et al.* utilized dual-energy micro CT to study the effects of single, high-dose RT on NP delivery [16]. One day following irradiation of the sarcomas with a single dose of 20 Gy, mice were administered PEGylated liposomal iodine (approximately 140 nm) and imaged immediately and 3 days later with CT at 80 and 40 kVp, to image calcium (bone and limb) and iodine (liposomes), respectively. The authors found that scans of irradiated tumors indicated increases in iodine concentration by day 4 compared to tumors that did not receive RT (Fig. 3). While CD31 assessment in resected tumors showed no significant change, evidence of increased permeability was present in irradiated tumors and not in controls. These studies showed that imaging with CT was able to detect increased NP retention in tumors for extended periods following single, high-dose RT, providing rationale for subsequent liposome-based therapy.

Imaging Improved NP Drug Delivery Following RT

While the effects of RT therapy on NP tracers have been imaged, the question remains of whether these effects would subsequently alter treatment with therapeutic NP. Lammers *et al.* utilized MR imaging and gamma scintigraphy to image the effect of RT on delivery of two NP copolymer drugs [21]. The authors chose to study HPMA copolymers with Dox or gemcitabine (gem), with the addition of Gd or I-131 for MR or gamma camera imaging, respectively, in a mouse model of prostate carcinoma. Twenty-four hours after a 20-Gy dose of RT, images of both copolymers showed enhanced localization to tumor, between 31 and 44 % in MR (Fig. 4), and 24–57 % with gamma camera. When observed for therapeutic efficacy, HPMA-Dox plus RT caused significant inhibition of the tumors, although increases in toxicity were also observed. HPMA-gem plus RT also showed significant tumor inhibition, with a slightly better toxicity profile. This study demonstrates that RT-augmented NP delivery measured with imaging may be used to predict and measure RT-enhanced efficacy of therapeutic NP.

Another study, performed by Appelbe *et al.*, imaged RT-induced enhancement of tumor-specific delivery of fluorescent PEG polymers (Angiosense750EX®) and PEGylated IONP (AngioSPARK680®) [25]. In breast tumor xenograft models, radiation with a single dose of 5 Gy induced an increase in accumulation of both probes by 1.2- to 3.3-fold compared to that of controls. Interestingly, in B16F10 mouse melanoma tumors, the authors found that a 5-Gy dose of RT plus Doxil® (Doxorubicin Liposomal, (Dox)) treatment was more effective at decreasing tumor growth than either therapy alone. Thus, the ability to image RT-

augmented delivery of NP can be indicative of therapeutic benefit when RT is combined with NP therapy. These studies provide strong evidence for the use of imaging in such studies to provide quick, non-invasive assessment of how and when RT can affect and improve NP treatments.

Hyperthermia and Thermal Ablation

Hyperthermia, or direct heating, of tumor tissues has been utilized in the clinic to enhance therapies like RT [65], as it can induce changes in the tumor microenvironment at lower temperatures, or cell death at high temperatures [66, 67]. However, multiple studies of tumor hyperthermia have suggested that its effects on tumor vasculature may make it a viable option for combination with NP therapy [68, 69]. Increased temperatures in tumor tissues can lead to increased vascular perfusion, as well as microvascular permeability [70]. These effects can help to improve NP delivery, with preclinical studies demonstrating enhanced NP efficacy due to hyperthermia [71]. Thus, studies have begun to utilize imaging to measure the effect of hyperthermia on NP delivery.

Sustained Low-Temperature Hyperthermia

In a study of cats with soft tissue sarcomas, Matteucci *et al.* imaged the delivery of Tc-99m-labeled liposomes with gamma scintigraphy, both with and without mild hyperthermia (< 50 °C) [17]. Although individual cats were treated with different doses of heat for 60 min, the authors found that liposomal accumulation was significantly enhanced following thermal ablation compared to scans of the same cats with no ablation. Based on the images, the authors noted that hyperthermia-induced improvements in liposome localization were measurable at time points greater than 200 min post-injection. The authors also found that ablation did not lead to any notable toxicity and did not cause enhanced localization of liposomes to non-tumor tissues.

Another study, performed in rats, was able to measure similar increases in Tc-99m-labeled liposome accumulation by gamma scintigraphy following mild hyperthermia (approximately 40 °C). Kleiter *et al.* observed increases in [^{99m}Tc]DSPE-PEG₂₀₀₀ in rat fibrosarcoma xenografts when heated with warm water *via* intratumoral catheter for 45 min [18]. When subsequently treated with Doxil, the authors found that resected tumors which had received catheter warming contained higher levels of Dox than control tumors. Hyperthermia induced by a water bath in mice with human melanoma xenografts resulted in increased liposome extravasation, as shown by Li *et al.* using fluorescent imaging (Fig. 5) [72]. The authors found that the increased deposition of liposomes due to hyperthermia (41 °C for 30 min) compared to that of controls could easily be imaged at 4 h post-injection. This trend continued in measurements as late as 48 h post-injection.

High-Temperature Thermal Ablation

Head *et al.* utilized short-term radiofrequency thermal ablation (temperatures maintained at 70 °C for 5 min) to augment delivery of Tc-99m-labeled liposomal Dox to head and neck squamous cell carcinoma tumor models in rats [19]. Similar to trends seen in the Kleiter study, the authors measured enhanced uptake of the labeled liposomes with gamma

scintigraphy, as well as with single-photon emission computed tomography (SPECT)/X-ray computed tomography (CT). Further, measurements of intratumoral Dox levels mimicked the results seen in the scans, confirming the utility of imaging to measure the enhanced delivery of these liposomes.

Targeting the ECM and Basement Membrane

Along with high tumor cell density, the components that comprise the tumor microenvironment represent a critical barrier to the delivery of NP [39, 40]. Multiple strategies have been proposed to alter the extracellular matrix and basement membrane components of solid tumors to facilitate NP delivery [73]. For example, treatment with losartan, an angiotensin II receptor antagonist, has been shown to inhibit collagen I in solid tumors and can improve the distribution and efficacy of NP therapies [74]. Ablation of hyaluronic acid in tumor stroma has also been shown to normalize IFP, which can improve drug delivery and improve therapeutic efficacy [75].

A study performed by Zheng *et al.* in a xenograft model of squamous cell carcinoma of the tongue utilized ultrasound and Tc-99m-labeled Doxil for SPECT to measure the effects of collagenase-2 on IFP and NP delivery [20]. The authors monitored the immediate effects of intravenous or intratumoral injection of collagenase-2 and found that both caused a measurable but transient drop in IFP as measured by ultrasound, which was greatest between 30 and 60 min, while increases in tumor blood flow peaked by 120 min. When injected immediately after intratumoral collagenase-2, [^{99m}Tc]Doxil showed significant increases in intratumoral localization by 20 h. Not only were the authors able to demonstrate a measurable effect of collagenase-2 treatment on IFP, they also showed that the effect of collagenase-2 on nanoparticle delivery could be imaged with SPECT early into treatment.

Conclusions and Perspectives

Although research of NP in cancer has encountered multiple challenges, imaging may be the most comprehensive tool to improve the transition of NP from the lab to the clinic. Further, as therapeutic techniques to improve NP delivery to tumors are identified, imaging can serve as a non-invasive means of measuring response early into and throughout treatments. The possible advantages of utilizing imaging to monitor therapeutically augmented NP delivery are fourfold: (1) Imaging can quickly assess the effect of various therapeutic interventions on NP delivery to streamline preclinical studies of combination therapies; (2) Non-invasive imaging can measure NP delivery at multiple time points, to allow for simpler evaluation of treatment/combination dosing schedules; (3) Simple and modifiable imaging protocols can be used to screen multiple therapeutic interventions and/or NP platforms which have already been clinically tested or approved, allowing for quicker clinical application of NP treatment regimens; (4) Imaging of NP delivery, as well as therapeutic effects on NP delivery, can provide a means by which physicians assess, modify, and monitor NP treatment in cancer patients for precision medicine with NP.

As demonstrated, imaging of augmented NP delivery can be achieved with a variety of imaging platforms and has been studied in a wide array of tumor models with multiple NP platforms. Although the EPR effect may be more complex in humans than has been seen in

animal models, it is also capable of being modulated with established therapies. The factors affecting NP delivery and retention in solid tumors are governed by dynamic conditions including vascular density and perfusion, tumor cell compactness, and stromal components. Each of these characteristics can be modified with therapeutic interventions in attempts to improve NP delivery. While the tumor environment can be difficult to assess and treat, imaging NP delivery can be the key to identifying the most successful methods of improving NP therapy in cancer.

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Fig. 1. DCE-MRI with liposomal Gd showing increased accumulation in tumors following Alk5 inhibition. Curves describing the concentration of Gd over time in colon 26 xenografts after IP injection of liposomal Gd (a) show increased initial area under the Gd curve (IAUGC) following two injections of A-83-01 compared to baseline (b). Reprinted with permission from Minowa *et al.* [12].

Fig. 2. Cyclophosphamide pre-treatment increases intratumoral accumulation HER-2-targeted liposomal Dox as measured by PET. PET with [^{64}Cu]MM-302 showed increased accumulation of liposomes in BT474-M3 xenografts after treatment with cyclophosphamide, compared to controls. Reprinted with permission from Geretti *et al.* [13].

Fig. 3.

Dual-energy CT images show increased uptake of liposomal iodine in tumors treated with single high-dose RT. DE micro CT quantitation of iodine in sarcoma tumor models measures accumulation of liposomal iodine before and after a single 20-Gy dose of radiation. Irradiated tumors showed increased concentrations of iodine following treatment (bottom) compared to those of control tumors (top). Reprinted with permission from Moding *et al.* [16].

Fig. 4.

Single high-dose RT increases intratumoral Gd-HPMA copolymers in tumors, as measured with MR. MR images with Gd-HPMA copolymer contrast of animals bearing rat prostate carcinoma tumors which received 20-Gy local RT (right tumor) compared to control (left tumor) (**a**). Gd concentration, as determined from MR, was found to be significantly higher in tumors treated with RT compared to that of controls ($*p < 0.05$) (**b**). Reprinted with permission from Lammers *et al.* [21].

Fig. 5.

Fluorescent images of liposomes show increased accumulation in tumors for 48 h after hyperthermia. In human melanoma xenografts in mice, enhanced accumulation of liposomes after water bath-induced hyperthermia (41 °C for 30 min) was measured with fluorescent imaging (a). Compared to controls, hyperthermia caused increased liposome accumulation for up to 48 h post-injection (b). Reprinted with permission from Li *et al.* [72].

Table 1.
Examples of nanoparticle platforms utilized for imaging in preclinical studies of delivery

Platform	Name	Tumor model	Modality	Therapeutic component	Ref.
Liposome	Liposomal GdDTPA	Colon	DCE-MRI	None	[12]
	MM-302	HER-2+ breast	PET	Dox; anti-HER-2 antibody fragment	[13]
	Fluorescently labeled liposomes	Murine colon	NiR imaging	None	[14]
	Fluorescent PEGylated siRNA-lipoplexes	Murine colon	NiR imaging	siBcl-2	[15]
	Liposomal iodine	Primary sarcoma	CT	None	[16]
	[^{99m} Tc] liposomes	Feline soft tissue sarcoma	Gamma camera	None	[17]
	[^{99m} Tc] DSPE-PEG ₂₀₀₀	Rat fibrosarcoma	Gamma camera	None	[18]
Copolymers	Tc-99m-labeled liposomal Dox	Head and neck; squamous cell carcinoma	SPECT	Dox	[19, 20]
	HPMA-Dox	Prostate	MRI	Dox	[21]
Dendrimer	HPMA-gencitabine	Prostate	Gamma camera	Gencitabine	[21]
	G8-Gd-D	Squamous cell carcinoma	MRI	None	[22]
Iron oxide nanoparticles	Ferumoxylol (Feraheme)	Murine mammary; pancreas	MRI	None	[23, 24]
	AngioSPARK680	Breast	NiR imaging	None	[25]
Macromolecular complexes	Albumin-(GdDTPA) ₃₀	Breast	DCE-MRI	None	[26]
	Gadofosveset trisodium (albumin bound)	Mouse mammary	MRI	None	[23]

Table 2.

Imaging NP for measuring therapeutically altered NP delivery

Therapeutic strategy	Treatment	Drug	NP imaging probe	Modality	Observed effect	Ref.
Targeting tumor vasculature	VEGFR inhibition	Axitinib	Albumin-(GdDTPA) ₃₀	DCE-MRI	Reduced vascular permeability of NP	[26]
	Alk5 inhibition	LY-364947	Ferumoxylol (Feraheme)	MRI	Modest improvement of enhancement throughout tumor	[23]
		A-83-01	Liposomal GdDTPA	DCE-MRI	Increased AUC of Gd accumulation in tumor	[12]
Tumor debulking	Cytotoxic therapy	Cyclophosphamide	[⁶⁴ Cu]MM-302 (HER-2-targeted liposomal Dox)	PET	Reduced IFP; increased liposomal delivery to tumors; improved T ₁ efficacy	[13]
		S-1 (tegafur)	Fluorescent-PEGylated liposomes	NiR imaging	Increased liposomal delivery; increased homogeneity	[14]
			Fluorescent siRNA lipoplexes	NiR imaging	Increased uptake of lipoplexes in tumors; improved therapeutic efficacy	[15]
Radiation	Single high-dose RT	N/A	G8-Gd-D	MRI	Increased enhancement at multiple time points	[22]
			Liposomal iodine	Dual-energy CT	Increased iodine concentrations in tumors; increased permeability	[16]
			HPMA-Dox (Gd)	MRI	Enhanced tumor localization; increased efficacy and toxicity	[21]
Hyperthermia/thermal ablation			HPMA-gemcitabine (¹³¹ I)	Gamma camera	Enhanced tumor localization; increased efficacy, modest toxicity	[21]
			AngioSPARK680	NiR imaging	Increased accumulation of probe in tumors; increased efficacy of subsequent DoxilT _x	[25]
Hyperthermia/thermal ablation	Microwave ablation	N/A	Tc-99m liposomes	Gamma camera	Increased liposome accumulation in tumor	[17]
	Warm water catheter	N/A	[^{99m} Tc]DSPE-PEG ₂₀₀₀	Gamma camera	Increased liposome accumulation in tumor; increased Dox delivery with subsequent DoxilT _x	[18]
	Radio-frequency thermal ablation	N/A	[^{99m} Tc]Liposomal Dox	Gamma camera; SPECT	Increased liposome delivery to tumor; increased levels of Dox in resected tumor tissues	[19]
Targeting ECM and BM	Collagen remodeling	Collagenase-2	[^{99m} Tc]Doxil	SPECT	Transient drop in IFP; increased intratumoral Doxil localization	[20]
		Losartan	Ferumoxylol	MRI	Increased tumor blood pool as measured with ferumoxylol; enhanced uptake of subsequent SMI drugs	[24]