Published in final edited form as:

Adv Drug Deliv Rev. 2014 September 30; 76: 39–59. doi:10.1016/j.addr.2014.07.001.

Image-guided interventional therapy for cancer with radiotherapeutic nanoparticles[☆]

William T. Phillips^{a,*}, Ande Bao^{a,b,1}, Andrew J. Brenner^c, and Beth A. Goins^a

^aDepartment of Radiology, University of Texas Health Science Center San Antonio, Mail Code 7800, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900, USA

^bDepartment of Otolaryngology—Head and Neck Surgery, University of Texas Health Science Center San Antonio, Mail Code 7777, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900, USA

^cCancer Therapy and Research Center, Mail Code 8232, 7979 Wurzbach Road, San Antonio, TX 78229, USA

Abstract

One of the major limitations of current cancer therapy is the inability to deliver tumoricidal agents throughout the entire tumor mass using traditional intravenous administration. Nanoparticles carrying beta-emitting therapeutic radionuclides that are delivered using advanced image-guidance have significant potential to improve solid tumor therapy. The use of image-guidance in combination with nanoparticle carriers can improve the delivery of localized radiation to tumors. Nanoparticles labeled with certain beta-emitting radionuclides are intrinsically theranostic agents that can provide information regarding distribution and regional dosimetry within the tumor and the body. Image-guided thermal therapy results in increased uptake of intravenous nanoparticles within tumors, improving therapy. In addition, nanoparticles are ideal carriers for direct intratumoral infusion of beta-emitting radionuclides by convection enhanced delivery, permitting the delivery of localized therapeutic radiation without the requirement of the radionuclide exiting from the nanoparticle. With this approach, very high doses of radiation can be delivered to solid tumors while sparing normal organs. Recent technological developments in image-guidance, convection enhanced delivery and newly developed nanoparticles carrying beta-emitting radionuclides will be reviewed. Examples will be shown describing how this new approach has promise for the treatment of brain, head and neck, and other types of solid tumors.

Keywords

Radionuclide therapy; Convection enhanced delivery; Imaging; Solid tumor; Liposomes; Rhenium-186; Drug delivery; Beta-emitting radionuclides

[☆]This review is part of the Advanced Drug Delivery Reviews theme issue on “Targeted Imaging”.

*Corresponding author at: Radiology Department, University of Texas Health Science Center San Antonio, MC 7800, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900 USA. Phillips@uthscsa.edu (W.T. Phillips).

¹Present Address: Department of Radiation Oncology, School of Medicine, Case Western Reserve University/University Hospitals Case Medical Center, 11100 Euclid Avenue, Cleveland, OH 44106, USA.

1. Introduction

1.1. Challenges in drug targeting and delivery to solid tumors of intravenously administered drugs

One of the major challenges of current cancer therapy is the inability to deliver intravenously administered tumoricidal drugs throughout the solid tumor mass. One reason for this is that intravenously administered drugs are inhibited in their intratumoral penetration by high interstitial pressures which prevent diffusion of drugs from the blood circulation into the tumor tissue [1–5]. This problem is compounded by the relatively rapid clearance of intravenously administered drugs from the blood circulation by kidneys and liver. In addition, drugs that do reach the solid tumor by diffusion are inhomogeneously distributed at the micro-scale. This problem of inadequate intratumoral drug levels cannot be overcome by simply administering larger systemic doses as toxicity to normal organs is generally the dose limiting factor. The use of nanoparticles for carrying anti-cancer drugs is one method for increasing the drug accumulation in tumor following intravenous administration since the nanoparticles can be passively targeted and accumulate in the tumor through the enhanced permeability and retention (EPR) effect [6–8]. However, even nanoparticulate drugs have poor penetration from the vascular compartment into the tumor and the nanoparticles that do penetrate are most often heterogeneously distributed [9–11]. Imaging methods at the micro-scale are being developed to better understand the heterogeneous pattern of nanoparticle accumulation in an attempt to develop new therapies [12–14].

1.2. Inclusion of imaging in drug delivery studies

Imaging is becoming an integral component of drug development as well as for monitoring drug delivery and the response of targeted processes to the therapy [15–17]. Imaging can be used to guide minimally invasive procedures such as guiding a needle for tumor biopsy which is much less invasive than collecting specific tumor samples surgically [18]. Companion imaging probes targeting molecular features determined from the biopsy sample can be integrated into the drug development process. In addition, the inclusion of a companion imaging probe during drug development can aid in determining the clearance kinetics and tissue distribution of the drug non-invasively using imaging modalities such as single photon emission computed tomography (SPECT), positron emission tomography (PET), X-ray computed tomography (CT), magnetic resonance imaging (MRI), ultrasound or optical methods [19]. This companion imaging probe can also be used to determine the likelihood of the drug reaching the tumor and to what extent. In this situation of personalized medicine, individual cancer patients can be stratified for promising drug treatment responses with this type of imaging. Drugs that have increased accumulation within the targeted site are likely to be more effective as compared with others with minimal accumulation at the target site [19]. This makes treatment more efficient and cost effective. Moreover, the Food and Drug Administration requires the availability of a companion diagnostic test to select patients for targeted therapies and in many cases this diagnostic is an imaging agent [20,21].

Nanoparticle-based drugs have an additional advantage over free drugs with their potential to be multifunctional carriers capable of carrying both therapeutic and diagnostic imaging probes (theranostic) in the same nanocarrier. These multifunctional nanoparticles can serve as theranostic agents and facilitate personalized treatment planning. Additionally, nanoparticles are less likely to be affected by inclusion of an imaging component within their structure unlike small molecules, peptides, oligonucleotides and proteins (monoclonal antibodies) which can more readily lose functionality by the addition of imaging probes. The design and testing of potential theranostic nanoparticles has been a burgeoning area of research in the past 15 years. An exhaustive review of these nanoparticle constructs is outside of the scope of this article and previous review articles covering this topic are available in the literature [16,22–29].

1.3. Image-guidance for enhancing drug delivery of intravenously administered nanoparticle-based drugs

Imaging can also be used for localization of the tumor to improve the placement of a catheter or external device within tumors to cause cell death through thermal ablation or oxidative stress secondary to reactive oxygen species. Image guided thermal ablation methods include radiofrequency (RF) ablation, microwave ablation or high intensity focused ultrasound (HIFU) [30–33]. Photodynamic therapy methods using external light devices to activate photosensitizing agents can also be used to treat superficial tumors or deeper tumors when used with endoscopic catheters.

A number of nanoparticle types including iron oxide particles, carbon nanotubes, gold nanoparticles, polymeric nanoparticles, and liposomes have been tested as combination therapies to improve the efficiency of the ablation through mechanisms such as increased tumor heating and enhanced cell death in the presence of nanoparticles as well as triggered release of anti-cancer agents from the nanoparticle during the ablative procedure. A recent review of nanoparticles applied to thermal ablation has been published by Manthe et al. [34].

1.4. Radiotherapeutic nanoparticles

Over the past 20 years, wide ranging investigations have been performed into the use of nanoparticles for imaging and therapy applications, including those with liposomes, solid lipid nanocapsules, polymeric nanoparticles, dendrimers, micelles, gold nanoparticles, quantum dots, iron oxide magnetic particles, and carbon nanotubes. Several review articles have been published describing these nanoparticles [35–46]. Despite the numerous investigations of nanoparticles for cancer therapy, there has been limited effort in the development of radiotherapeutic nanoparticles for image-guided radionuclide therapies. To date, the majority of investigations of nanoparticles with radiotherapeutic nanoparticles have focused on beta (β)-emitting radionuclides. This is most likely due to their much greater penetration range in tissue (1–4 mm in average tissue penetration), especially in comparison with alpha (α)-emitters (10–100 μ m in average tissue penetration). Table 1 describes the β -emitting radionuclides that have properties suitable for use in image-guided nanoparticle radionuclide therapy with the corresponding nanoparticles that have been prepared. When considering techniques for radiolabeling nanoparticles, cost, convenience and the ability to prepare radiolabeled nanoparticles with high radiolabeling efficiency and radiolabel stability

are important factors. Stability of the radionuclide within the nanoparticle is very important. The radionuclide needs to stay associated with the nanoparticle for a minimal period of several days in order for adequate therapy to be delivered to the tumor otherwise the advantageous distribution characteristics of the nanoparticle will be lost. For historical purposes, Table 1 also includes phosphorous-32 (P-32) colloidal particles, the majority of which are in the micro-particle size range.

1.5. Ideal theranostic properties of certain beta-emitting radionuclides

As noted in Table 1, many beta-emitting radiotherapeutic nuclides used to label nanoparticles also emit a low ratio of gamma photon (~5–15 gamma photons for every 100 beta-emissions). This ratio is considered ideal because it is a high enough ratio of gamma photons for easy monitoring of intratumoral and organ distribution within the body without being so high as to deliver a significant radiation dose to the whole body or to nearby persons. Certain therapeutic radionuclides not only have an ideal ratio of gamma photons, but their photon energy is also in the ideal range for nuclear imaging, ranging from 80 to 200 keV. This energy range is ideal because it is a high enough energy for many of the emitted photons to escape the body, but a low enough energy to be easily stopped by nuclear imaging camera detectors. This is why the most commonly used diagnostic nuclear medicine imaging radionuclide is the pure gamma photon emission radionuclide, technetium-99m (^{99m}Tc), which has an ideal photon energy of 140 keV. The gamma photon emitted by these therapeutic radionuclide containing nanoparticles can be used to localize and quantitate the distribution of the nanoparticles with SPECT nuclear imaging. Therefore, these radionuclides are intrinsically theranostic in nature and this feature has greatly facilitated therapeutic nanoparticle development and these theranostic properties should prove valuable in clinical radiotherapeutic nanoparticle applications. One good example of a therapeutic radionuclide with ideal theranostic properties is rhenium-186 (^{186}Re). It has ~1 gamma photon for every 10 beta-emissions and these gamma photons have a photopeak energy of 137 keV.

2. Image-guidance for thermal ablation combined with intravenous nanoparticles

2.1. Radiofrequency ablation combined with chemotherapeutic nanoparticles

Image-guidance can be used to direct focal thermal therapy into solid tumors. This thermal ablation can be combined with intravenously administered nanoparticles to increase the effectiveness of the local tumor therapy. For example, image guidance is routinely used to direct a radiofrequency (RF) emitting needle probe into a solid tumor to induce short duration heating of tumor tissue to $>50\text{ }^{\circ}\text{C}$. When long circulating nanoparticles are injected intravenously immediately following this image-guided RF thermal damage, a greatly increased accumulation of the nanoparticles in the region of the thermal ablation occurs. This increased nanoparticle accumulation is most likely due to the increased leakage of nanoparticles into the tumor as well as their increased retention within the region associated with the thermally induced inflammation which has been described as an EPR effect [6,8]. When nanoparticles carry anti-cancer agents, the size of the tumor ablation can be significantly increased as compared to thermal tumor ablation alone [79]. Studies have

shown that the size of the tumor ablation zone is much larger when the drug is carried by a nanoparticle as compared to when the drug is free [80].

The potential to induce an increased tumor ablation zone has important therapeutic implications for solid tumor therapy. For example, although RF ablation is widely used in clinical practice, particularly for liver tumor therapy, it is often impossible to completely ablate the entire tumor due to decreased efficacy of this therapy at the highly vascularized margins of the tumors, particularly in tumors that are larger than 3 cm in diameter [81–83]. Although it is possible to achieve local control of liver metastasis in 80–90% of cases with RF ablation if the tumor is less than 2.5 cm in diameter [80], if the tumor is >2.5 cm, local tumor recurrence is common [80]. For this reason, combinations of image-guided thermal ablation with intravenously administered nanoparticles carrying a variety of anti-cancer agents has been investigated as a method to increase the effectiveness of tumor therapy [79,80, 84–86].

Extensive investigations include combinations of thermal ablation and different chemotherapeutic agents in both free form and contained within nanoparticles. In an initial study, Ahmed and Goldberg examined RF ablation combined with different formulations of chemotherapeutic agents [80]. In this study, intravenously administered nanoparticles containing doxorubicin administered at the time of image-guided RF ablation were found to have an increased amount of coagulated tumor tissue and more doxorubicin was deposited within the ablated tumor [80]. In another study by the same group, the tumor destruction diameter for RF ablation alone was 6.7 ± 0.6 mm compared with 11.4 ± 1.1 mm for RF ablation combined with free doxorubicin and 13.1 ± 1.5 mm ($P < 0.001$) for RF ablation combined with liposomal doxorubicin [79]. The greater tumor destruction found by combining intravenously administered liposomal doxorubicin with thermal ablation therapy was considered to be most likely due to a combination of increased intratumoral drug accumulation and improved cytotoxicity related to thermal effects of the RF ablation [80]. These studies were the first to show the importance of lipid nanoparticles as carriers of the drug when using thermal ablation since the nanoparticle liposome carrier was associated with an increased accumulation of doxorubicin in the tumor while non-encapsulated free doxorubicin did not have increased tumor uptake following RF ablation [80]. In fact, some studies have shown that RF ablation followed by intravenous liposomal doxorubicin was associated with intratumoral doxorubicin uptake that was 5.6-fold greater as compared with unablated tumors in a breast tumor model [87]. In addition to increased tumor cytotoxicity secondary to the increased intratumoral concentrations of doxorubicin, evidence also suggests that the lipid component of liposomes may be contributing to increased tumor damage secondary to formation of lipid hydro-peroxide leading to enhanced oxidative stress [88]. Nanoparticle size can influence the intratumoral drug accumulation and tissue coagulation. Greater tumor coagulation was observed with liposomes of 100 nm diameter compared with either 20 nm or 250 nm liposomes containing doxorubicin [84].

The significantly increased RF ablation-induced uptake of liposomal doxorubicin within tumor tissue was also shown in a head and neck model using imaging with liposomal doxorubicin radiolabeled with ^{99m}Tc or ^{186}Re [89,90]. These reports used a radiolabeling method for loading a commercial liposomal doxorubicin formulation with high activities

of ^{99m}Tc or ^{186}Re by taking advantage of the same pH gradient used to load doxorubicin into the liposomes [7]. Head et al. demonstrated that radiolabeled liposomal doxorubicin had a 3-fold increased accumulation in tumors treated with RF ablation and that intratumoral doxorubicin levels correlated with the quantitatively measured activity in the tumor using non-invasive nuclear imaging [5].

2.2. Radiofrequency ablation combined with radiotherapeutic nanoparticles

Building on the prior work with combination image-guided RF ablation and liposomal doxorubicin, Soundararajan et al. investigated the addition of radiotherapeutic nanoparticle therapy to the combination of RF ablation and doxorubicin nanoparticles by radiolabeling the liposomal doxorubicin with ^{186}Re [86,91]. Cancer therapy with these dual therapeutic carrier liposomes has been previously termed chemoradionuclide therapy [90]. When these dual therapeutic nanoparticles are combined with a third therapy of RF ablation, a further increased amount of tumor tissue can be effectively treated [86]. As can be observed in Fig. 1A, tumors treated with triple therapy had increased tumor accumulation of the ^{186}Re -liposomal doxorubicin which corresponded to greatly decreased tumor growth compared to all single and double therapy combinations (Fig. 1B) and resulted in the highest percentage of complete response to therapy (Fig. 1C). The triple therapy animals also had increased survival as compared to either the RF alone, liposomal doxorubicin alone or various combinations of dual therapies [86]. The mechanism behind the increased response of adding local radiation to the chemotherapy is unknown, but may be due to the synergistic effects of radiation with chemotherapy or due to the local 2 mm beta-particle radiation enhancing the penetration of the drug into the tumor. Although detailed studies of the mechanism need to be performed [85], triple therapy with chemoradiation nanoparticles appears promising for image-guided cancer therapy and clinical trials of this therapy are deserving.

A significant advantage of adding ^{186}Re to this therapy is that ^{186}Re has an ~10% emission of a gamma photon of 137 keV in the ideal energy range for nuclear imaging. The ability to image the distribution of the ^{186}Re -liposomes permits quantification of their location within the tumor and within the body. This theranostic ability of ^{186}Re -liposomal doxorubicin is shown in Fig. 1A in images of rats with head and neck tumors obtained over serial time points out to 5 days. In the rat treated with RF ablation (lower panel), it can be observed that the tumor uptake was significantly higher and there was prolonged retention in the tumor as compared with a rat with an unablated tumor that was also administered the same dose of ^{186}Re -liposomal doxorubicin. This ability to localize and quantify distribution permits quantification of the dose of liposomes, radionuclide and doxorubicin that accumulates in a specifically treated tumor enabling both chemo and radiation dosimetry.

2.3. High intensity focused ultrasound combined with nanoparticle therapeutics

Another area of significant research is the application of HIFU to improve the drug delivery and triggered drug release from nanoparticles. Several good reviews on the use of HIFU in nanoparticle drug delivery have been published [34,92–94]. Ultrasound technology can impart both thermal and mechanical effects to improve drug delivery from nanocarriers. For mechanical effects, gas bubbles in the mm size range are used to increase the permeability

of nanoparticles through the vessel wall or blood brain barrier following intravenous infusion or through sonoporation of cell membranes, thereby enhancing drug and gene transport into the cell [95–106]. Thermal effects from HIFU have been used for tumor ablation and mild hyperthermia. One approach is to use ultrasound image guidance to localize the tumor for HIFU therapy following intravenous infusion of the nanocarrier. The increased heating in the area causes increased permeability of the nanocarrier in the tumor. Various types of nanoparticles are under study for combination therapies with HIFU including polymeric micelles, nanobubbles, nanodroplets, microemulsions and liposomes including specially designed temperature sensitive liposomes [107–113]. To track the drug delivery in many of the studies, the nanocarriers are designed to contain various contrast agents including MRI, fluorescent and radiolabeled agents for non-invasive imaging [114–116]. Increased permeability was shown with ^{99m}Tc -liposomes with more accumulation of the ^{99m}Tc -liposomes in cat fibrosarcomas treated with HIFU hyperthermia [117]. Other groups have also tracked the accumulation of liposomes after HIFU with radiolabeled liposomes [118,119]. Several researchers are combining MRI to monitor drug release following heating of the temperature sensitive liposomes [120,121]. An advantage of MRI is that it can provide chemodosimetry or dose painting feedback to quantify the drug from the signal of MRI contrast within the tumor [92,122,123].

3. Intratumoral administration of nanoparticle drugs

The ability to adequately treat solid tumors at their primary site locally remains an important problem worthy of the development of new therapeutic approaches. Although cancer is generally considered to be a systemic disease, inability to control local cancer invasion remains an important cause of death in ~30% of all cancer patients [124, 125]. It is also likely that inadequately treated local cancer is associated with the development of cancer drug resistance which subsequently leads to locoregional or distant metastasis that go on to result in the death of the patient.

To address the problems of inadequate solid tumor targeting and drug delivery, numerous technologies using direct image-guided intratumoral administration of therapeutic agents are currently undergoing evaluation to avoid the need for drug delivery via systemic administration. Nanoparticles carrying therapeutic radionuclides have recently been shown to have important advantages for use in intratumoral solid tumor therapy. These recent developments using intratumorally administered radiotherapeutic nanoparticles as a viable option for use in cancer patients will be one focus of this review article.

3.1. Convection enhanced drug delivery

To improve drug delivery to solid tumors, an alternative to intravenous drug delivery has been developed known as convection enhanced delivery (CED). CED for cancer therapy is an approach that uses the direct injection of agents into solid tumors [126,127]. CED is performed by using image-guidance to place a catheter tip directly into solid tumor parenchyma while therapeutic agents are infused through the catheter tip under constant pressure over an extended time. The resulting pressure gradient moves therapeutic agents through the interstitial spaces of the targeted tumor by bulk flow, which results in the injected agent being more evenly distributed at higher concentrations over a larger area than

is possible without CED [126,128]. By the use of CED, a much higher tumor-to-normal tissue concentration of therapeutic agents is achieved as compared with intravenous administration, which relies solely on the diffusion of drug from the blood into the tumor. Studies have shown that CED also may have important advantages in comparison with simple intratumoral bolus injection [129]. CED was originally described in an animal model in 1994 [130] when Bobo et al. demonstrated that CED enhanced the volume of distribution of a radioactively labeled large molecule in the brain while also achieving concentrations in brain tissue that were orders of magnitude greater than systemic levels [130].

The majority of current investigations using CED have been in the brain due to the limited ability of intravenously administered drugs to cross the blood–brain barrier. Intracerebral CED has been investigated for a variety of disease processes including Parkinson's and Alzheimer's diseases in addition to its use to treat brain tumors [131,132]. For brain cancer some of the potential drugs that are being tested by CED include immunotoxins, liposomal agents and gene therapies [22,126,132–139]. CED also has important potential for use in all types of solid tumors, since all solid tumors tend to have high tumor interstitial pressure, limiting drug diffusion from the blood [1]. Therefore, solid tumors of all types are candidates for image-guided nanoparticle therapy, particularly those tumors that are not amenable to surgical removal or when the patient is not a surgical candidate. To date, intratumoral nanoparticle delivery has been investigated with head and neck tumors, prostate and melanoma tumors [48–50,64] in addition to much prior research of CED for brain tumors [128,140–145]. In addition to these solid tumors, it is the author's opinion that CED delivery with nanoparticle therapeutics also could have applications in lung, liver and breast tumors.

3.2. CED nanoparticle therapeutic as possible adjuvant therapy

CED administration of nanoparticle therapeutics has important promise as an adjuvant for solid tumor therapy. One adjuvant approach is to use CED administered nanoparticles to completely treat the margins of the tumor where residual invasive tumor cells are likely to reside. This can be done by CED administration of nanoparticles along the surgical margins of a tumor cavity during the time of the surgery as proposed by Sampson et al. [129]. CED administered nanoparticles along the surgical margins may have a more homogeneous distribution along the margins of the surgery to treat infiltrative residual tumors than CED administered nanoparticles directly into the tumor [129].

This adjuvant approach has been investigated in a head and neck solid tumor model. In this study, Wang et al. administered ^{186}Re -radiotherapeutic nanoparticles directly into residual tumor as a model of surgical tumor resection with residual tumor in an intraoperative procedure [146]. In this model, the post-surgical adjuvant therapy was very effective. Control untreated residual tumors had average tumor volumes of 290% of initial volumes at the end of the study (35 days post-treatment) while ^{186}Re -nanoparticle treated residual tumors had tumor volumes of only ~26% of their initial volumes. All groups showed consistent increases in body weight and there was no significant systemic toxicity observed in any of the animals. With excellent tumor suppression and minimal side-effect profile, the authors concluded that intraoperative use of radiotherapeutic nanoparticles may play a role

in the management of positive surgical margins in advanced head and neck squamous cell tumors.

CED nanoparticle therapeutics may also have potential in a neoadjuvant role of treating the central regions of the poorly vascularized tumors prior to administration of systemic therapy. This would have the potential of treating the central portions of the solid tumor that are difficult for intravenous drugs to reach while also potentially increasing intravenous nanoparticle delivery of drugs to the outer portions of the tumor due to EPR effects associated with the CED therapy-induced inflammation.

3.3. CED challenges

Although CED has significant advantages in cancer drug delivery, in initial clinical research it has faced significant challenges related to the 1) inability to verify the distribution and retention of the CED administered drugs, 2) inability to accurately locate the catheter within the tumor, 3) backflow of CED administered drug along the catheter [147], and 4) lack of use of carrier agents, such as nanoparticles, to improve the distribution and retention of therapeutic agents within the tumor. Many of these technical limitations have been discussed in more detail by several authors [134,148]. In the last 10 years, significant progress has been made in all of these areas including 1) significant improvements in image-guidance technologies, 2) improvements in catheter design [132], 3) development of new nanoparticles and 4) development of techniques to improve the intratumoral distribution and retention of CED infused agents based on newly gained scientific understandings of the many factors that influence CED therapy [140]. Another important area where progress has been made is the in vivo imaging technology to monitor the CED infusate distribution. Some of the tracers used in conjunction with the therapeutic agent are radiolabeled albumin, and gadolinium alone or conjugated to albumin or liposomes [135,141, 149–151]. A recent article by Allard et al. provides a thorough review of the current state of our knowledge on the delivery parameters and nanoparticle characteristics for successful convection enhanced delivery of nanoparticles to brain [152]. In recent years, many of the challenges associated with CED therapy have been addressed and these new approaches will be discussed in the following sections of this article.

3.4. Advantages of using β -emitting radionuclides with intratumoral CED nanoparticle therapy

β -Emitting radionuclides provide significant unique advantages for use with nanoparticles in the treatment of cancer. The path length of β -particles in tissue depends on the β -energy of each particular radionuclide [20]. For therapeutic β -emitting radionuclides, the β -particles have path lengths of 1–5 mm in tissue, creating a micro-field of therapy around each nanoparticle in which they are contained (Fig. 2). The 1–5 mm average path length is optimal for delivering high radiation absorbed doses throughout the tumor; while limiting the radiation absorbed dose to a very low level in the surrounding tissue. Considering that the average cell diameter in the human body is ~25–50 μ m, the micro-field around a radiolabeled nanoparticle with an average β -emission path length of 2 mm, such as ^{186}Re , can potentially treat a micro-field of 40–80 tumor cells extending radially in all directions from the nanoparticle. Higher energy β -radionuclides such as yttrium-90 (^{90}Y) and

rhenum-188 (^{188}Re), with average β -emission path lengths of 4 mm, can treat a micro-field of 80–160 cells in all directions. This micro-field greatly decreases the need for homogeneity of the actual nanoparticle distribution resulting from CED infusion. As one investigator has stated for effective cancer therapy, β -emitting radionuclides only need to remain within an “effective range” of the tumor cells for a “sufficient period” of time [54]. In contrast, the requirements for nanoparticle distribution homogeneity are much stricter for successful chemotherapeutic drug delivery, in which, generally, the drugs need to escape from the nanoparticle carrier and eventually reach every single tumor cell.

Because β -emitting radiotherapeutic nanoparticles are well dispersed and retained in solid tumors, they can deliver very high radiation doses to the tumor while greatly sparing adjacent normal tissue. In studies in our lab, glioma tumors in rats were treated with absorbed doses as high as 1700 Gray (Gy) following CED intratumoral administration of ^{186}Re -liposome nanoparticles without significant toxicity to adjacent normal brain tissue due to the relatively short path length of the β -particle radiation [142]. Initially, 50–65% of the ^{186}Re -liposome nanoparticles were retained in the brain tumor and only very slowly left the brain over a 5-day time period. The radiation dose delivered to normal organs was also very low because 35–50% of the nanoparticles that initially escaped from the brain tumor were taken up by the liver and spleen which resulted in low doses of radiation being delivered to these organs as determined from serial whole body quantitative scintigraphic imaging. These organs, with weights several hundred times larger than the CED treated tumor, received much lower radiation doses than the tumor because the concentration of any radiation delivered on a per organ weight basis is much lower than the highly focal β -radiation activity concentrated in the tumor.

The 1700 Gy radiation dose that was delivered to the tumor is much higher than can be safely delivered to tumors with external beam radiation where maximum delivered doses are generally no more than 70 Gy due to the limitation of radiation toxicity to the normal organs through which the external beam of radiation passes. Although locally applied β -emitting radiation delivered with nanoparticles can be considered a type of brachytherapy, this type of brachytherapy is very different from current clinical brachytherapy in which metallic seeds carrying low energy photon radiation are implanted in a stationary location within tumors such as prostate cancer [153]. Because photon radiation emitting agents are used with this standard brachytherapy, there is potential for significant radiation toxicity to be delivered in normal organs adjacent to the implanted seeds. Similar to external beam radiation therapy, current clinical brachytherapy use radionuclides with photon radiation path lengths of 1–3 cm which limits the total tumor radiation dose that can be delivered to generally no more than 110–145 Gy [154].

Another inherent advantage of certain β -emitting radionuclides is that they also can act as theranostic agents. Radionuclides such as ^{186}Re and ^{188}Re emit gamma photons in a ratio of approximately 10–15% of the number of β -emissions. This relatively low ratio of gamma photons can be used for SPECT imaging to determine the location of the radiation dose delivered to specific regions within the body on a quantitative basis. β -Emitting radionuclides which also emit gamma photons for diagnostic imaging purposes are shown in Table 1. This theranostic capability of specific β -emitting therapeutic radionuclides has

proven very useful in the development of rhenium-nanoparticle intratumoral therapies, permitting visualization and quantification of the distribution of these nanoparticles within the tumor and in the various organs of the body on a percent injected dose basis using non-invasive imaging [64,66,91,142,146,155].

3.5. Recent technological progress in image-guidance

Over the last decade, significant improvements have occurred in clinical imaging technology. Magnetic resonance imaging (MRI) scanners with high strength magnetic fields of 3 T or greater have become commonly available in clinical radiology facilities providing physicians with images with much higher resolution and contrast. Localization of tumors based on physiologic processes such as tumor glucose metabolism using positron emission tomography (PET) imaging fused with high resolution X-ray computed tomography (CT) cameras has become available in many hospitals. Recent progress in this clinical diagnostic imaging technology permits ever more accurate localization of pathological processes based on both anatomic structures and physiologic processes. These recent improvements in diagnostic PET/CT imaging cameras and methods has increased the accuracy in the placement of needles and catheters into tumors for more accurate biopsy assessment of tumor masses [156].

PET/CT guided biopsy of a glucose-avid tumor region has increased the accuracy of biopsy sampling and new methods of improving localization by PET/CT are currently under development [157]. The most recent advancement in clinical imaging is the introduction of PET/MRI systems that offer the potential of improved anatomic resolution and tissue characterization with MRI, and the ability to fuse high resolution MRI images with important physiologic processes such as cell division and glucose metabolism using simultaneously acquired PET images [158,159]. In addition to providing high resolution imaging that does not require ionizing radiation, MRI also provides the ability to monitor the intratumoral distribution of CED administered nanoparticles either after treatment or in real time during treatment using imaging nanoparticles that contain MRI contrast agents. PET/MRI image co-registration allows the development of CED infusion protocols to ensure that the most metabolically active regions of a solid tumor received a high concentration of nanoparticles. Nanoparticles are ideally suited for use with these hybrid imaging systems as their versatility allows the same nanoparticle to carry different diagnostic and therapeutic agents within the same nanoparticle and thereby take advantage of the strengths of each type of imaging modality and/or various combinations of different therapeutic agents [114,160]. An example of a multifunctional nanoparticle that can carry therapeutic agents as well as MRI, optical and PET imaging agents for monitoring intratumoral CED delivery of liposomes will be described in a later section of this article.

Building on these image-guidance technological improvements related to biopsy, image-guided cancer therapies have become increasingly popular in clinical practice. Common clinical applications of image-guided cancer therapy in current clinical use include image-guided placement of probes within liver tumor for their thermal ablation [161–163] and the image-guided delivery of chemotherapeutic and β -emitting radiotherapeutic microspheres administered intra-arterially guided by fluoroscopic angiography [164].

4. Advantages of nanoparticles for intratumoral CED administration

Nanoparticles have been found to have significant advantages when used for CED delivery which offer the promising potential to greatly enhance the efficacy of image-guided CED solid tumor therapy. Their principle advantages of enhanced dispersion and retention (EDR) will be reviewed in sections 4.1 and 4.2.

4.1. Nanoparticles have improved retention within the tumor

Specific nanoparticle carriers can have greatly improved retention of therapeutic agents within the tumor following CED administration in comparison with naked, i.e. non-nanoparticle associated, molecules. For brain CED studies, free drugs in solution quickly leave the brain through the blood and cerebrospinal fluid. Much of the research on CED nanoparticle delivery for brain cancer therapy has been in normal brain rather than tumors. Studies by Bankiewicz's group have demonstrated the very prolonged retention of liposomal drugs following CED administration in a rat U87 tumor model [159]. CED administered topotecan encapsulated within liposomes had a tumor clearance half-life of 1.5 days versus free topotecan which had a tumor clearance half-life of 0.1 days following CED administration [144,149].

In a study published by the authors, the tumor retention and effectiveness of ^{186}Re -liposomes were compared with a ^{186}Re -radiolabeled lipophilic small molecule (^{186}Re -N,N-bis[2-mercapto-ethyl]-N',N'-diethyl-ethylenediamine (BMEDA)) and free ^{186}Re -perrhenate after intratumoral infusion in a head and neck tumor xenograft rat model [64]. This study was specifically designed to show the advantages of the CED administered liposome carrier for use in intratumoral radionuclide therapy as compared with CED administered small molecules. At 4 h following CED administration, a significantly higher amount of ^{186}Re activity (42% of the administered dose) was retained in the tumor as compared with the naked ^{186}Re -perrhenate (only 0.2% tumor retention) and ^{186}Re -BMEDA (only 1.7% tumor retention). At 20 h, 39.7% of the ^{186}Re -liposomes were still present in the tumor whereas only 0.1% of ^{186}Re -perrhenate and 0.7% of ^{186}Re -BMEDA were retained. This difference in retention is graphically shown in Fig. 3A. Based on this prolonged tumor retention time, the radiation absorbed dose delivered to the tumor was much greater with ^{186}Re -liposomes (526 Gy) versus ^{186}Re -perrhenate (3.3 Gy) and ^{186}Re -BMEDA (13.4 Gy).

Not surprisingly, the greatly improved tumor retention and higher radiation absorbed dose delivered to the tumor by the ^{186}Re -liposomes was associated with a significantly greater therapeutic response in the group of rats infused with ^{186}Re liposomes, with virtually no observable therapeutic effect noted in the ^{186}Re perrhenate or ^{186}Re -BMEDA groups. With ^{186}Re -liposomes, there was an 87% reduction in average tumor volume with the ^{186}Re -liposomes whereas ^{186}Re -perrhenate, ^{186}Re -BMEDA or control unlabeled liposomes all had a greater than 395% increase in tumor volume as shown in Fig. 3B. Tumor responses in individual animals administered ^{186}Re liposomes are shown in Fig. 3C. Interestingly, when examined by individual treated animal, half of the tumors appeared to be completely suppressed over the 47 days of the study whereas the other half had a gradual tumor regrowth at a much slower rate than the initial growth rate of the untreated control tumors.

Several observations can be made regarding this study. First, it is important to note that the initial size of this tumor treated by CED is ~2 g which is in the size range of human tumors. Thus, CED administered ^{186}Re -liposomes are likely to have the ability to control human tumors of this size if a good CED intratumoral nanoparticle distribution can be achieved. Second, in examining the individual tumor responses as shown in Fig. 3C, it is likely that the three recurrent tumors did not receive a complete coverage of the tumor, yet the ^{186}Re -liposome treated tumors regrew at a much slower rate when compared to the initial rapid growth rate that the control untreated tumors had. A similar finding of slow recurrent tumor growth was also observed in a study by our group of ^{186}Re -liposome glioma brain tumors [142]. Although the cause of the slower tumor regrowth is not known, one possibility is that an immune response was induced. An immune response to radiotherapeutic nanoparticles administered intratumorally has been recently described with a ^{188}Re -lipid nanocapsules used for treatment of a rat glioma tumor [165]. Third, since these animals demonstrated no observable toxicity to the treatment either locally or systemically in terms of hematology or weight changes, the animals could have likely tolerated another local CED administration of the ^{186}Re -liposomes with the possibility of further prolongation of survival or cure.

Improved tumor retention was also clearly shown with another type of intratumorally administered nanoparticle, gold nanoshells. In this study by Xie et al., gold nanoshells administered intratumorally, had a 5-fold greater retention in the solid tumor at 48 h post-injection as compared with either an un-modified small molecule or a much larger molecular weight pegylated molecule [15]. An interesting finding of this study [47] is that the larger pegylated ^{64}Cu -radiolabeled molecules did not have good retention in the tumor in comparison with the gold nanoshells or in comparison with prior observations by our group of CED intratumorally administered radiolabeled liposomes [64,91,142].

These studies demonstrate the prolonged retention of nanoparticles in tumors following intratumoral CED administration. In a study in which liposome encapsulated MRI contrast agents were administered to normal brain tissue, these contrast agents cleared from the brain over a prolonged half-life of ~60 h [166]. It has been suggested that this prolonged retention is due to the uptake of liposome encapsulated agents by perivascular cells, most likely perivascular macrophages [166]. These perivascular cells are said to have relatively short half-lives as they are replenished by circulating monocytes [167], however their retention and circulation time within tumors is likely to be long enough for significant radiation to be delivered to brain tumors considering the previously observed 60 hour half -life of CED administered liposomes within brain tumor and the documented retention of CED administered liposomes within perivascular macrophages for at least 2 days [166]. However, it could also be that the nanoparticles are taken up by any of the high concentration of microglia in brain tumors which have a slow turnover [168].

In general, the high concentration of phagocytic cells in brain tumors, are likely responsible for the increased retention of CED administered nanoparticles in brain tumors, thus making these phagocytic cells very promising targets for radiotherapeutic nanoparticles. These phagocytic cells in the brain have been termed monocyte derived cells of the brain (MDCB) [169]. It has been shown that the higher the concentration of MDCB in glioblastoma tumors, the worse a patient's prognosis [170]. MDCB are thought to be associated with three

functions within gliomas including 1) promotion of tumor invasion and growth, 2) promotion of angiogenesis vasculogenesis and 3) immune suppression. Because tumors have been shown to have a higher concentration of phagocytic MDCB than normal brain [171], tumor tissue may have a longer retention of nanoparticles than normal brain. Considering that intratumoral retention time is a very important factor affecting the total radiation absorbed dose delivered from therapeutic radionuclides, nanoparticles could act as “Trojan horses” that are phagocytized by the very MDCB that are both targeted cells and responsible for their increased retention within the tumor. In the case of radiotherapeutic nanoparticles, their 2 mm beta radiation could be delivered to surrounding stem-cells within the tumor further increasing their antitumor effectiveness.

4.2. Nanoparticles have excellent dispersion through the tumor with CED administration

Work by our group and others have shown that intratumorally administered nanoparticles have excellent dispersion through solid tumors when administered by CED [166,172]. Based on work by Bao et al. using scintigraphic imaging to track the distribution of agents directly injected into tumors, nanoparticles infused by CED can have much greater spread throughout a local tissue region while also maintaining significant retention of ~50–60% of the nanoparticles in this local infused region [64,65]. This advantageous spread of nanoparticles through a solid tumor can be seen when radiolabeled liposomes were infused directly into a solid tumor as compared to the infusion of the small molecule, BMEDA (Fig. 4) [17]. The small BMEDA molecules had decreased local spread and greatly decreased local retention which was likely due to a more rapid absorption into blood capillaries.

In the case of brain cancer, most of the nanoparticle distribution studies have focused on the dispersion in normal brain tissue. Magnetic resonance imaging (MRI) of liposomes has shown that the volume of liposomes infused by CED into the normal brain tissue is highly correlated with the total brain tissue distribution volume. In these studies, the brain distribution volumes are approximately 3-fold greater than the volume of liposomes infused [173].

Interestingly, positively charged cationic liposomes, that were shown to have a 50 fold higher binding constant for normal brain tissue had approximately 3.5 times shorter dispersion distance in tissue as compared with neutral and negatively charged liposomes [166]. Based on a review of previous studies, Allard et al. proposed optimal nanoparticle properties including a particle size of <100 nm, neutral or negative surface charge and a steric coating to reduce binding of the nanoparticles to brain cells [152].

5. History of intratumorally administered radiotherapeutic nanoparticles

Prior to the development of nanoparticle-based therapeutic radionuclide agents, a moderate amount of research was performed with micron-sized particles carrying β -emitting radionuclides administered intratumorally [58,125]. These early studies with β -emitting particles are instructive regarding the potential for nanoparticle radionuclide therapy and they have been previously reviewed by McCready and Cornes [125]. Chromic phosphate particles (600–1300 nm in diameter) radiolabeled with the β -emitting radionuclide, phosphorus-32 (^{32}P), was one of the first particles investigated for direct intratumoral

radionuclide therapy. ^{32}P has a relatively long half-life of 14.29 days and a maximum β -emission energy of 1.7 MeV giving it an average tissue path length of ~ 3 mm. The large and variable size range of ^{32}P -chromic phosphate is due to the tendency of the colloidal particle to aggregate and form particles in the micron size range in a relatively uncontrolled fashion. Particles of this size range are not likely to have a significant amount of dispersion distance through the tumor when injected intratumorally; however, they are likely to have good intratumoral retention. In fact, the large ^{32}P -chromic phosphate particles were chosen by the investigators because in preliminary studies, the ^{32}P -chromic phosphate particles showed “virtually no diffusion from infiltrated regions to non-infiltrated regions”, emphasizing tumor retention over penetration within the tumor.

In spite of this minimal dispersion distance, these initial studies with ^{32}P -chromic phosphate in humans demonstrated very promising results for image-guided cancer therapy in patients. In a Phase II study of 17 patients by Firusian et al. [60], patients with chemotherapy-resistant tumors were intratumorally administered ^{32}P -chromic phosphate by ultrasound image-guidance. An encouraging response rate of 71% was reported with complete remission in 41% of the patients [60]. A wide variety of tumors were treated in this study including lymph node metastasis from adenocarcinoma of the breast, squamous cell carcinoma of the lungs, metastasis of the liver from adenocarcinoma of the stomach and colon, and primary hepatocellular carcinoma. Minimal side effects of the treatment were noted. Among the 17 cases treated, thrombocytopenia was noted in 1 patient that was treated with 3 doses of ^{32}P -chromic phosphate. In 2 other patients with superficial lymph node metastasis, transient erythema and a burning sensation without late effects was noted [60]. Interestingly, many of the treated tumors in this study developed a central cystic necrotic region which the authors attributed to the “unavoidable inhomogeneous accumulation of activity.”

In a follow-up article, this group reported a study of 14 patients with refractory head and neck cancer treated with intratumoral ^{32}P -chromic phosphate particles [61]. Eight of the 14 patients had a partial response to treatment associated with remarkable regression. Follow up pathologic investigation of treated tumors demonstrated a central cystic lesion surrounded by a centrifugal necrotic region and a marginal fibrotic structure. Three of the treated patients developed grade I/II thrombocytopenia. It is likely that this thrombocytopenia is related to the avidity of ^{32}P for bone and that the ^{32}P -chromic phosphate particles were degraded to free ^{32}P and then depositing in bone adjacent to active bone marrow platelet formation. Overall, this initial work with intratumorally administered β -emitting radionuclide carrying particles was considered successful in patients with chemotherapy resistant tumors and this work brought attention to the potential of intratumoral radionuclide particle therapy.

Table 2 reviews previous work with intratumorally administered nanoparticle carriers of radionuclides in which studies have been performed.

5.1. Liposomes

5.1.1. Head and neck cancer—The potential use of nanoparticles for delivery of therapeutic beta-emitting radionuclides intratumorally was first described by Bao et al. in

2006 (Fig. 3) [65]. This article used the diagnostic imaging agent, technetium-99m (^{99m}Tc), to study the distribution of intratumorally administered liposomal nanoparticles by SPECT/CT imaging in a rat tumor model. This article describes the promising intratumoral distribution and retention of the ^{99m}Tc -labeled liposomes within solid tumors and pointed out the promise of liposomes as carriers for the therapeutic β -emitting radionuclides of rhenium including ^{186}Re and ^{188}Re . In a previous article published in 2003, Bao et al. had already shown that the same chemistry for labeling liposomes with ^{99m}Tc was also effective for labeling liposomes with ^{186}Re [66]. Intratumorally administered liposomes carrying radionuclides had significantly improved intratumoral dispersion and retention as compared with the lipophilic small molecule, BMEDA. Based on this work, liposomes were shown to be ideal candidates for CED administration of therapeutic radionuclides in addition to other drugs. Liposomes and lipid nanoparticles are by nature bio-tolerable, due to naturally occurring metabolic pathways for lipids.

In a follow-up study in 2008, the author's group showed therapeutic efficacy of 100 nm ^{186}Re -liposomes in an intraoperative model of head and neck cancer [146]. This model was designed to treat the situation of residual tumor following cancer surgery. Rats with an initial tumor size of >3 g had a majority of their tumor removed leaving an ~1 gram tumor remnant which was intratumorally infused with ^{186}Re -liposomes as compared with a sham surgical control administered an unlabeled liposome formulation. At the end of the study at 35 days, the ^{186}Re -liposome-treated tumors were ~25% of their post-surgical baseline volume, while the control tumors were 290% of their baseline volume. A total of 8 animals were treated with ^{186}Re -liposomes; 4 treated with neutral liposomes and 4 with cationic liposomes. Both types of liposomes appeared equally effective and at the end of the study, all animals had smaller tumors than they had at post-surgical baseline. There was no significant difference in weight gain or white blood cell counts in the treated animals as compared with the control animals. This study was the first to show therapeutic efficacy of intratumorally administered radiotherapeutic liposomes for solid cancers.

Intratumoral CED therapy of a solid head and neck tumor model with ^{186}Re -liposomes was previously described in Section 4.1 in regard to intratumoral retention.

Excellent results were found using ^{186}Re -liposomes administered by CED to head and neck tumor xenografts of 2 g in size at the time of initial therapy (see Fig. 3).

5.1.2. Glioblastoma—In 2012, our group showed promising results in the use of CED administered ^{186}Re -liposomes for the treatment of glioblastoma in U87 rat glioma model and in the more aggressive U251 rat glioma model [142,174]. Glioblastoma is an ideal target for local nanoparticle radionuclide therapy due to its poor prognosis with current therapies and local uncontrolled growth as the cause of death with 90% of tumor recurrences occurring within 2 cm of the resection. In the initial portion of the study, the ability to perform non-invasive diagnostic imaging was very useful for determination of the volume and rates to be used for the intratumoral CED infusion. Follow determination of ideal CED parameters, a wide variety of ^{186}Re -liposome radiation absorbed doses were examined and a significant therapeutic benefit was noted in U87 tumors with doses of greater than 100 Gy with >50% of the animals surviving until the end of the study at 120 days. No significant

toxicity was noted in doses up to 1500 Gy. Serial MRI images clearly demonstrated the therapeutic effect of the ^{186}Re -liposomes particularly in comparison with control images as shown in Fig. 5A.

In a follow up of the more aggressive U251 tumor, rats treated with ^{186}Re -liposomes, carrying a mean dose of 1700 Gy, were compared with control rats (Fig. 5B). No control rats survived past 35 days post tumor implantation while 83% of the treated rats were still alive at 130 days post implantation. Histology did not reveal U251 tumor cells in any of the surviving rats.

These pre-clinical results in this aggressive glioma model show the promising potential of CED administered ^{186}Re -liposomes for glioblastoma therapy. Based on extensive pre-clinical work with ^{186}Re -liposomes demonstrating their efficacy for solid tumor therapy, ^{186}Re -liposomes appear to be promising therapeutic agents for image-guided CED treatment of glioblastoma and the local control of other types of solid tumors.

5.2. Solid lipid nanocapsules

In 2008, Allard et al. described the use of solid lipid nanocapsules as carriers for the therapeutic radionuclide, ^{188}Re , for the treatment of malignant gliomas [69]. ^{188}Re has a β -emission with twice the energy of ^{186}Re and an average tissue path length of 4 mm. Rats with 9L brain tumors were treated with ^{188}Re -nanocapsules at 6 days after implantation. The survival time was increased by 80% in the treated rats as compared with the control rats with 33% of the rats being long-term survivors. This study was the first to show the *in vivo* efficacy of ^{188}Re internal radiotherapy for the treatment of brain malignancy.

5.3. Radioactive gold nanoparticles

In 2008, Khan et al. described the use of an intratumorally administered radioactive gold-198 (^{198}Au) dendrimer composite nanodevice in the treatment of a mouse melanoma model [48]. ^{198}Au has a half-life of 2.7 days and an average β -radiation tissue path length of 2 mm, similar to ^{186}Re . Eight days after treatment, there was a statistically significant 45% reduction in tumor volume when compared with untreated groups and those injected with the “cold” nanodevice. No toxicity was observed in the treated mice.

In 2010, radioactive gold-198 (^{198}Au) nanoparticles were investigated in a model of prostate cancer [49]. These gold nanoparticles had a hydrodynamic diameter of 85 nm.

On day 8 after implantation in the flank of mice, tumors were treated with ^{198}Au gold nanoparticles and tumor growth was compared to control animals receiving injections of phosphate buffered saline. Three weeks after treatment, the tumors of the treated animals were 82% smaller in comparison with the tumor volume of the control animals. At the end of the 3-week period, 19% of the initially infused ^{198}Au still remained in the tumor. On pathological examination, the tumor consisted mostly of necrotic tissue, indicating extensive tumor cell killing. This group also functionalized the ^{198}Au -nanoparticles by adding epigallocatechin-gallate (EGCG) to the surface of the ^{198}Au -nanoparticles to specifically target the laminin receptor which is over-expressed on prostate cancer cells. In a mouse prostate cancer model, there was an 80% reduction in tumor volume at 28 days after

treatment with the intratumorally administered EGCg-¹⁹⁸Au-nanoparticles [50]. A recent review of the investigations of intratumorally administered functionalized radioactive gold nanoparticles in tumor therapy has been published [175].

5.4. Metallofullerene nanoparticles radiolabeled with lutetium-177 (¹⁷⁷Lu)

In 2011, a multimodal β -emitting metallofullerene-based nanoplatform was developed for brain tumor brachytherapy. This nanoplatform carried the β -emitting radionuclide of ¹⁷⁷Lu [54] while also carrying the MRI contrast agent, gadolinium. ¹⁷⁷Lu has an average β -radiation tissue penetration distance of 0.7 mm and a radionuclide half-life of 6.7 days. When administered by CED to mice with intracranial U87 brain tumors implanted 8 days previously, the treated mice had survival times of >2.5 times the control untreated mice. At the end of the study at 60 days, small but viable tumor remained present in the brain of the treated mice; however, the small size suggests that the growth rate of the tumor was very significantly slowed. Immediately after CED infusion, 60% of the infused dose remained in the region of the brain tumor. Follow-up MRI images demonstrated an extended retention of the metallofullerenes within the tumor region with 25% of the administered metallofullerenes retained at 52 days [54]. In contrast, free chelated gadolinium injected intratumorally cleared from brain rapidly with <1% of the infused dose retained in the brain at 24 h. The size of these metallofullerene particles in vivo was not easily determined as in vitro studies of their particle size ranged from 1 to 20 nm depending on the pH of the medium with larger clustering of metallofullerenes observed at lower pH. One of the limitations of this study noted by the authors was that there was no comparison of the therapeutic efficacy of the ¹⁷⁷Lu-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) chelator alone, however based on the studies by our group, it is likely that ¹⁷⁷Lu-DOTA administered alone would not be effective for brain tumor therapy due to its rapid clearance from the tumor [47,64]. A second limitation of the study is that the initial tumors were very small at the time of treatment. At 8 days post implantation, tumors were not well visualized on the baseline MRI images and represent less of a therapeutic challenge in comparison with larger brain tumors.

6. Future directions for image-guided nanoparticle therapy

6.1. Development of methods to improve CED coverage of and retention within a solid tumor

Future research will be needed to investigate convenient and effective methods to achieve complete coverage of a solid tumor. Using current CED technology, complete coverage of the tumor with CED infused nanoparticles will likely require that multiple infusions be administered at spaced intervals within the same tumor. Based on current work by the authors using standard CED systems, nanoparticle tissue coverage from CED is estimated at ~1–2 g of tumor tissue. Current research is being conducted to increase CED tissue coverage using a variety of techniques [128,140,143,176–179].

6.1.1. Improvement in catheter design—In the last decade, there has been significant improvement in the design of CED catheters to allow greater CED coverage of an infused tumor. These improvements included the use of catheters with tips of less than 1 mm in

diameter to decrease tissue trauma [180], and the use of stepped and recessed step catheters to facilitate high flow infusions with great volume of distributions and eliminate backflow along the catheter track [140]. However, despite these advancements, there is still much room for further improvements in catheter design such as the development of multiple pore and multipronged catheters that will cover larger tumor volumes from a single infusion.

6.1.2. Use of non-invasive image guidance to improve solid tumor coverage

6.1.2.1. Advanced imaging for monitoring intratumoral nanoparticle distribution and tumor response:

To date, only a few studies have examined in a quantitative fashion the intratumoral retention and intratumoral distribution of nanoparticles [64,65,142,146]. Non-invasive imaging is ideally suited to monitor tumor retention and distribution of CED administered agents and to correlate tumor response to the delivery and retention of nanoparticles in individual patients. With radiotherapeutic nanoparticles which emit gamma photons as well as therapeutic beta particles, it will be possible to correlate the distribution and retention of intratumorally administered agents with the therapeutic response of the tumor in individual patients. In the future, it may be possible to determine the distribution and therapeutic dose delivered to a treated tumor in real time using advanced imaging techniques.

In addition, physiologic imaging of the tumor response to CED administered radionuclide therapy can be done using PET imaging. Rapid assessment of tumor treatment efficacy is possible with a variety of PET radiopharmaceuticals. For example, markers of cell division such as fluorine-18 (^{18}F)-fluorothymidine could provide feedback, feasibly within a 1-week time period following the intratumoral CED nanoparticle radiotherapy. An advantage of ^{18}F -fluorothymidine is that it specifically demonstrates regions in the tumor of rapid cell division; and unlike, ^{18}F -fluorodeoxyglucose (^{18}F -FDG) is not increased typically in sites of inflammation. With the advent of clinical PET/MRI high resolution imaging scanners, focal regions of residual tumor could be retreated with specifically targeted image-guidance and without fear of local toxicity, which is a major advantage of β -emitting radionuclides due to their limited path length of the β -particle radiation.

Image-guidance is likely to play a very important role in the placement and real-time changes in the location of the CED catheter with a tumor undergoing treatment with nanoparticles that can be imaged. The best location of placement for the CED treatment of solid tumors is not known due to very little research that addresses this problem. Because the intratumoral pressure is highest in the center of the tumor which tend to move nanoparticles out towards the tumor rim, the central regions of the tumor would appear to be an important targeted region for CED infusion. Most of the current research with CED has been performed in small animal models of normal brain or with tumors, and which is not likely to translate well to human studies. Restriction of this prior work to small animals is likely due to the minimal availability of large animal brain tumor models [141,148]. It is the author's belief that the best approach will be to use real-time imaging, likely MRI, to ensure that nanoparticles have covered as much of the tumor as possible, including the core and rim of the tumor, until more knowledge is gained from future clinical real-time intratumoral distribution studies. Because current distribution patterns are not entirely predictable, most

researchers have emphasized the importance of real-time image guidance that adjusts the placement of the CED infusion during the treatment so as to paint as much of the tumor as possible [129,141, 143,147].

6.1.2.2. Prediction of nanoparticle distribution based on tumor microstructure as determined from prior high resolution anatomic imaging: High resolution MRI scanners with very high strength magnetic fields have been recently described for use in brain imaging, providing micro-anatomic details and an in-plane resolution of 0.23 mm in human brain [181]. It may be possible to use this high resolution, anatomically detailed MRI imaging of tumor microanatomy to plan and predict distribution of CED administered nanoparticles within tumors. Extensive pre-therapy planning could be performed to determine the precise locations of catheter placements and infusion volumes and rates using computer algorithms. Actual distributions could be monitored with real-time high resolution MRI [143] with the possibility of on-the-fly adjustment of CED infusions to ensure total tumor volume coverage.

6.1.2.3. Development of treatment planning systems for CED catheter planning and real-time imaging to monitor CED tumor coverage: To achieve full tumor coverage, planning systems using computer based algorithms could be developed to pre-select the site of CED infusions based on known CED volumes of distribution and potentially using microstructural information of the tumor obtained from high resolution MRI imaging prior to treatment. Feasibility of this real-time approach has already been shown in primate brain using liposomes carrying MRI contrast agents [143]. This liposomal MRI contrast made it possible to monitor the intratumoral distribution of nanoparticles administered by CED using MRI. Nanoparticles carrying both MRI contrast and β -emitting radiotherapeutic isotopes could be administered under intraoperative MRI-guidance in real-time to ensure the proper distribution within the tumor. This real time application of MRI image guidance for CED administration could ensure that the entire tumor is covered with radiotherapeutic nanoparticles. This real-time image-guidance is similar to the current use of advanced MRI and PET/CT for “dose painting” by external beam radiation therapy [182].

6.1.2.4. Potential for image-guided robotically administered CED: Radiotherapeutic nanoparticles have potential to be delivered into solid tumors by robots using image-guidance to achieve complete tumor coverage. Robotic placement of catheters using image guidance has recently been described as a method for obtaining brain stem biopsies with promising results based on image-guidance and frameless stereotactic localization of the biopsy needle by the robot [183]. In one study, 15 patients (five children and ten adults) underwent biopsies of brain stem lesions using a frameless image-guided robot. The biopsy procedure required four stages: image acquisition, preoperative planning, patient-to-image registration, and operative procedure. There was no operative mortality. The investigators concluded that the use of frameless stereotactic techniques for brain stem biopsies could increase the number of biopsies and therefore improve the diagnostic yield and accuracy of the technique.

Feasibility of using robots for delivering a CED therapy has also recently been demonstrated. In this study, robotic delivery of carboplatin into a diffuse pontine glioma was

performed [179]. This procedure used stereotactic planning software to develop the catheter placement approach. In this procedure, a transfrontal trajectory for catheter implantation was planned for positioning of a recessed-step catheter. The catheter was implanted using a specially designed robot for brain catheter placement. Carboplatin was infused into the tumor while real-time T2-weighted MRI was performed, facilitating estimation of the total volume of infusate distribution [179].

6.2. Further investigations into the ideal characteristics of nanoparticles for CED with enhanced volume of distribution and tumor retention

More in-depth knowledge is required regarding the effect of nanoparticle size on the volume of distribution achieved during CED administration. How the size of the nanoparticle relates to the volume of distribution, and perhaps, more importantly, the retention of the nanoparticle within the tumor are areas for further research. Also, the effect of surface charge and the addition of specific surface receptors on the CED volume of distribution are thought to be important parameters to consider. In a study by Szoka's group, liposomes with cationic surface charge had less dispersion distance than liposomes with neutral or negative surface charges [73]. Initial studies examining these characteristics have been performed, but further more detailed studies are needed. Non-invasive imaging in clinical studies with MRI and nuclear imaging (PET and SPECT) can be used to examine different nanoparticle characteristics on volume of distribution and retention in targeted tumor tissue.

6.2.1. Investigations of the most ideal size of nanoparticles for CED

administration—Only a few studies have been performed specifically looking at the most ideal size of nanoparticles for CED drug carriers. Theoretically, the size of the nanoparticle would be smaller than the pore size of the extracellular matrix of the tissue being treated. The majority of the research investigating extracellular matrix pore size has been for brain drug delivery. The pore size of the extracellular matrix of normal brain extracellular space has been reported to be 38–64 nm in size [184] and the pore size of the extracellular space of brain tumor was found to be larger at 70–100 nm in size [185]. Zhou et al. have suggested that nanoparticles for the treatment of brain tumors by CED should be 60–80 nm in diameter to allow access to the brain tumor while sparing the normal brain [185]. Zhou et al. developed a 74 ± 18 nm PLGA nanoparticle and compared its CED volume of distribution to 150 ± 30 nm diameter PLGA nanoparticle in normal rat brain [186]. The mean volume of distribution of the small 74 nm nanoparticles was ~7 times greater than the 150 nm nanoparticles. These 74 nm nanoparticles were also studied in a normal pig brain model and the volume of distribution (Vd)/volume of infusion (Vi) ratio was 3.5 with a total volume of distribution of 1.18 cm^3 which was considered to be clinically relevant due to the fact that most glioblastomas recur within 2 cm of their original location.

The type of nanoparticle may also play a role in their distance of penetration by CED. For example, liposomes which have some deformability could potentially squeeze through pores without losing their integrity. In a study investigating different types of CED administered liposomes, liposome nanoparticles of up to 200 nm in size and of neutral or negative charge were shown to have a similar radius of penetration when injected into normal rat brain [166]. No significant differences in the radius of penetration were noted for neutral

liposomes of 85 nm in diameter or negatively charged liposomes of 83 nm in diameter. Neutral liposomes of 197 nm had a slightly smaller radius of penetration than the 85 nm liposomes. Nanoparticles larger than 200 nm began to be retained near the point of infusion. In this study, 10 kDa dextran molecules penetrated significantly further in tissue as compared to 83 nm liposome nanoparticles when administered by CED, however their time of retention in the tumor was not assessed [166].

Although studies are limited, it appears that tissues outside of the brain may have significantly large extracellular space pores. Although not the same as extracellular space pores, the vascular pore cutoff sizes in tumors grown subcutaneously (200–1,200 nm) was much larger compared to 100–380 nm for intracranially grown tumors [187]. The possibility of substantially larger pore cutoff sizes in the extracellular matrix of tumors outside the brain could make image-guided delivery to these tumors more rapid and easier to accomplish. In a rat head and neck tumor, the CED infusion rate was 0.5 ml/min which is 100–200 times more rapid than in the typical CED infusion protocols in brain CED applications. In this head and neck model, non-invasive imaging demonstrated fairly homogeneous distribution throughout the tumor. Certainly, much more investigation of nanoparticle CED applications in tumors other than brain are needed.

6.2.2. Multifunctional nanoparticles for tracking CED distribution within tumors—An article by Li et al. and authors of this paper have shown that the same liposome can serve as a carrier not only for the therapeutic radionuclides of rhenium, ^{186}Re and ^{188}Re , but simultaneously also for a therapeutic drug, as well as 4 different types of imaging contrast agents including an MRI contrast agent, PET and SPECT nuclear diagnostic imaging agents and optical contrast agents as shown in Fig. 6 [160]. The ability to perform multiple types of imaging on the same nanoparticles will allow studies investigating the distribution and retention of nanoparticles initially in vivo using non-invasive imaging and later at the histological level using optical imaging.

6.3. Therapeutic ultrasound to enhance the convection of intratumoral nanoparticles

One promising approach under investigation is the use of therapeutic ultrasound-assisted CED [178]. With this approach, the vibration of the ultrasound probe during convection enhanced delivery results in an enhanced rate of intratumoral administration and a larger volume of distribution of infused agents. Studies in the rat brain using a novel, low profile ultrasound transducer positioned in the brain along with the CED catheter demonstrated that the volume of the CED infusion could greatly increase the volume of distribution of Evans Blue dye by ~3-fold in normal brain. From histological examination of the brain, no changes were noted that could be attributed to the perturbation from ultrasound application. Certainly, further investigations of this promising CED delivery technique are needed. The challenges for this ultrasound technology and CED cancer therapy in general were concisely addressed in a commentary by J.H. Sampson Jr. [148].

6.4. Investigations into different β -emitting radionuclides for intratumoral image-guided therapy

Further investigations can be performed with different radionuclides for potential use in radionuclide nanoparticle therapy. For different applications, different tumor types and different tumor locations in the body, there may be specific advantages in using radionuclides with different half-lives and different beta energies. For example, the shorter half-life and longer tissue path length of ^{188}Re may have specific advantages for tumor treatment where the longer tissue path length will prove advantageous. Liposomes have already been shown to be stably labeled with ^{188}Re by Chen and Chang et al. [73,188,189] who have reported promising results of ^{188}Re -liposomes for intravenously administered anti-tumor applications. There could also be important cost advantages to specific radionuclides. For instance, the majority of radionuclides for therapy are made from a reactor and used in a fairly short time frame of less than a week. The rapid use is associated with significant production cost and expedited shipping. Other therapeutic radionuclides are produced using generator technology. ^{188}Re (half-life of 17 h, tissue path length of 4 mm) can be produced from a tungsten-188 (^{188}W)/ ^{188}Re generator similar to the current production of $^{99\text{m}}\text{Tc}$ for diagnostic imaging [190]. The availability of generator-produced therapeutic β -emitting radionuclides could significantly lower the cost of radionuclide nanoparticle therapy. The $^{188}\text{W}/^{188}\text{Re}$ generator could be used for up to 6 months with doses capable of treating many patients being eluted from this generator every 2 days. This radionuclide also has a photon with an ideal SPECT imaging photon energy of 155 keV. The low cost of this small, highly portable $^{188}\text{W}/^{188}\text{Re}$ -generator for use in tumor therapy with liposome nanoparticles on a per-patient basis could make radionuclide nanoparticle cancer therapy feasible in locations without access to advanced technology. Although this therapy is ideally delivered with MRI and PET image-guidance, the recent advancements in less expensive ultrasound imaging could also be used for radiotherapeutic nanoparticle therapy and palliation of ultrasound accessible tumors. Diagnostic imaging ultrasound for image-guidance delivery in cancer chemotherapy has been recently described [191].

6.5. Nanoparticles as ideal carriers to target intratumoral macrophages/microglia

Many tumors have been shown to have high concentrations of macrophages and in many tumor types, it has been shown that higher macrophage concentration within tumors is associated with a poorer prognosis [170,192–196]. In recent years, macrophages located within the tumor have become recognized as important cells supporting tumor growth [169,197–199], and this information has led to macrophages becoming important targets for the treatment of solid tumors including gliomas [198]. Because nanoparticles are prone to opsonization by blood and other body fluids [200], they serve as natural carriers that target macrophages due to the inherent phagocytic response of macrophages to particles [200]. It is well known that the majority of nanoparticles are taken up by macrophages in the liver, spleen and bone marrow following intravenous nanoparticle administration. Although not well studied, nanoparticle uptake by intratumoral macrophages is also highly likely to occur following intratumoral nanoparticle administration.

Very few investigations have been performed to determine the ultimate cellular location of nanoparticles following intratumoral CED administration. Only one recent study related to

cellular location could be found by the authors in which 223 nm and 193 nm PLGA nanocapsules were administered intratumorally [201]. Histological examination at 48 h post-intratumoral infusion demonstrated that the PLGA nanoparticles were located exclusively in intratumoral macrophages.

6.5.1. Macrophage/microglia in glioblastoma tumor—Glioblastoma tumors are heavily infiltrated by cells of microglia and macrophages located in the tumor stroma, termed monocyte derived cells of the brain (MDCB). These MDCB can comprise up to 30% of the total glioblastoma tumor mass [202]. Increasing evidence has also shown that MDCB assist in glioma cell growth, motility and invasion [203]. MDCB have been shown to be closely associated with a subpopulation of glioma cells known as glioma stem-like cells (GSLCs) [22]. Recent studies show that these GSLCs are much more efficient at forming tumors in vivo and are likely to be substantially more invasive than the bulk of tumor cells [204]. GSLCs also appear to be the most resistant to radiation therapy [205].

A strategy to target microglia/macrophage stromal cells has been suggested to improve glioblastoma therapy [203]. An advantage of targeting the microglia/macrophages within glioblastoma is that they are genetically normal cells that have undergone a phenotypic transformation that under normal circumstances would be physiologic which would make them less likely to develop resistance to a therapy that targets them [203].

Nanoparticles carrying beta-emitting radionuclides have particular promise for effectively delivering therapy to both MDCB and associated GSLCs. Phagocytic MDCB in the glioblastoma tumor stroma are highly likely to phagocytose intratumorally delivered beta-emitting nanoparticles and radiation therapy would not only be delivered to these MDCB but the beta-particle radiation traveling through 2–4 mm of tumor tissue will also treat GSLCs. Because the local dose delivered can be much higher than with standard external beam radiation therapy, radiation resistance of GSLC would be overcome by the very high local dose delivered. In previous studies with ^{186}Re -liposome nanoparticle administered intratumorally, the dose of radiation delivered to the tumor was >1500 Gy [142]. A high radiation dose delivered to MDCB and GSLCs may provide one explanation for the surprising effective results obtained in the rat glioma tumor models with >80% of treated animals having long term survival with no residual evidence of tumor at the termination of the study [142].

6.5.2. Macrophages/microglia as intratumoral transporters of nanoparticles—

The high macrophage concentration in tumors, particularly along the growing margins of the tumor may be an important component of the natural retention of nanoparticles in tumors following their CED administration, thus providing a mechanism administered liposome nanoparticles within tumor as compared to normal brain (unpublished observation by our group) [206]. It is also possible that macrophages within a tumor could carry intratumorally administered nanoparticles to the most active growing regions of the solid tumor, including the invasive margins of the tumor. This possibility is graphically shown in Fig. 7.

Intratumoral macrophages could take up the CED administered nanoparticles and serve as a carrier to transport the nanoparticles to actively growing sites in the tumor.

This possibility merits further research to determine if local intratumoral macrophage transport is occurring. Macrophages have already been proposed to be carriers of therapeutic agents by preloading macrophages *ex vivo* with therapeutic agents and administering them intravenously [207]. This approach assumes mobility of the macrophages within the tumor. The loading of intratumoral macrophages with nanoparticles may be a simpler approach to achieve the same goal.

Certainly, more detailed investigations of the location of intra-tumorally administered nanoparticles over time post CED infusion will be important to develop a better understanding of intratumoral nanoparticle therapies, particularly those using β -emitting radionuclides. A significant advantage of this macrophage or tumor phagocytosis in regard to β -emitting radiotherapeutic nanoparticles, are that the physical location of the β -emitting radionuclides does not require release from the interior of the macrophage for effective treatment of a 40–100 cell diameter sphere of tumor cells surrounding the phagocytic cell and radiation damage to these phagocytic cells is not instantaneous, permitting time, likely days, for the macrophage to move within the tumor after phagocytosis of the β -emitting radionuclide nanoparticle. Evidence for the prolonged survival of tumor cells and likely associated macrophages is provided by a recent study using ^{186}Re -liposomes to treat an intracranial glioma xenograft. In this study, optical methods were used to non-invasively assess the viability of luciferase transfected-glioma cells following ^{186}Re -liposome intratumoral treatment (Fig. 8A). After a mean absorbed dose of 1700 Gy of ^{186}Re -liposomes, tumor cell death gradually occurred over a 12 day period (Fig. 8B). Considering the multi-day half-life of most β -emitting radionuclides, the slow death of tumor cells and tumor associated macrophages, beta-emission therapy could be transported by intratumoral phagocytic cells to the most rapidly growing tumor regions for an extended period prior to the death of the macrophage from β -radiation therapy.

6.6. Potential for combination of chemotherapy with radionuclide nanoparticle therapy, i.e. chemo-radionuclide therapy

As described previously regarding image-guided RF ablation, studies with intravenously administered liposome nanoparticles containing both ^{186}Re and doxorubicin demonstrated an improved tumor response in a large solid tumor model in rats as compared to liposomes containing only doxorubicin [86,90,208]. A nanoparticle containing both ^{188}Re and doxorubicin for the intravenous treatment of a colon cancer model has also shown a synergistic tumor treatment efficacy of this combination [209]. These studies demonstrate the significant promise that locally applied combination chemo-radionuclide carrying nanoparticles could be more effective in treating solid tumors by image-guided local application compared to each agent being applied separately. In glioblastoma therapy, ^{186}Re -liposomes could be combined with traditional intravenous glioblastoma chemotherapy with agents such as temozolimide and this has been proposed as a promising direction for future work [174].

6.7. Potential for immunostimulation of radiotherapeutic nanoparticles

In a recent study, intratumorally administered solid lipid nano-capsules carrying ^{188}Re have been shown to induce an anti-tumor immune response following local application in a rat

brain tumor model [165]. This immune response has been attributed to the unique combination of the immunostimulatory properties of nanoparticles with the ability of ionizing radiation to induce immunogenic tumor cell death [165]. The authors of the paper have proposed that further strategies such as nanoparticle modification with immunostimulants could be used to increase an induced immune response against the tumor. Further investigations of the immune response induced by local nanoparticle radionuclide therapy appear promising. We agree and note that local beta-emission immunoradiotherapy has appealing potential in that the radiation therapy is mainly limited to the local tumor region, while appearing to have limited toxicity to the remaining immune system, thus providing potential of a significant immune response against anti-genic tumor tissue released in the local region following radionuclide nanoparticle therapy.

7. Conclusions

Image-guided radiotherapeutic nanoparticles have significant potential for solid tumor cancer therapy. The current success of this therapy in animals is most likely due to the improved accumulation, retention and dispersion of nanoparticles within solid tumor following image-guided therapies as well as the micro-field of the β -particle which reduces the requirement of perfectly homogeneous tumor coverage. It is also possible that the intratumoral distribution of nanoparticles may benefit from their uptake by intratumoral macrophages although more research is required to determine the importance of this aspect of intratumoral radionuclide nanoparticle therapy. This new approach to cancer therapy is a fertile ground for many new technological developments as well as for new understandings in the basic biology of cancer therapy. The clinical success of this approach will depend on progress in many areas of interdisciplinary research including imaging technology, nanoparticle technology, computer and robot assisted image-guided application of therapies, radiation physics and oncology. Close collaboration of a wide variety of scientists and physicians including chemists, nanotechnologists, drug delivery experts, radiation physicists, robotics and software experts, toxicologists, surgeons, imaging physicians, and oncologists will best facilitate the implementation of this novel approach to the treatment of cancer in the clinical environment. Image-guided nanoparticle therapies including those with β -emission radionuclide nanoparticles have excellent promise to significantly impact clinical cancer therapy and advance the field of drug delivery.

Acknowledgments

This research was supported in part by NIH grants 5 P30 CA054174-16 and R01 CA131039. The authors also appreciate Jonathan Sumner for his help in figure preparation.

References

1. Stohrer M, Boucher Y, Stangassinger M, Jain RK. Oncotic pressure in solid tumors is elevated. *Cancer Res.* 2000; 60:4251–4255. [PubMed: 10945638]
2. Lammers T, Kiessling F, Hennink WE, Storm G. Drug targeting to tumors: principles pitfalls and (pre-) clinical progress. *J. Control. Release.* 2012; 161:175–187. [PubMed: 21945285]
3. Ernsting MJ, Murakami M, Roy A, Li SD. Factors controlling the pharmacokinetics biodistribution and intratumoral penetration of nanoparticles. *J. Control. Release.* 2013; 172:782–794. [PubMed: 24075927]

4. Brown JM, Giaccia AJ. The unique physiology of solid tumors: opportunities (and problems) for cancer therapy. *Cancer Res.* 1998; 58:1408–1416. [PubMed: 9537241]
5. Minchinton AI, Tannock IF. Drug penetration in solid tumours. *Nat. Rev. Cancer.* 2006; 6:583–592. [PubMed: 16862189]
6. Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. *J. Control. Release.* 2000; 65:271–284. [PubMed: 10699287]
7. Iyer AK, Khaled G, Fang J, Maeda H. Exploiting the enhanced permeability and retention effect for tumor targeting. *Drug Discov. Today.* 2006; 11:812–818. [PubMed: 16935749]
8. Torchilin V. Tumor delivery of macromolecular drugs based on the EPR effect. *Adv. Drug Deliv. Rev.* 2011; 63:131–135. [PubMed: 20304019]
9. Bhagat M, Halligan S, Sofou S. Nanocarriers to solid tumors: considerations on tumor penetration and exposure of tumor cells to therapeutic agents. *Curr. Pharm. Biotechnol.* 2012; 13:1306–1316. [PubMed: 22201591]
10. Holback H, Yeo Y. Intratumoral drug delivery with nanoparticulate carriers. *Pharm. Res.* 2011; 28:1819–1830. [PubMed: 21213021]
11. Ishida T, Kiwada H. Alteration of tumor microenvironment for improved delivery and intratumor distribution of nanocarriers. *Biol. Pharm. Bull.* 2013; 36:692–697. [PubMed: 23649327]
12. Herzog E, Taruttis A, Beziere N, Lutich AA, Razansky D, Ntziachristos V. Optical imaging of cancer heterogeneity with multispectral optoacoustic tomography. *Radiology.* 2012; 263:461–468. [PubMed: 22517960]
13. Mikhail AS, Eetezadi S, Ekdawi SN, Stewart J, Allen C. Image-based analysis of the size- and time-dependent penetration of polymeric micelles in multicellular tumor spheroids and tumor xenografts. *Int. J. Pharm.* 2014; 464:168–177. [PubMed: 24440400]
14. Rygh CB, Qin S, Seo JW, Mahakian LM, Zhang H, Adamson R, Chen JQ, Borowsky AD, Cardiff RD, Reed RK, Curry FR, Ferrara KW. Longitudinal investigation of permeability and distribution of macromolecules in mouse malignant transformation using PET. *Clin. Cancer Res.* 2011; 17:550–559. [PubMed: 21106723]
15. Lammers T, Kiessling F, Hennink WE, Storm G. Nanotheranostics and image-guided drug delivery: current concepts and future directions. *Mol. Pharm.* 2010; 7:1899–1912. [PubMed: 20822168]
16. Lanza GM, Moonen C, Baker JR Jr, Chang E, Cheng Z, Grodzinski P, Ferrara K, Hynynen K, Kelloff G, Lee YE, Patri AK, Sept D, Schnitzer JE, Wood BJ, Zhang M, Zheng G, Farahani K. Assessing the barriers to image-guided drug delivery. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* 2014; 6:1–14. [PubMed: 24339356]
17. Qin S, Fite BZ, Gagnon MK, Seo JW, Curry FR, Thorsen F, Ferrara KW. A physiological perspective on the use of imaging to assess the in vivo delivery of therapeutics. *Ann. Biomed. Eng.* 2014; 42:280–298. [PubMed: 24018607]
18. Gupta S, Madoff DC. Image-guided percutaneous needle biopsy in cancer diagnosis and staging. *Tech. Vasc. Interv. Radiol.* 2007; 10:88–101. [PubMed: 18070687]
19. Kircher MF, Hricak H, Larson SM. Molecular imaging for personalized cancer care. *Mol. Oncol.* 2012; 6:182–195. [PubMed: 22469618]
20. Abi-Jaoudeh N, Duffy AG, Greten TF, Kohn EC, Clark TW, Wood BJ. Personalized oncology in interventional radiology. *J. Vasc. Interv. Radiol.* 2013; 24:1083–1092. (quiz 1093). [PubMed: 23885909]
21. Lee JM, Han JJ, Altwerger G, Kohn EC. Proteomics and biomarkers in clinical trials for drug development. *J. Proteome.* 2011; 74:2632–2641.
22. Al-Jamal WT, Kostarelos K. Liposomes: from a clinically established drug delivery system to a nanoparticle platform for theranostic nanomedicine. *Acc. Chem. Res.* 2011; 44:1094–1104. [PubMed: 21812415]
23. Bhojani MS, Van Dort M, Rehemtulla A, Ross BD. Targeted imaging and therapy of brain cancer using theranostic nanoparticles. *Mol. Pharm.* 2010; 7:1921–1929. [PubMed: 20964352]

24. Fernandez-Fernandez A, Manchanda R, McGoron AJ. Theranostic applications of nanomaterials in cancer: drug delivery image-guided therapy and multifunctional platforms. *Appl. Biochem. Biotechnol.* 2011; 165:1628–1651. [PubMed: 21947761]
25. Janib SM, Moses AS, MacKay JA. Imaging and drug delivery using theranostic nanoparticles. *Adv. Drug Deliv. Rev.* 2010; 62:1052–1063. [PubMed: 20709124]
26. Jokerst JV, Gambhir SS. Molecular imaging with theranostic nanoparticles. *Acc. Chem. Res.* 2011; 44:1050–1060. [PubMed: 21919457]
27. Kelkar SS, Reineke TM. Theranostics: combining imaging and therapy. *Bioconjug. Chem.* 2011; 22:1879–1903. [PubMed: 21830812]
28. Namiki Y, Fuchigami T, Tada N, Kawamura R, Matsunuma S, Kitamoto Y, Nakagawa M. Nanomedicine for cancer: lipid-based nanostructures for drug delivery and monitoring. *Acc. Chem. Res.* 2011; 44:1080–1093. [PubMed: 21786832]
29. Xie J, Lee S, Chen X. Nanoparticle-based theranostic agents. *Adv. Drug Deliv. Rev.* 2010; 62:1064–1079. [PubMed: 20691229]
30. Appelbaum L, Mahgerefteh SY, Sosna J, Goldberg SN. Image-guided fusion and navigation: applications in tumor ablation. *Tech. Vasc. Interv. Radiol.* 2013; 16:287–295. [PubMed: 24238384]
31. Kagadis GC, Katsanos K, Karnabatidis D, Loudos G, Nikiforidis GC, Hendee WR. Emerging technologies for image guidance and device navigation in interventional radiology. *Med. Phys.* 2012; 39:5768–5781. [PubMed: 22957641]
32. Knavel EM, Brace CL. Tumor ablation: common modalities and general practices. *Tech. Vasc. Interv. Radiol.* 2013; 16:192–200. [PubMed: 24238374]
33. Wood BJ, Locklin JK, Viswanathan A, Kruecker J, Haemmerich D, Cebal J, Sofer A, Cheng R, McCreedy E, Cleary K, McAuliffe MJ, Glossop N, Yanof J. Technologies for guidance of radiofrequency ablation in the multimodality inter-ventional suite of the future. *J. Vasc. Interv. Radiol.* 2007; 18:9–24. [PubMed: 17296700]
34. Manthe RL, Foy SP, Krishnamurthy N, Sharma B, Labhasetwar V. Tumor ablation and nanotechnology. *Mol. Pharm.* 2010; 7:1880–1898. [PubMed: 20866097]
35. Wankhede M, Bouras A, Kaluzova M, Hadjipanayis CG. Magnetic nanoparticles: an emerging technology for malignant brain tumor imaging and therapy. *Expert. Rev. Clin. Pharmacol.* 2012; 5:173–186. [PubMed: 22390560]
36. Sanna V, Pala N, Sechi M. Targeted therapy using nanotechnology: focus on cancer. *Int. J. Nanomedicine.* 2014; 9:467–483. [PubMed: 24531078]
37. Provenzale JM, Silva GA. Uses of nanoparticles for central nervous system imaging and therapy. *AJNR Am. J. Neuroradiol.* 2009; 30:1293–1301. [PubMed: 19617446]
38. Phillips WT, Goins BA, Bao A. Radioactive liposomes. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* 2009; 1:69–83. [PubMed: 20049780]
39. Nduom EK, Bouras A, Kaluzova M, Hadjipanayis CG. Nanotechnology applications for glioblastoma. *Neurosurg. Clin. N. Am.* 2012; 23:439–449. [PubMed: 22748656]
40. Mukerjee A, Ranjan AP, Vishwanatha JK. Combinatorial nanoparticles for cancer diagnosis and therapy. *Curr. Med. Chem.* 2012; 19:3714–3721. [PubMed: 22680922]
41. Menon JU, Jadeja P, Tambe P, Vu K, Yuan B, Nguyen KT. Nanomaterials for photo-based diagnostic and therapeutic applications. *Theranostics.* 2013; 3:152–166. [PubMed: 23471164]
42. Liu Y, Zhang N. Gadolinium loaded nanoparticles in theranostic magnetic resonance imaging. *Biomaterials.* 2012; 33:5363–5375. [PubMed: 22521487]
43. Li J, Gupta S, Li C. Research perspectives: gold nanoparticles in cancer theranostics. *Quant. Imaging Med. Surg.* 2013; 3:284–291. [PubMed: 24404441]
44. Huynh NT, Passirani C, Saulnier P, Benoit JP. Lipid nanocapsules: a new platform for nanomedicine. *Int. J. Pharm.* 2009; 379:201–209. [PubMed: 19409468]
45. Gong H, Peng R, Liu Z. Carbon nanotubes for biomedical imaging: the recent advances. *Adv. Drug Deliv. Rev.* 2013; 65:1951–1963. [PubMed: 24184130]
46. Akhter S, Ahmad MZ, Ahmad FJ, Storm G, Kok RJ. Gold nanoparticles in theranostic oncology: current state-of-the-art. *Expert Opin. Drug Saf.* 2012; 9:1225–1243.

47. Xie H, Goins B, Bao A, Wang ZJ, Phillips WT. Effect of intratumoral administration on biodistribution of ^{64}Cu -labeled nanoshells. *Int. J. Nanomedicine*. 2012; 7:2227–2238. [PubMed: 22619558]
48. Khan MK, Minc LD, Nigavekar SS, Kariapper MS, Nair BM, Schipper M, Cook AC, Lesniak WG, Balogh LP. Fabrication of $\{^{198}\text{Au}0\}$ radioactive composite nanodevices and their use for nanobrachytherapy. *Nanomedicine*. 2008; 4:57–69. [PubMed: 18249156]
49. Chanda N, Kan P, Watkinson LD, Shukla R, Zambre A, Carmack TL, Engelbrecht H, Lever JR, Katti K, Fent GM. Radioactive gold nanoparticles in cancer therapy: therapeutic efficacy studies of GA-198AuNP nanoconstruct in prostate tumor-bearing mice. *Nanomed. Nanotechnol. Biol. Med.* 2010; 6:201–209.
50. Shukla R, Chanda N, Zambre A, Upendran A, Katti K, Kulkarni RR, Nune SK, Casteel SW, Smith CJ, Vimal J, Boote E, Robertson JD, Kan P, Engelbrecht H, Watkinson LD, Carmack TL, Lever JR, Cutler CS, Caldwell C, Kannan R, Katti KV. Laminin receptor specific therapeutic gold nanoparticles (198AuNP-EGCg) show efficacy in treating prostate cancer. *Proc. Natl. Acad. Sci. U. S. A.* 2012; 109:12426–12431. [PubMed: 22802668]
51. Hamoudeh M, Fessi H, Salim H, Barbos D. Holmium-loaded PLLAnanoparticles for intratumoral radiotherapy via the TMT technique: preparation characterization and stability evaluation after neutron irradiation. *Drug Dev. Ind. Pharm.* 2008; 34:796–806. [PubMed: 18651284]
52. Zielhuis SW, Seppenwoolde JH, Mateus VA, Bakker CJ, Krijger GC, Storm G, Zonnenberg BA, van het Schip AD, Koning GA, Nijsen JF. Lanthanide-loaded liposomes for multimodality imaging and therapy. *Cancer Biother. Radiopharm.* 2006; 21:520–527. [PubMed: 17105424]
53. Yang W, Barth RF, Wu G, Huo T, Tjarks W, Ciesielski M, Fenstermaker RA, Ross BD, Wikstrand CJ, Riley KJ, Binns PJ. Convection enhanced delivery of boronated EGF as a molecular targeting agent for neutron capture therapy of brain tumors. *J. Neuro-Oncol.* 2009; 95:355–365.
54. Shultz MD, Wilson JD, Fuller CE, Zhang J, Dorn HC, Fatouros PP. Metallofullerene-based nanoplatform for brain tumor brachytherapy and longitudinal imaging in a murine orthotopic xenograft model. *Radiology*. 2011; 261:136–143. [PubMed: 21813738]
55. Arora G, Shukla J, Ghosh S, Maulik SK, Malhotra A, Bandopadhyaya G. PLGA nanoparticles for peptide receptor radionuclide therapy of neuroendocrine tumors: a novel approach towards reduction of renal radiation dose. *PLoS One*. 2012; 7:e34019. [PubMed: 22442740]
56. Helbok A, Decristoforo C, Dobrozemsky G, Rangger C, Diederer E, Stark B, Prassl R, von Guggenberg E. Radiolabeling of lipid-based nanoparticles for diagnostics and therapeutic applications: a comparison using different radiometals. *J. Lipo-some Res.* 2010; 20:219–227.
57. Zubillaga MB, Boccio JR, Nicolini JO, Ughetti R, Lanari E, Caro RA. Use of colloids of chromic $[^{32}\text{P}]$ phosphate in treatment of solid tumors. *Nucl. Med. Biol.* 1996; 23:907–910. [PubMed: 8971858]
58. Order SE, Siegel JA, Principato R, Zeiger LE, Johnson E, Lang P, Lustig R, Wallner PE. Selective tumor irradiation by infusional brachytherapy in nonresectable pancreatic cancer: a phase I study. *Int. J. Radiat. Oncol. Biol. Phys.* 1996; 36:1117–1126. [PubMed: 8985034]
59. Morton ME. Colloidal chromic radiophosphate in high yields for radiotherapy. *Nucleonics*. 1952; 10:92–97.
60. Firusian N, Dempke W. An early phase II study of intratumoral P-32 chromic phosphate injection therapy for patients with refractory solid tumors and solitary metastases. *Cancer*. 1999; 85:980–987. [PubMed: 10091778]
61. Alimi KA, Firusian N, Dempke W. Effects of intralesional 32-P chromic phosphate in refractory patients with head and neck tumours. *Anticancer Res.* 2007; 27:2997–3000. [PubMed: 17695485]
62. Moringlane JR, Alexander C, Kirsch CM. Successful low-dose intracavitary irradiation of a Rathke's cleft cyst with colloidal rhenium-186. *Minim. Invasive Neurosurg.* 2001; 44:218–220. [PubMed: 11830781]
63. Goins B, Bao A, Phillips WT. Techniques for loading technetium-99m and rhenium-186/188 radionuclides into pre-formed liposomes for diagnostic imaging and radionuclide therapy. *Methods Mol. Biol.* 2010; 606:469–491. [PubMed: 20013416]

64. French JT, Goins B, Saenz M, Li S, Garcia-Rojas X, Phillips WT, Otto RA, Bao A. Interventional therapy of head and neck cancer with lipid nanoparticle-carried rhenium 186 radionuclide. *J. Vasc. Interv. Radiol.* 2010; 21:1271–1279. [PubMed: 20478719]
65. Bao A, Phillips WT, Goins B, Zheng X, Sabour S, Natarajan M, Ross Woolley F, Zavaleta C, Otto RA. Potential use of drug carried-liposomes for cancer therapy via direct intratumoral injection. *Int. J. Pharm.* 2006; 316:162–169. [PubMed: 16580161]
66. Bao A, Goins B, Klipper R, Negrete G, Phillips WT. ^{186}Re -liposome labeling using ^{186}Re -SNS/S complexes: in vitro stability imaging and biodistribution in rats. *J. Nucl. Med.* 2003; 44:1992–1999. [PubMed: 14660726]
67. Vanpouille-Box C, Lacoëuille F, Belloche C, Lepareur N, Lemaire L, LeJeune JJ, Benoit JP, Menei P, Couturier OF, Garcion E, Hindre F. Tumor eradication in rat glioma and bypass of immunosuppressive barriers using internal radiation with (^{188}Re) -lipid nanocapsules. *Biomaterials.* 2011; 32:6781–6790. [PubMed: 21705077]
68. Ballot S, Noiret N, Hindre F, Denizot B, Garin E, Rajerison H, Benoit JP. $^{99\text{m}}\text{Tc}/^{188}\text{Re}$ -labelled lipid nanocapsules as promising radiotracers for imaging and therapy: formulation and biodistribution. *Eur. J. Nucl. Med. Mol. Imaging.* 2006; 33:602–607. [PubMed: 16450136]
69. Allard E, Hindre F, Passirani C, Lemaire L, Lepareur N, Noiret N, Menei P, Benoit JP. ^{188}Re -loaded lipid nanocapsules as a promising radiopharmaceutical carrier for internal radiotherapy of malignant gliomas. *Eur. J. Nucl. Med. Mol. Imaging.* 2008; 35:1838–1846. [PubMed: 18465130]
70. Tsai CC, Chang CH, Chen LC, Chang YJ, Lan KL, Wu YH, Hsu CW, Liu IH, Ho CL, Lee WC, Ni HC, Chang TJ, Ting G, Lee TW. Biodistribution and pharmacokinetics of ^{188}Re -liposomes and their comparative therapeutic efficacy with 5-fluorouracil in C26 colonic peritoneal carcinomatosis mice. *Int. J. Nanomedicine.* 2011; 6:2607–2619. [PubMed: 22114492]
71. Huang FY, Lee TW, Kao CH, Chang CH, Zhang X, Lee WY, Chen WJ, Wang SC, Lo JM. Imaging autoradiography. and biodistribution of (^{188}Re) -labeled PEGylated nanoliposome in orthotopic glioma bearing rat model. *Cancer Biother. Radiopharm.* 2011; 26:717–725. [PubMed: 22145660]
72. Hsu CW, Chang YJ, Chang CH, Chen LC, Lan KL, Ting G, Lee TW. Comparative therapeutic efficacy of rhenium-188 radiolabeled-liposome and 5-fluorouracil in LS-174T human colon carcinoma solid tumor xenografts. *Cancer Biother. Radiopharm.* 2012; 27:481–489. [PubMed: 23067100]
73. Chen MH, Chang CH, Chang YJ, Chen LC, Yu CY, Wu YH, Lee WC, Yeh CH, Lin FH, Lee TW, Yang CS, Ting G. MicroSPECT/CT imaging and pharmacokinetics of ^{188}Re -(DXR)-liposome in human colorectal adenocarcinoma-bearing mice. *Anticancer Res.* 2010; 30:65–72. [PubMed: 20150618]
74. Cui W, Zhang Y, Xu X, Shen YM. Synthesis and ^{188}Re radiolabelling of dendrimer polyamide amine (PAMAM) folic acid conjugate. *Med. Chem.* 2012; 8:727–731. [PubMed: 22548334]
75. Maggioni D, Arosio P, Orsini F, Ferretti AM, Orlando T, Manfredi A, Ranucci E, Ferruti P, D'Alfonso G, Lascialfari A. Superparamagnetic iron oxide nanoparticles stabilized by a poly(amidoamine)-rhenium complex as potential theranostic probe. *Dalton Trans.* 2014; 43:1172–1183. (2003). [PubMed: 24169854]
76. Tang QS, Chen DZ, Xue WQ, Xiang JY, Gong YC, Zhang L, Guo CQ. Preparation and biodistribution of ^{188}Re -labeled folate conjugated human serum albumin magnetic cisplatin nanoparticles (^{188}Re -folate-CDDP/HSA MNPs) in vivo. *Int. J. Nanomedicine.* 2011; 6:3077–3085. [PubMed: 22163161]
77. Li Z, Zhang G, Shen H, Zhang L, Wang Y. Synthesis and cell uptake of a novel dualmodality (^{188}Re) -HGRGD (D) F-CdTe QDs probe. *Talanta.* 2011; 85:936–942. [PubMed: 21726721]
78. Peng CL, Shih YH, Lee PC, Hsieh TM, Luo TY, Shieh MJ. Multimodal image-guided photothermal therapy mediated by ^{188}Re -labeled micelles containing a cyanine-type photosensitizer. *ACS Nano.* 2011; 5:5594–5607. [PubMed: 21671580]
79. Goldberg SN, Girnan GD, Lukyanov AN, Ahmed M, Monsky WL, Gazelle GS, Huertas JC, Stuart KE, Jacobs T, Torchilin VP, Halpern EF, Kruskal JB. Percutaneous tumor ablation: increased necrosis with combined radio-frequency ablation and intravenous liposomal doxorubicin in a rat breast tumor model. *Radiology.* 2002; 222:797–804. [PubMed: 11867804]

80. Ahmed M, Goldberg SN. Combination radiofrequency thermal ablation and adjuvant IV liposomal doxorubicin increases tissue coagulation and intratumoural drug accumulation. *Int. J. Hyperth.* 2004; 20:781–802.
81. Decadt B, Siriwardena AK. Radiofrequency ablation of liver tumours: systematic review. *Lancet Oncol.* 2004; 5:550–560. [PubMed: 15337485]
82. Goldberg SN, Grassi CJ, Cardella JF, Charboneau JW, Dodd GD 3rd, Dupuy DE, Gervais DA, Gillams AR, Kane RA, Lee FT Jr, Livraghi T, McGahan J, Phillips DA, Rhim H, Silverman SG, Solbiati L, Vogl TJ, Wood BJ, Vedantham S, Sacks D. Image-guided tumor ablation: standardization of terminology and reporting criteria. *J. Vasc. Interv. Radiol.* 2009; 20:S377–S390. [PubMed: 19560026]
83. Lencioni R, Crocetti L, Pina MC, Cioni D. Percutaneous image-guided radiofrequency ablation of liver tumors. *Abdom. Imaging.* 2009; 34:547–556. [PubMed: 19030918]
84. Ahmed M, Lukyanov AN, Torchilin V, Tournier H, Schneider AN, Goldberg SN. Combined radiofrequency ablation adjuvant liposomal chemotherapy: effect of chemotherapeutic agent nanoparticle size and circulation time. *J. Vasc. Interv. Radiol.* 2005; 16:1365–1371. [PubMed: 16221908]
85. Goldberg SN. Science to practice: Which approaches to combination interventional oncologic therapy hold the greatest promise of obtaining maximal clinical benefit? *Radiology.* 2011; 261:667–669. [PubMed: 22095989]
86. Soundararajan A, Dodd GD 3rd, Bao A, Phillips WT, McManus LM, Prihoda TJ, Goins BA. Chemoradionuclide therapy with ¹⁸⁶Re-labeled liposomal doxorubicin in combination with radiofrequency ablation for effective treatment of head and neck cancer in a nude rat tumor xenograft model. *Radiology.* 2011; 261:813–823. [PubMed: 22025735]
87. Monsky WL, Kruskal JB, Lukyanov AN, Girnun GD, Ahmed M, Gazelle GS, Huertas JC, Stuart KE, Torchilin VP, Goldberg SN. Radio-frequency ablation increases intratumoral liposomal doxorubicin accumulation in a rat breast tumor model. *Radiology.* 2002; 224:823–829. [PubMed: 12202721]
88. Solazzo SA, Ahmed M, Schor-Bardach R, Yang W, Girnun GD, Rahmanuddin S, Levchenko T, Signoretti S, Spitz DR, Torchilin V, Goldberg SN. Liposomal doxo-rubicin increases radiofrequency ablation-induced tumor destruction by increasing cellular oxidative and nitrate stress and accelerating apoptotic pathways. *Radiology.* 2010; 255:62–74. [PubMed: 20160000]
89. Head HW, Dodd GD 3rd, Bao A, Soundararajan A, Garcia-Rojas X, Prihoda TJ, McManus LM, Goins BA, Santoyo CA, Phillips WT. Combination radiofrequency ablation and intravenous radiolabeled liposomal Doxorubicin: imaging and quantification of increased drug delivery to tumors. *Radiology.* 2010; 255:405–414. [PubMed: 20413753]
90. Soundararajan A, Bao A, Phillips WT, McManus LM, Goins BA. Chemoradionuclide therapy with ¹⁸⁶Re-labeled liposomal Doxorubicin: toxicity dosimetry and therapeutic response. *Cancer Biother. Radiopharm.* 2011; 26:603–614. [PubMed: 21834653]
91. Bao A, Goins B, Klipper R, Negrete G, Phillips WT. Direct ^{99m}Tc labeling of pegylated liposomal doxorubicin (Doxil) for pharmacokinetic and non-invasive imaging studies. *J. Pharmacol. Exp. Ther.* 2004; 308:419–425. [PubMed: 14610219]
92. Grull H, Langereis S. Hyperthermia-triggered drug delivery from temperature-sensitive liposomes using MRI-guided high intensity focused ultrasound. *J. Control. Release.* 2012; 161:317–327. [PubMed: 22565055]
93. Lai CY, Fite BZ, Ferrara KW. Ultrasonic enhancement of drug penetration in solid tumors. *Front. Oncol.* 2013; 3:204. [PubMed: 23967400]
94. Phenix CP, Togtema M, Pichardo S, Zehbe I, Curiel L. High intensity focused ultrasound technology its scope and applications in therapy and drug delivery. *J. Pharm. Pharm. Sci.* 2014; 17:136–153. [PubMed: 24735765]
95. Hynynen K, McDannold N, Vykhodtseva N, Jolesz FA. Noninvasive MR imaging-guided focal opening of the blood-brain barrier in rabbits. *Radiology.* 2001; 220:640–646. [PubMed: 11526261]
96. Ji G, Yang J, Chen J. Preparation of novel curcumin-loaded multifunctional nanodroplets for combining ultrasonic development and targeted chemotherapy. *Int. J. Pharm.* 2014; 466:314–320. [PubMed: 24657138]

97. Kedar U, Phutane P, Shidhaye S, Kadam V. Advances in polymeric micelles for drug delivery and tumor targeting. *Nanomedicine*. 2010; 6:714–729. [PubMed: 20542144]
98. Klibanov AL, Hughes MS, Villanueva FS, Jankowski RJ, Wagner WR, Wojdyla JK, Wible JH, Brandenburger GH. Targeting and ultrasound imaging of microbubble-based contrast agents. *MAGMA*. 1999; 8:177–184. [PubMed: 10504045]
99. Manzoor AA, Lindner LH, Landon CD, Park JY, Simnick AJ, Dreher MR, Das S, Hanna G, Park W, Chilkoti A, Koning GA, ten Hagen TL, Needham D, Dewhirst MW. Overcoming limitations in nanoparticle drug delivery: triggered intra-vascular release to improve drug penetration into tumors. *Cancer Res*. 2012; 72:5566–5575. [PubMed: 22952218]
100. Oerlemans C, Bult W, Bos M, Storm G, Nijssen JF, Hennink WE. Polymeric micelles in anticancer therapy: targeting imaging and triggered release. *Pharm. Res*. 2010; 27:2569–2589. [PubMed: 20725771]
101. Rapoport NY, Kennedy AM, Shea JE, Scaife CL, Nam KH. Controlled and targeted tumor chemotherapy by ultrasound-activated nanoemulsions/microbubbles. *J. Control. Release*. 2009; 138:268–276. [PubMed: 19477208]
102. Yang FY, Wong TT, Teng MC, Liu RS, Lu M, Liang HF, Wei MC. Focused ultrasound and interleukin-4 receptor-targeted liposomal doxorubicin for enhanced targeted drug delivery and antitumor effect in glioblastoma multiforme. *J. Control. Release*. 2012; 160:652–658. [PubMed: 22405901]
103. Yin T, Wang P, Li J, Wang Y, Zheng B, Zheng R, Cheng D, Shuai X. Tumor-penetrating codelivery of siRNA and paclitaxel with ultrasound-responsive nanobubbles hetero-assembled from polymeric micelles and liposomes. *Biomaterials*. 2014; 35:5932–5943. [PubMed: 24746965]
104. Yudina A, de Smet M, Lepetit-Coiffe M, Langereis S, Van Ruijssevelt L, Smirnov P, Bouchaud V, Voisin P, Grull H, Moonen CT. Ultrasound-mediated intracellular drug delivery using microbubbles and temperature-sensitive liposomes. *J. Control. Release*. 2011; 155:442–448. [PubMed: 21689699]
105. Zhu L, Torchilin VP. Stimulus-responsive nanopreparations for tumor targeting. *Integr. Biol. (Camb.)*. 2013; 5:96–107. [PubMed: 22869005]
106. Suzuki R, Maruyama K. Effective in vitro and in vivo gene delivery by the combination of liposomal bubbles (bubble liposomes) and ultrasound exposure. *Methods Mol. Biol*. 2010; 605:473–486. [PubMed: 20072902]
107. Dicheva BM, ten Hagen TL, Li L, Schipper D, Seynhaeve AL, van Rhooen GC, Eggermont AM, Lindner LH, Koning GA. Cationic thermosensitive liposomes: a novel dual targeted heat-triggered drug delivery approach for endothelial and tumor cells. *Nano Lett*. 2013; 13:2324–2331. [PubMed: 22616659]
108. Dromi S, Frenkel V, Luk A, Traughber B, Angstadt M, Bur M, Poff J, Xie J, Libutti SK, Li KC, Wood BJ. Pulsed-high intensity focused ultrasound and low temperature-sensitive liposomes for enhanced targeted drug delivery and antitumor effect. *Clin. Cancer Res*. 2007; 13:2722–2727. [PubMed: 17473205]
109. Needham D, Dewhirst MW. The development and testing of a new temperature-sensitive drug delivery system for the treatment of solid tumors. *Adv. Drug Deliv. Rev*. 2001; 53:285–305. [PubMed: 11744173]
110. Sirsi SR, Borden MA. State-of-the-art materials for ultrasound-triggered drug delivery. *Adv. Drug Deliv. Rev*. 2014; 72C:3–14. [PubMed: 24389162]
111. Yatvin MB, Weinstein JN, Dennis WH, Blumenthal R. Design of liposomes for enhanced local release of drugs by hyperthermia. *Science (New York N.Y.)*. 1978; 202:1290–1293.
112. Zhao YZ, Du LN, Lu CT, Jin YG, Ge SP. Potential and problems in ultrasound-responsive drug delivery systems. *Int. J. Nanomedicine*. 2013; 8:1621–1633. [PubMed: 23637531]
113. Frenkel V, Etherington A, Greene M, Quijano J, Xie J, Hunter F, Dromi S, Li KC. Delivery of liposomal doxorubicin (Doxil) in a breast cancer tumor model: investigation of potential enhancement by pulsed-high intensity focused ultrasound exposure. *Acad. Radiol*. 2006; 13:469–479. [PubMed: 16554227]

114. Koning GA, Krijger GC. Targeted multifunctional lipid-based nanocarriers for image-guided drug delivery. *Anticancer Agents Med. Chem.* 2007; 7:425–440. [PubMed: 17630918]
115. Kheirloom A, Lai CY, Tam SM, Mahakian LM, Ingham ES, Watson KD, Ferrara KW. Complete regression of local cancer using temperature-sensitive liposomes combined with ultrasound-mediated hyperthermia. *J. Control. Release.* 2013; 172:266–273. [PubMed: 23994755]
116. Li L, ten Hagen TL, Hossann M, Suss R, van Rhooen GC, Eggermont AM, Haemmerich D, Koning GA. Mild hyperthermia triggered doxorubicin release from optimized stealth thermosensitive liposomes improves intratumoral drug delivery and efficacy. *J. Control. Release.* 2013; 168:142–150. [PubMed: 23524188]
117. Matteucci ML, Anyarambhatla G, Rosner G, Azuma C, Fisher PE, Dewhirst MW, Needham D, Thrall DE. Hyperthermia increases accumulation of technetium-99m-labeled liposomes in feline sarcomas. *Clin. Cancer Res.* 2000; 6:3748–3755. [PubMed: 10999769]
118. de Smet M, Langereis S, van den Bosch S, Bitter K, Hijnen NM, Heijman E, Grull H. SPECT/CT imaging of temperature-sensitive liposomes for MR-image guided drug delivery with high intensity focused ultrasound. *J. Control. Release.* 2013; 169:82–90. [PubMed: 23598044]
119. Oerlemans C, Nijssen F, van Amersfoort M, van Bloois L, Heijman E, Luijten P, Mali W, Storm G. A novel approach to identify non-palpable breast lesions combining fluorescent liposomes and magnetic resonance-guided high intensity focused ultrasound-triggered release. *Eur. J. Pharm. Biopharm.* 2011; 77:458–464. [PubMed: 21195760]
120. de Smet M, Heijman E, Langereis S, Hijnen NM, Grull H. Magnetic resonance imaging of high intensity focused ultrasound mediated drug delivery from temperature-sensitive liposomes: an in vivo proof-of-concept study. *J. Control. Release.* 2011; 150:102–110. [PubMed: 21059375]
121. Negussie AH, Yarmolenko PS, Partanen A, Ranjan A, Jacobs G, Woods D, Bryant H, Thomasson D, Dewhirst MW, Wood BJ, Dreher MR. Formulation and characterisation of magnetic resonance imageable thermally sensitive liposomes for use with magnetic resonance-guided high intensity focused ultrasound. *Int. J. Hyperthermia.* 2011; 27:140–155. [PubMed: 21314334]
122. Ponce AM, Viglianti BL, Yu D, Yarmolenko PS, Michelich CR, Woo J, Bally MB, Dewhirst MW. Magnetic resonance imaging of temperature-sensitive liposome release: drug dose painting and antitumor effects. *J. Natl. Cancer Inst.* 2007; 99:53–63. [PubMed: 17202113]
123. Viglianti BL, Ponce AM, Michelich CR, Yu D, Abraham SA, Sanders L, Yarmolenko PS, Schroeder T, MacFall JR, Barboriak DP, Colvin OM, Bally MB, Dewhirst MW. Chemodosimetry of in vivo tumor liposomal drug concentration using MRI. *Magn. Reson. Med.* 2006; 56:1011–1018. [PubMed: 17029236]
124. Iacobuzio-Donahue CA, Fu B, Yachida S, Luo M, Abe H, Henderson CM, Vilardell F, Wang Z, Keller JW, Banerjee P, Herman JM, Cameron JL, Yeo CJ, Halushka MK, Eshleman JR, Raben M, Klein AP, Hruban RH, Hidalgo M, Laheru D. DPC4 gene status of the primary carcinoma correlates with patterns of failure in patients with pancreatic cancer. *J. Clin. Oncol.* 2009; 27:1806–1813. [PubMed: 19273710]
125. McCready VR, Cornes P. The potential of intratumoural unsealed radioactive source therapy. *Eur. J. Nucl. Med.* 2001; 28:567–569. [PubMed: 11383859]
126. Fiandaca MS, Berger MS, Bankiewicz KS. The use of convection-enhanced delivery with liposomal toxins in neurooncology. *Toxins.* 2011; 3:369–397. [PubMed: 22069714]
127. Raghavan R, Brady ML, Rodriguez-Ponce MI, Hartlep A, Pedain C, Sampson JH. Convection-enhanced delivery of therapeutics for brain disease, and its optimization. *Neurosurg. Focus.* 2006; 20:E12. [PubMed: 16709017]
128. Saito R, Bringas JR, McKnight TR, Wendland MF, Mamot C, Drummond DC, Kirpotin DB, Park JW, Berger MS, Bankiewicz KS. Distribution of liposomes into brain and rat brain tumor models by convection-enhanced delivery monitored with magnetic resonance imaging. *Cancer Res.* 2004; 64:2572–2579. [PubMed: 15059914]
129. Sampson JH, Akabani G, Friedman AH, Bigner D, Kunwar S, Berger MS, Bankiewicz KS. Comparison of intratumoral bolus injection and convection-enhanced delivery of radiolabeled antitenascin monoclonal antibodies. *Neurosurg. Focus.* 2006; 20:E14. [PubMed: 16709019]

130. Bobo RH, Laske DW, Akbasak A, Morrison PF, Dedrick RL, Oldfield EH. Convection-enhanced delivery of macromolecules in the brain. *Proc. Natl. Acad. Sci. U. S. A.* 1994; 91:2076–2080. [PubMed: 8134351]
131. Barua NU, Miners JS, Bienemann AS, Wyatt MJ, Welser K, Tabor AB, Hailes HC, Love S, Gill SS. Convection-enhanced delivery of neprilysin: a novel amyloid-beta-degrading therapeutic strategy. *J. Alzheimers Dis.* 2012; 32:43–56. [PubMed: 22751177]
132. Vogelbaum MA, Iannotti CA. Convection-enhanced delivery of therapeutic agents into the brain. *Handb. Clin. Neurol.* 2012; 104:355–362. [PubMed: 22230453]
133. Ferguson S, Lesniak MS. Convection enhanced drug delivery of novel therapeutic agents to malignant brain tumors. *Curr. Drug Deliv.* 2007; 4:169–180. [PubMed: 17456036]
134. Juratli TA, Schackert G, Krex D. Current status of local therapy in malignant gliomas—a clinical review of three selected approaches. *Pharmacol. Ther.* 2013; 139:341–358. [PubMed: 23694764]
135. Mehta AI, Choi BD, Raghavan R, Brady M, Friedman AH, Bigner DD, Pastan I, Sampson JH. Imaging of convection enhanced delivery of toxins in humans. *Toxins.* 2011; 3:201–206. [PubMed: 22069706]
136. Rainov NG. A phase III clinical evaluation of herpes simplex virus type 1 thymidine kinase and ganciclovir gene therapy as an adjuvant to surgical resection and radiation in adults with previously untreated glioblastoma multiforme. *Hum. Gene Ther.* 2000; 11:2389–2401. [PubMed: 11096443]
137. Rainov NG, Gorbatyuk K, Heidecke V. Clinical trials with intracerebral convection-enhanced delivery of targeted toxins in malignant glioma. *Rev. Recent Clin. Trials.* 2008; 3:2–9. [PubMed: 18474010]
138. Voges J, Reszka R, Gossmann A, Dittmar C, Richter R, Garlip G, Kracht L, Coenen HH, Sturm V, Wienhard K, Heiss WD, Jacobs AH. Imaging-guided convection-enhanced delivery and gene therapy of glioblastoma. *Ann. Neurol.* 2003; 54:479–487. [PubMed: 14520660]
139. Sawyer AJ, Saucier-Sawyer JK, Booth CJ, Liu J, Patel T, Piepmeier JM, Saltzman WM. Convection-enhanced delivery of camptothecin-loaded polymer nanoparticles for treatment of intracranial tumors. *Drug deliv. Transl. Res.* 2011; 1:34–42. [PubMed: 21691426]
140. Barua NU, Gill SS, Love S. Convection-enhanced drug delivery to the brain: therapeutic potential and neuropathological considerations. *Brain Pathol.* 2014; 24(2):117–127. [PubMed: 23944716]
141. Dickinson PJ, LeCouteur RA, Higgins RJ, Bringas JR, Roberts B, Larson RF, Yamashita Y, Krauze M, Noble CO, Drummond D, Kirpotin DB, Park JW, Berger MS, Bankiewicz KS. Canine model of convection-enhanced delivery of liposomes containing CPT-11 monitored with real-time magnetic resonance imaging: laboratory investigation. *J. Neurosurg.* 2008; 108:989–998. [PubMed: 18447717]
142. Phillips WT, Goins B, Bao A, Vargas D, Gutierrez JE, Trevino A, Miller JR, Henry J, Zuniga R, Vecil G, Brenner AJ. Rhenium-186 liposomes as convection-enhanced nanoparticle brachytherapy for treatment of glioblastoma. *Neuro-Oncol.* 2012; 14:416–425. [PubMed: 22427110]
143. Saito R, Krauze MT, Bringas JR, Noble C, McKnight TR, Jackson P, Wendland MF, Mamot C, Drummond DC, Kirpotin DB, Hong K, Berger MS, Park JW, Bankiewicz KS. Gadolinium-loaded liposomes allow for real-time magnetic resonance imaging of convection-enhanced delivery in the primate brain. *Exp. Neurol.* 2005; 196:381–389. [PubMed: 16197944]
144. Saito R, Krauze MT, Noble CO, Drummond DC, Kirpotin DB, Berger MS, Park JW, Bankiewicz KS. Convection-enhanced delivery of Ls-TPT enables an effective continuous low-dose chemotherapy against malignant glioma xenograft model. *Neuro-Oncol.* 2006; 8:205–214. [PubMed: 16723630]
145. Saito R, Krauze MT, Noble CO, Tamas M, Drummond DC, Kirpotin DB, Berger MS, Park JW, Bankiewicz KS. Tissue affinity of the infusate affects the distribution volume during convection-enhanced delivery into rodent brains: implications for local drug delivery. *J. Neurosci. Methods.* 2006; 154:225–232. [PubMed: 16472868]
146. Wang SX, Bao A, Herrera SJ, Phillips WT, Goins B, Santoyo C, Miller FR, Otto RA. Intraoperative ¹⁸⁶Re-liposome radionuclide therapy in a head and neck squamous cell carcinoma

- xenograft positive surgical margin model. *Clin. Cancer Res.* 2008; 14:3975–3983. [PubMed: 18559620]
147. Sampson JH, Archer G, Pedain C, Wembacher-Schroder E, Westphal M, Kunwar S, Vogelbaum MA, Coan A, Herndon JE, Raghavan R, Brady ML, Reardon DA, Friedman AH, Friedman HS, Rodriguez-Ponce MI, Chang SM, Mittermeyer S, Croteau D, Puri RK. Poor drug distribution as a possible explanation for the results of the PRECISE trial. *J. Neurosurg.* 2010; 113:301–309. [PubMed: 20020841]
 148. Sampson JH Jr. Editorial: convection-enhanced delivery. *J. Neurosurg.* 2012; 117:1126–1127. (discussion 1127). [PubMed: 22998062]
 149. Grahn AY, Bankiewicz KS, Dugich-Djordjevic M, Bringas JR, Hadaczek P, Johnson GA, Eastman S, Luz M. Non-PEGylated liposomes for convection-enhanced delivery of topotecan and gadodiamide in malignant glioma: initial experience. *J. Neuro-Oncol.* 2009; 95:185–197.
 150. Mehta AI, Choi BD, Ajay D, Raghavan R, Brady M, Friedman AH, Pastan I, Bigner DD, Sampson JH. Convection enhanced delivery of macromolecules for brain tumors. *Curr. Drug Discov. Technol.* 2012; 9:305–310. [PubMed: 22339074]
 151. Sampson JH, Brady M, Raghavan R, Mehta AI, Friedman AH, Reardon DA, Petry NA, Barboriak DP, Wong TZ, Zalutsky MR, Lally-Goss D, Bigner DD. Colocalization of gadolinium-diethylene triamine pentaacetic acid with high-molecular-weight molecules after intracerebral convection-enhanced delivery in humans. *Neurosurgery.* 2011; 69:668–676. [PubMed: 21430586]
 152. Allard E, Passirani C, Benoit JP. Convection-enhanced delivery of nanocarriers for the treatment of brain tumors. *Biomaterials.* 2009; 30:2302–2318. [PubMed: 19168213]
 153. Stone NN, Stock RG. Permanent seed implantation for localized adenocarcinoma of the prostate. *Curr. Urol. Rep.* 2002; 3:201–206. [PubMed: 12084189]
 154. Skowronek J. Brachytherapy in the therapy of prostate cancer — an interesting choice. *Contemp. Oncol.* 2013; 17:407–412.
 155. Hrycushko BA, Li S, Goins B, Otto RA, Bao A. Direct intratumoral infusion of liposome encapsulated rhenium radionuclides for cancer therapy: effects of non-uniform intratumoral dose distribution. *Med. Phys.* 2011; 38:1339. [PubMed: 21520844]
 156. Haaga JR, Alfidi RJ. Precise biopsy localization by computer tomography. *Radiology.* 1976; 118:603–607. [PubMed: 1251009]
 157. Shyn PB, Tatli S, Sahni VA, Sadow CA, Forgione K, Mauri G, Morrison PR, Catalano PJ, Silverman SG. PET/CT-guided percutaneous liver mass biopsies and ablations: targeting accuracy of a single 20 s breath-hold PET acquisition. *Clin. Radiol.* 2014; 69:410–415. [PubMed: 24411824]
 158. Queiroz MA, Hullner M, Kuhn F, Huber G, Meerwein C, Kollias S, von Schulthess G, Veit-Haibach P. PET/MRI and PET/CT in follow-up of head and neck cancer patients. *Eur. J. Nucl. Med. Mol. Imaging.* 2014; 41(6):1066–1075. <http://dx.doi.org/10.1007/s00259-014-2707-9>. [PubMed: 24577950]
 159. Seemann MD. Whole-body PET/MRI: the future in oncological imaging. *Technol. Cancer Res. Treat.* 2005; 4:577–582. [PubMed: 16173829]
 160. Li S, Goins B, Zhang L, Bao A. Novel multifunctional theranostic liposome drug delivery system: construction characterization multimodality MR near-infrared fluorescent and nuclear imaging. *Bioconjug. Chem.* 2012; 23:1322–1332. [PubMed: 22577859]
 161. Hong K, Georgiades CS, Geschwind JF. Technology insight: image-guided therapies for hepatocellular carcinoma— intra-arterial and ablative techniques. *Nat. Clin. Pract. Oncol.* 2006; 3:315–324. [PubMed: 16757969]
 162. McGhana JP, Dodd GD 3rd. Radiofrequency ablation of the liver: current status. *AJR Am. J. Roentgenol.* 2001; 176:3–16. [PubMed: 11133529]
 163. Clasen S, Rempp H, Hoffmann R, Graf H, Pereira PL, Claussen CD. Image-guided radiofrequency ablation of hepatocellular carcinoma (HCC): is MR guidance more effective than CT guidance? *Eur. J. Radiol.* 2014; 83:111–116. [PubMed: 24161781]
 164. Memon K, Lewandowski RJ, Riaz A, Salem R. Yttrium 90 microspheres for the treatment of hepatocellular carcinoma. *Recent Results Cancer Res.* 2013; 190:207–224. [PubMed: 22941023]

165. Vanpouille-Box C, Hindre F. Nanovectorized radiotherapy: a new strategy to induce anti-tumor immunity. *Front. Oncol.* 2012; 2:136. [PubMed: 23087900]
166. MacKay JA, Deen DF, Szoka FC Jr. Distribution in brain of liposomes after convection enhanced delivery; modulation by particle charge particle diameter and presence of steric coating. *Brain Res.* 2005; 1035:139–153. [PubMed: 15722054]
167. Williams KC, Hickey WF. Traffic of hematogenous cells through the central nervous system. *Curr. Top. Microbiol. Immunol.* 1995; 202:221–245. [PubMed: 7587365]
168. Vilhardt F. Microglia: phagocyte and glia cell. *Int. J. Biochem. Cell Biol.* 2005; 37:17–21. [PubMed: 15381143]
169. Kushchayev SV, Kushchayeva YS, Wiener PC, Badie B, Preul MC. Monocyte-derived cells of the brain and malignant gliomas: the double face of Janus. *World Neurosurg.* 2014 in press).
170. Lu-Emerson C, Snuderl M, Kirkpatrick ND, Goveia J, Davidson C, Huang Y, Riedemann L, Taylor J, Ivy P, Duda DG, Ancukiewicz M, Plotkin SR, Chi AS, Gerstner ER, Eichler AF, Dietrich J, Stemmer-Rachamimov AO, Batchelor TT, Jain RK. Increase in tumor-associated macrophages after antiangiogenic therapy is associated with poor survival among patients with recurrent glioblastoma. *Neuro-Oncol.* 2013; 15:1079–1087. [PubMed: 23828240]
171. Roggendorf W, Strupp S, Paulus W. Distribution and characterization of microglia/ macrophages in human brain tumors. *Acta Neuropathol.* 1996; 92:288–293. [PubMed: 8870831]
172. Bao A, Goins B, Klipper R, Negrete G, Mahindaratne M, Phillips WT. A novel liposome radiolabeling method using ^{99m}Tc -”SNS/S” complexes: in vitro and in vivo evaluation. *J. Pharm. Sci.* 2003; 92:1893–1904. [PubMed: 12950007]
173. Saito R. Distribution of liposomes into brain and rat brain tumor models by convection-enhanced delivery monitored with magnetic resonance imaging. *Cancer Res.* 2004; 64:2572–2579. [PubMed: 15059914]
174. James CD. Nanoparticles for treating brain tumors: unlimited possibilities. *Neuro-Oncol.* 2012; 14:389. [PubMed: 22436865]
175. Kannan R, Zambre A, Chanda N, Kulkarni R, Shukla R, Katti K, Upendran A, Cutler C, Boote E, Katti KV. Functionalized radioactive gold nanoparticles in tumor therapy. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* 2012; 4:42–51. [PubMed: 21953803]
176. Richardson RM, Kells AP, Martin AJ, Larson PS, PA Starr, Piferi PG, Bates G, L Tansey, Rosenbluth KH, Bringas JR, Berger MS, Bankiewicz KS. Novel platform for MRI-guided convection-enhanced delivery of therapeutics: preclinical validation in nonhuman primate brain. *Stereotact Funct Neurosurg.* 2011; 89:141–151. [PubMed: 21494065]
177. Motion JP, Huynh GH, Szoka FC Jr, Siegel RA. Convection and retro-convection enhanced delivery: some theoretical considerations related to drug targeting. *Pharm Res.* 2011; 28(3):472–479. (Epub 2010 Oct 21) <http://dx.doi.org/10.1007/s11095-010-0296-2>. [PubMed: 20963628]
178. Lewis GK Jr. Schulz ZR, Pannullo SC, Southard TL, Olbricht WL. Ultrasound-assisted convection-enhanced delivery to the brain in vivo with a novel transducer cannula assembly: laboratory investigation. *J. Neurosurg.* 2012; 117:1128–1140. [PubMed: 22998056]
179. Barua NU, Lowis SP, Woolley M, O’Sullivan S, Harrison R, Gill SS. Robot-guided convection-enhanced delivery of carboplatin for advanced brainstem glioma. *Acta Neurochir.* 2013; 155:1459–1465. [PubMed: 23595829]
180. Chen MY, Lonser RR, Morrison PF, Governale LS, Oldfield EH. Variables affecting convection-enhanced delivery to the striatum: a systematic examination of rate of infusion cannula size, infusate concentration and tissue-cannula sealing time. *J. Neurosurg.* 1999; 90:315–320. [PubMed: 9950503]
181. Kirov II, Hardy CJ, Matsuda K, Messinger J, Cankurtaran CZ, Warren M, Wiggins GC, Perry NN, Babb JS, Goetz RR, George A, Malaspina D, Gonen O. In vivo 7 Tesla imaging of the dentate granule cell layer in schizophrenia. *Schizophr. Res.* 2013; 147:362–367. [PubMed: 23664589]
182. Houweling AC, Wolf AL, Vogel WV, Hamming-Vrieze O, van Vliet-Vroegindeweij C, van de Kamer JB, van der Heide UA. FDG-PET and diffusion-weighted MRI in head-and-neck cancer patients: implications for dose painting. *Radiother. Oncol.* 2013; 106:250–254. [PubMed: 23395065]

183. Haegelen C, Touzet G, Reyns N, Maurage CA, Ayachi M, Blond S. Stereotactic robot-guided biopsies of brain stem lesions: experience with 15 cases. *Neurochirurgie*. 2010; 56:363–367. [PubMed: 20728907]
184. Thorne RG, Nicholson C. In vivo diffusion analysis with quantum dots and dextrans predicts the width of brain extracellular space. *Proc. Natl. Acad. Sci. U. S. A.* 2006; 103:5567–5572. [PubMed: 16567637]
185. Zhou J, Atsina KB, Himes BT, Strohbehn GW, Saltzman WM. Novel delivery strategies for glioblastoma. *Cancer J.* 2012; 18:89–99. [PubMed: 22290262]
186. Zhou J, Patel TR, Sirianni RW, Strohbehn G, Zheng MQ, Duong N, Schafbauer T, Huttner AJ, Huang Y, Carson RE, Zhang Y, Sullivan DJ Jr, Piepmeyer JM, Saltzman WM. Highly penetrative drug-loaded nanocarriers improve treatment of glioblastoma. *Proc. Natl. Acad. Sci. U. S. A.* 2013; 110:11751–11756. [PubMed: 23818631]
187. Hobbs SK, Monsky WL, Yuan F, Roberts WG, L Griffith, Torchilin VP, Jain RK. Regulation of transport pathways in tumor vessels: role of tumor type and micro-environment. *Proc. Natl. Acad. Sci. U. S. A.* 1998; 95:4607–4612. [PubMed: 9539785]
188. Chang YJ, Chang CH, Chang TJ, Yu CY, Chen LC, Jan ML, Luo TY, Lee TW, Ting G. Biodistribution pharmacokinetics and microSPECT/CT imaging of ¹⁸⁸Re-bMEDA-liposome in a C26 murine colon carcinoma solid tumor animal model. *Anticancer Res.* 2007; 27:2217–2225. [PubMed: 17695506]
189. Chen LC, Chang CH, Yu CY, Chang YJ, Wu YH, Lee WC, Yeh CH, Lee TW, Ting G. Pharmacokinetics micro-SPECT/CT imaging and therapeutic efficacy of (188)Re-DXR-liposome in C26 colon carcinoma ascites mice model. *Nucl. Med. Biol.* 2008; 35:883–893. [PubMed: 19026950]
190. Knapp FF Jr. Rhenium-188—a generator-derived radioisotope for cancer therapy. *Cancer Biother. Radiopharm.* 1998; 13:337–349. [PubMed: 10851424]
191. Solorio L, Exner AA. Applications of ultrasound for image-guided drug delivery in cancer chemotherapy. *Ther. Deliv.* 2013; 4:785–789. [PubMed: 23883123]
192. Campbell MJ, Tonlaar NY, Garwood ER, Huo D, Moore DH, Khramtsov AI, A Au, Baehner F, Chen Y, Malaka DO, Lin A, Adeyanju OO, Li S, Gong C, McGrath M, Olopade OI, Esserman LJ. Proliferating macrophages associated with high grade hormone receptor negative breast cancer and poor clinical outcome. *Breast Cancer Res. Treat.* 2011; 128:703–711. [PubMed: 20842526]
193. Kang JC, Chen JS, Lee CH, Chang JJ, Shieh YS. Intratumoral macrophage counts correlate with tumor progression in colorectal cancer. *J. Surg. Oncol.* 2010; 102:242–248. [PubMed: 20740582]
194. Pedersen MB, Danielsen AV, Hamilton-Dutoit SJ, Bendix K, Norgaard P, Moller MB, Steiniche T, d'Amore F. High intratumoural macrophage content is an adverse prognostic feature in anaplastic large cell lymphoma. *Histopathology*. 2014 in press <http://dx.doi.org/10.1111/his.12407>.
195. Qian BZ, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. *Cell.* 2010; 141(1):39–51. <http://dx.doi.org/10.1016/j.cell.2010.03.014>. [PubMed: 20371344]
196. Tang X, Mo C, Wang Y, Wei D, Xiao H. Anti-tumour strategies aiming to target tumour-associated macrophages. *Immunology*. 2013; 138:93–104. [PubMed: 23113570]
197. Capece D, Fischietti M, Verzella D, Gaggiano A, Ciccirelli G, Tessitore A, Zazzeroni F, Alesse E. The inflammatory microenvironment in hepatocellular carcinoma: a pivotal role for tumor-associated macrophages. *BioMed. Res. Int.* 2013; 2013:187204. [PubMed: 23533994]
198. da Fonseca AC, Badie B. Microglia and macrophages in malignant gliomas: recent discoveries and implications for promising therapies. *Clin. Dev. Immunol.* 2013; 2013:264124. [PubMed: 23864876]
199. Eljaszewicz A, Wiese M, Helmin-Basa A, Jankowski M, Gackowska L, Kubiszewska I, Kaszewski W, Michalkiewicz J, Zegarski W. Collaborating with the enemy: function of macrophages in the development of neoplastic disease. *Mediat. Inflamm.* 2013; 2013:831387.
200. Moghimi SM, Parhamifar L, Ahmadvand D, Wibroe PP, Andresen TL, Farhangrazi ZS, Hunter AC. Particulate systems for targeting of macrophages: basic and therapeutic concepts. *J. Innate Immun.* 2012; 4:509–528. [PubMed: 22722900]

201. Zweit J. Radionuclides and carrier molecules for therapy. *Phys. Med. Biol.* 1996; 41:1905–1914. [PubMed: 8912370]
202. Watters JJ, Schartner JM, Badie B. Microglia function in brain tumors. *J. Neurosci. Res.* 2005; 81:447–455. [PubMed: 15959903]
203. Coniglio SJ, Segall JE. Review: molecular mechanism of microglia stimulated glioblastoma invasion. *Matrix Biol.* 2013; 32:372–380. [PubMed: 23933178]
204. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB. Identification of human brain tumour initiating cells. *Nature.* 2004; 432:396–401. [PubMed: 15549107]
205. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD, Rich JN. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature.* 2006; 444:756–760. [PubMed: 17051156]
206. Huysentruyt LC, Akgoc Z, Seyfried TN. Hypothesis: are neoplastic macrophages/ microglia present in glioblastoma multiforme? *ASN Neuro.* 2011; 3
207. Madsen SJ, Baek SK, Makkouk AR, Krasieva T, Hirschberg H. Macrophages as cell-based delivery systems for nanoshells in photothermal therapy. *Ann. Biomed. Eng.* 2012; 40:507–515. [PubMed: 21979168]
208. Soundararajan A, Bao A, Phillips WT, Perez R 3rd, Goins BA. [(186)Re] Liposomal doxorubicin (Doxil): in vitro stability pharmacokinetics imaging and biodistribution in a head and neck squamous cell carcinoma xenograft model. *Nucl. Med. Biol.* 2009; 36:515–524. [PubMed: 19520292]
209. Chang YJ, Chang CH, Yu CY, Chang TJ, Chen LC, Chen MH, Lee TW, Ting G. Therapeutic efficacy and microSPECT/CT imaging of ¹⁸⁸Re-DXR-liposome in a C26 murine colon carcinoma solid tumor model. *Nucl. Med. Biol.* 2010; 37:95–104. [PubMed: 20122674]

Fig. 1.

(A) Planar gamma camera images of rats bearing head and neck tumor acquired following intravenous infusion of rhenium-186 (Re-186) liposomal doxorubicin (Doxil) without (upper panel) or in combination with RF ablation therapy (lower panel) over a 5 day period. There is increased accumulation of rhenium-186 Doxil in the tumor following RF ablation. Panel B demonstrates the greatly decreased change in tumor volume with the triple therapy of RF ablation (RFA) with liposomes carrying rhenium-186 and doxorubicin as compared with dual and single therapies. Panel C demonstrates the greatly increased percentage of complete

response to therapy with triple therapy as compared with dual and single therapy combinations. Complete response was defined as >20% decrease in tumor volume at the end of the study.

Fig. 2. Illustration depicting the β -radiation from radiotherapeutic nanoparticles following intratumoral CED administration. A significant advantage of β -emitting radionuclides for use with CED-administered nanoparticles is that the path length of the β -radiation extends the treatment to a micro-field of 40–80 tumor diameter distances from the location of the nanoparticle in all directions.

Fig. 3.

Tumor retention (A) and therapy response (B–C) of ^{186}Re -liposomes compared with free ^{186}Re -perrhenate, ^{186}Re -BMEDA or unlabeled liposome controls following intratumoral CED administration in 2-gram head and neck tumor xenografts. Tumor retention of ^{186}Re -liposomes was much greater than the free ^{186}Re -perrhenate or the small chelator molecule, ^{186}Re -BMEDA. This improved tumor retention of the ^{186}Re -liposomes as well as its improved intratumoral dispersion resulted in a significant therapeutic response that was not observed when the same amount of ^{186}Re activity was administered in the chemical form of either free ^{186}Re -perrhenate or ^{186}Re -BMEDA (***) $p < 0.001$ for ^{186}Re -liposomes versus the ^{186}Re and liposome control groups). When the therapeutic response of individual animals to the ^{186}Re -liposomes was examined, ~hal fo fthe animals appeared to have a complete response while the other half had tumors that re-grew at a much slower rate than that of control animals.

Fig. 4. SPECT/CT images of rats with head and neck tumor xenografts acquired 20 h after the intratumoral administration of ^{99m}Tc -liposomes (A, C) versus ^{99m}Tc -BMEDA chelator (B, D). In the sagittal SPECT images superimposed on co-registered CT images (A, B), note the white color located centrally at the site of the intratumoral administration of the ^{99m}Tc -BMEDA chelator representing a very high local concentration of activity, which indicates the poor dispersion capability of small molecules from CED administration. In contrast, the broad range distribution of radioactive liposomes shows the advantages of the use of liposome nanoparticles for improved drug distribution coverage in the solid tumor. Trans-axial SPECT images of the excised tumor intratumorally infused with ^{99m}Tc -liposomes (Fig.

3C) or ^{99m}Tc -BMEDA (Fig. 3D) also show the improved distribution of ^{99m}Tc -liposome nanoparticles.

Fig. 5.

MRI-based therapy response monitoring (A) and survival (B) of rats with intracranial glioblastoma xenografts after treatment with ^{186}Re -liposomes. MRI images of intracranial tumors reveal comparable pretreatment U87 brain tumor size in control animals and in animals treated with intratumoral CED administration of ^{186}Re -liposome nanoparticles. Tumors in control animals had progressive growth by day 14 post-tx, whereas ^{186}Re -liposome-treated tumors gradually decreased over time and disappeared at last time point studied. Survival was greatly extended for the rats treated with ^{186}Re -liposomes. Four of the five animals treated by CED infusion of ^{186}Re -liposomes showed no evidence of U251 tumor re-growth at the end of the study, approximately 100 days post-treatment. All control animals died by 17 days after the time of the start of treatment in the experimental animals.

Fig. 6.

Multimodal MRI (A), PET (B), near infrared optical (C) images of head and neck tumor acquired following intratumoral CED administration and schematic diagram of multifunctional liposomes demonstrate the versatility of nanoparticles for multifunctional imaging and therapy applications. These multiple images were obtained from the same liposome nanoparticle formulation. Intratumoral distribution of the multifunctional liposome nanoparticles was non-invasively acquired with MR imaging through gadolinium-DOTA (A), PET imaging with ^{64}Cu -labeling on DOTA group (B), and optical imaging with IRDye on headgroup (C). Multifunctional liposomes can be very useful in pre-clinical research and clinical applications by providing different means of monitoring the distribution and retention of the nanoparticles. The strength of MR imaging is its ability to provide high resolution distribution non-invasively. The strength of PET imaging is its ability to provide quantitative data over time on the distribution and retention of nanoparticles within the tumor. The strength of near infrared optical imaging is in its use for non-invasive imaging in small animals, its potential use in real-time surgical applications and its use for distribution studies at the optical microscopic level using histological slides.

Fig. 7.

Illustration of the proposed mechanism of intratumoral macrophage involvement in β -radiation treatment with nanoparticles. These 3 images illustrate a possible mechanism by which nanoparticles could be carried within the tumor by intratumoral macrophages to reach the rapidly growing invasive margins of the tumor. In A, the nanoparticles administered intratumorally by CED results in dispersion through the tumor. In B, dispersed nanoparticles are phagocytized by intratumoral macrophages in the dispersed locations throughout the tumor. Panel C shows migration of mobile macrophages to regions at the invasive margins of the tumor while delivering therapy to a 3D field via β -radiation treatment surrounding the macrophage.

Fig. 8.

Bioluminescence optical imaging can be used to detect viable luciferase gene transfected U251 tumor cells non-invasively (A) and quantify tumor cell luciferase activity in the brain of rats (B) following intratumoral CED administration of ^{186}Re -liposomes. Note that tumor cell death in response to β -emission radionuclide therapy is a relatively slow process which provides time for extensive movement of intratumoral macrophages that have ingested the ^{186}Re -liposome nanoparticles to regions of active growth in the tumor. This slow tumor death also suggests the possibility that intratumoral treatment with ^{186}Re -liposome nanoparticles could induce an immune response to the tumor.

Table 1
Properties of β -emitting radionuclides and their use in radiotherapeutic nanoparticle preparation.

Radionuclide	Average β energy (MeV)	Mean path length (mm)	Half-life (days)	Gamma energy (keV) (% decay)	Production method	Nanoparticle radiolabeled
Copper-67	^{67}Cu 0.141	0.71	2.6	185 (48.7%)	Reactor	No ^{67}Cu . Gold nanoshells have been labeled with diagnostic PET imaging ^{64}Cu [47]
Gold-198	198Au 0.315	1.6	2.7	412 (95.6%)	Reactor	Gold dendrimer nanodevice [48] Gold nanoparticle [49,50]
Holmium-166	166Ho 0.666	3.2	1.1	81 (6.7%);	Reactor	Poly-L-lactide nanoparticles [51] Liposomes [52]
Iodine-131	131I 0.182	0.91	8.0	365 (82%)	Reactor	Boronated dendrimer [53]
Lutetium-177	177Lu 0.133	0.67	6.7	113 (6.4%)	Reactor	Metallofullerene [54] Poly(DL-lactide-co-glycolide) nanoparticles [55] Liposomes [56]
Phosphorus-32	32P 0.695	2.9	14	N.A.	Reactor	Chromic phosphate colloid [57–61]
Rhenium-186	186Re 0.362	1.8	3.8	137 (9.8%)	Reactor	Colloid [62] Liposomes [38,63–66]
Rhenium-188	188Re 0.764	3.5	0.71	155 (15.6%)	Tungsten-188/rhenium-188 generator	Solid lipid nanocapsules [67–69] Liposomes [70–73] Dendrimer [74] Iron oxide [75] Albumin magnetic nanoparticle [76] Quantum dot [77] Micelle [78]
Yttrium-90	90Y 0.935	3.9	2.7	N.A.	Reactor	None

Table 2

Previous research with intratumorally administered radiotherapeutic nanoparticle.

Agent	Size (nm)	Tumor treated	Species (number)	Radio activity mCi (MBq)	Results	Toxicity	Reference
P-32 chromic phosphate colloid	600–1300	Pancreatic	Human (N = 28)	4–60 (148–2220)/tumor	Median survival – 12 months without metastasis and 6.9 months with metastasis	Grade IV thrombocytopenia (2 patients) Grade III neutropenia (1 patient) No significant toxicity when dose limited to 2 infusions of 30 mCi each	[58]
P-32 chromic phosphate colloid	600–1300	Variety of solid tumors	Human (N = 17)	2–15 (74–555)/tumor	Therapy response – 71% Complete response – 41%	Minimal	[60]
P-32 chromic phosphate colloid	600–1300	Head and neck	Human (N = 14)	2–12 (74–444)/tumor	Partial response – 51%	Grade I/II thrombocytopenia (3 patients)	[61]
Au-198 gold nanoparticle (epigallocatechin-gallate targeted)	120–130	Prostate xenograft	Mice (N = 7)	0.136 (5)/tumor	80% decrease in tumor volume at Day 28	No clinical signs	[50]
Au-198 gold nanoparticle (Gum Arabic glycoprotein coated)	85	Prostate xenograft	Mice (N = 7)	0.408 (15)/tumor	82% decrease in tumor volume at day 21	Transient weight loss No systemic toxicity	[49]
Au-198 gold dendrimer nanodevice	10–29	Mouse melanoma	Mice (N = 14)	0.354 (13)/tumor (N = 7) 0.0744 (2.75)/tumor (N = 7)	45% decrease in tumor volume at day 8	No clinical toxicity	[48]
Lu-177 metallofullerene	20	Glioblastoma intracranial xenograft	Nude mice (N = 10)	0.03 (1.11)/tumor	At day 52 90% of treated mice survived compared with no control mice	Not determined	[54]
Re-188 nanocapsules	55.5 ± 11.5	Rat 9L gliosarcoma	Rat (N = 19)	0.03–0.12 (1.1–4.4)/tumor	For 2.8 MBq group, 33% of rats survived to day 100, an increase in median survival of 80% compared with control groups.	Side effects of lethargy and 25% weight loss observed in 4.4 MBq group. Slight body weight loss observed in 2.8 and 3.7 MBq groups until day 12.	[69]
Re-186 liposomes	135.1 ± 18	Head & neck xenograft	Nude rat (N = 6)	5 (185)/cm ³ tumor	87.7% decrease in tumor volume on day 42	Mild body weight loss until day 5 No systemic toxicity observed	[64]

Agent	Size (mm)	Tumor treated	Species (number)	Radio activity mCi (MBq)	Results	Toxicity	Reference
Re-186 liposomes	109 ± 11	Head & neck xenograft remnant	Nude rat (N = 8)	4.75 (176)/rat	Remnant tumor volume increased with 25.6% compared with 288% increase for unlabeled liposome control.	Mild body weight loss until day 5 No systemic toxicity	[146]
Re-186 liposomes	108 ± 26	Glioblastoma intracranial xenograft	Nude rat (U87 N = 18; U251 N = 5)	0.025–0.125 (0.92–4.6)/tumor	Median survival for U87 treated group was 126 days compared with 49 days for unlabeled liposome control group. Median survival was not reached at 120 days for U251 treated group.	No clinical or microscopic toxicity	[142]