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# Targeted Imaging and Therapy of Brain Cancer using Theranostic Nanoparticles

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## Abstract

The past decade has seen momentous development in brain cancer research in terms of novel imaging-assisted surgeries, molecularly targeted drug-based treatment regimens or adjuvant therapies and in our understanding of molecular footprints of initiation and progression of malignancy. However, mortality due to brain cancer has essentially remained unchanged in the last three decades. Thus, paradigm-changing diagnostic and therapeutic reagents are urgently needed. Nanotheranostic platforms are powerful tools for imaging and treatment of cancer. Multifunctionality of these nanovehicles offers a number of advantages over conventional agents. These include targeting to a diseased site thereby minimizing systemic toxicity, the ability to solubilize hydrophobic or labile drugs leading to improved pharmacokinetics and their potential to image, treat and predict therapeutic response. In this article, we will discuss the application of newer theranostic nanoparticles in targeted brain cancer imaging and treatment.

### Keywords

Nanoparticle; MRI; Photodynamic therapy; F3 and RGD peptides; toxicity; cancer therapy

## Brain Tumors: Current status

It is estimated that, in 2010, more than 1.5 million men and women in the US will be diagnosed with cancer and more than half a million will die of it.[1] Of these, there will be 22,020 cases of brain cancer with more than 13,000 associated fatalities.[2] Malignant glioma poses a special challenge due to difficulties in early stage detection and the close proximity of several anatomical structures essential for proper motor, cognitive, reflexive and other functions.[3,4] Gliomas constitute a major clinical problem because of their high rate of occurrence and aggressive phenotype leading to extremely poor prognosis. Gliomas represent 78% of all malignant brain tumors and in males between the age of 20–39 years, are the most common cause of cancer-related death.[2,4] Invasive biopsy is routinely utilized to assess histological type, classification, grade and potential aggressiveness of brain cancer and also for determination of the type of drug regimen employed for treatment.[5,6] This is further aided by newer imaging techniques such as CT, PET, ultrasound and MRI.[7–11] However, for some brain tumors the quantification of the actual tumor volume is difficult as peritumoral edema does not readily provide for precise discrimination of tumor margins.[12] The use of contrast agent may help overcome this deficiency and allow estimates of tumor margins from the largest cross-sectional area.[13–15] However, such tumor enhancement using contrast agents is possible only in patients with a compromised blood-brain barrier (BBB).[12]

Three main modalities used in the treatment of brain tumors include surgery, radiation therapy and chemotherapy.[16] Surgery is the primary mode of treatment of most brain cancers[17] but also poses the greatest challenge as crucial structures near the tumor bed may often control a critical function. During surgical resection, neoplastic tissue that is easily

detected radiographically is often visually indistinguishable from normal brain, complicating removal of the tumor tissue. This has led to the development of a number of image-assisted surgery protocols.[18,19] To further improve the eradication of tumor cells, adjuvant radiation and chemotherapy regimens have been developed. However, these treatments are often limited by side effects or the emergence of therapy-resistant populations. Additionally, radiation therapy results in a delayed but well-documented decline in cognitive function risk of secondary malignancy or metastatic disease[20,21] in adults, and it is documented to interfere with brain development in children.[20, 21] The chemotherapeutic agents used for brain cancer therapy often have a low therapeutic index and systemic side effects. Thus, although the past decade has seen dramatic advances in surgical modalities and development of novel molecular target based adjuvant therapies for brain tumors, a significant improvement in patient outcome is yet to be realized.[22] This suggests that, in order to rein in the death toll associated brain cancer or cancer in general, paradigm-changing modalities for detection and cure of cancer are urgently needed.

## Theranostic Nanoparticles for imaging and treatment of Tumors

Nanotechnology in cancer, an interdisciplinary field involving oncologists, chemists and engineers, deals with construction of unique submicrometer colloidal particle systems and their application in various aspects of cancer imaging, diagnosis and treatment.[16,23] In the recent past, nanotechnology has garnered much attention due to its potential application in cancer, and the National Cancer Institute has constituted an Alliance of Nanotechnology in Cancer[25,24] with focus on the development of novel nanoplatform-based diagnostics, therapeutics and preventive agents. Nanoparticles are colloidal particles (10–100 nm in size) typically synthesized in either aqueous or organic phases.[16,23,26–28] Due to their small size, nanoparticles can easily flow through blood capillaries and enter the target cancer cells.[16,23,27] Nanoplatform-based delivery systems offer a number of advantages over conventional drug delivery systems.[16,27,29–31] First, the nanoparticles can be engineered to provide designed functionalities using standard procedures in nanotechnology. The type and the number of linkers within and on the surface of nanoparticles and the size of the nanoparticle itself can be modulated to control the loading/releasing of the encapsulated or covalently linked drug components or to add surface coating. Second, they can improve the efficacy of existing imaging and treatment regimens. The ability to deliver contrast or therapeutic agents selectively to tumors at effective concentrations is a key factor for the efficacy of cancer detection and therapy. In terms of selective delivery, nanoparticles have a potential inherent advantage over small molecules as small molecules can diffuse into normal brain as well as tumor tissue but nanoparticles have a more limited/selective delivery via the leaky vasculature within solid tumors (see below). Third, nanoparticles can increase the maximum tolerated dose of the drug by using nontoxic (biocompatible) polymers to separate the therapeutic agent from being released within normal tissues thus reducing overall systemic toxicity. Additionally, encapsulation of drugs within nanoplatforms can provide a significant advantage when employing poorly soluble, poorly absorbed or labile agents by incorporating them in the matrix of the nanoparticle during the formulation/ synthetic process. Such encapsulation also aids in preventing premature inactivation or degradation of drugs during plasma transport as the interaction between the drug and the blood components is minimized. Additionally, the release of the drug at the site of action can also be manipulated by active targeting such as peptide targeting (see Figure 1), thereby leading to increased therapeutic efficacy through an increased localized release of the therapeutic agent resulting in a higher concentration of agent within the tumor mass with a concomitant overall reduction in undesired side effects.[32]

A unique attribute of nanoplatform-based delivery systems is their multifunctionality, is., nanoparticles can be designed that carry multiple components which include (see Figure 2)

(1) imaging agents, (2) therapeutic agents, (3) targeting ligands, and (4) “cloaking” agents that avoid interference with the immune system.[33–41] Targeted nanoparticle-based treatment technologies with diagnostic capabilities are referred to as theranostic agents as they form a class of agents which can serve diagnostic and therapeutic functions simultaneously. In the current state of technology, tumor detection and therapy are mostly performed separately. A more efficient and effective method can be achieved with theranostic nanoparticles, which would integrate the efforts for detection, treatment and follow-up monitoring of tumor response, and assist in the decision-making process for the need for further treatment. This concept has drawn interest in the cancer research community and has led to investigations to develop and translate this innovative nanoparticle-based strategy for cancer diagnosis and treatment into clinical practice. [37,42,43]

## Enhanced Permeability and Retention (EPR) Effect and Selective Retention of Theranostic Nanoparticles at Tumor Site

Solid tumors have a diffusion-limited maximal size of about 2 mm and will remain at this size until angiogenesis occurs. Neoangiogenesis provides tumor cells access to oxygen and nutrients for rapid proliferation.[44] As the cancer grows without checks and balances, the neovascularization is also leaky with defective architecture and impaired lymphatic drainage. The leaky vasculature of solid tumors offers critical advantages to nanoparticle-based imaging and therapeutic agents as they are selectively retained allowing them an easy access to the tumor’s interior.[45,46] This phenomenon is known as the enhanced permeability and retention (EPR) effect[47] and is not observed with small molecular weight substances, such as conventional chemotherapeutic agents. Small molecular weight compounds do not discriminate tumor tissue from normal tissue as their uptake and distribution is achieved by free diffusion. For EPR to work, the size and surface properties of drug delivery nanoparticles must be controlled to avoid uptake by the reticuloendothelial system (RES). To maximize circulation times and targeting ability, the optimal size should be less than 100 nm in diameter and the surface should be hydrophilic to circumvent clearance by macrophages within the RES. A hydrophilic nanoparticle surface can provide for a safeguard against plasma protein adsorption, and can be achieved by hydrophilic polymer coatings such as PEG, poloxamines, poloxamers, and polysaccharides or the use of branched or block copolymers.[48,49] The covalent linkage of amphiphilic copolymers (polylactic acid, polycaprolactone, and polycyanonacrylate) chemically coupled to PEG[49,50] is generally preferred, since it avoids aggregation and ligand desorption when in contact with blood components. [51]

An alternative strategy to passive targeting is to utilize the unique tumor microenvironment in a scheme called tumor-activated prodrug therapy. The drug is conjugated to a tumor-specific molecule and remains inactive until it reaches the target.[52] Mansour et al.[53] exploited the matrix metalloproteinase-2 (MMP-2) protease overexpression in melanoma by creating a water-soluble maleimide derivative of doxorubicin (DOX) and incorporating an MMP-2-specific peptide sequence (Gly-Pro-Leu-Gly-Ile-Ala-Gly-Gln) that binds selectively to the cysteine-34 of circulating albumin. The albumin-doxorubicin conjugate is cleaved efficiently and specifically by MMP-2, releasing doxorubicin. Other factors explored as drug-release triggers for site specific delivery are pH and redox potential at the tumor site.[54] Another example of the use of nanoparticles in cancer diagnosis is the application of nanoparticle-based evaluation of the safety of a hepatic arterial infusion of Rexin-G as an intervention for stage IV metastatic pancreatic cancer. This formulation has been approved for clinical trials. [55, 56]

## Molecular Targeting of Theranostic Nanoparticles to Tumor Site

Molecular targeting of nanoparticles can be achieved by conjugation of a specific ligand that recognizes and interacts with a unique molecular recognition site on the tumor cells leading to increased accumulation of nanoparticles within the tumor milieu.[57] In the recent past, due to human genome sequencing and development of differential expression profiling technologies such as serial analysis of gene expression(SAGE), subtractive proteomic mapping, and *in vivo* phage display, there has been an explosion of information in identification of biomarkers with the potential to be exploited as tumor specific targeting agents.[23,24] This investigation allows for development of nanoagents that can be utilized for specifically targeting tumors. Targeted nanoparticles with a pay load of drug will deliver the drug specifically to the cancer cell minimizing the unwanted distribution of the drug in healthy tissues. A wide variety of ligands, including antibodies, peptides, polysaccharides, aptamers, and drugs have been used as targeting components.[57] Interactions of lectin proteins and glycoproteins are examples of other high affinity interactions that have been exploited for targeted delivery.[58]

Targeting the tumor vasculature is one of the key strategies used to specifically deliver drug directly to the tumor vasculature for inhibition of tumor growth. Folkman et al. first reported the possibilities of this type of tumor treatment in 1989.[59] The FDA approved the first vascular targeted drug for the treatment of age-related macular degeneration in 1999. Multifunctional nanoparticles capable of targeting glioma cells, detectable by both magnetic resonance imaging and fluorescence microscopy, have been reported.[60] These nanomaterials were synthesized by coating iron oxide nanoparticles with a covalently bound bifunctional poly(ethylene glycol) (PEG) polymer, which were subsequently functionalized with chlorotoxin and the near-infrared fluorescing Cyanine dyes. Both MR imaging and fluorescence microscopy showed significant preferential uptake of the nanoparticle conjugates by glioma cells. In addition, Moffat et al. reported the use of PEGylated, superparamagnetic iron oxide (SPIO) encapsulated nanoparticles in imaging of glioma cells.[31] This novel polyacrylamide (PAM) SPIO nanoparticle platform provided an extremely large T2 and T2* relaxivity of between 620 and 1140 $s^{-1}$ $mM^{-1}$.[31] Administration of PAM nanoparticles into rats bearing orthotopic 9L gliomas allowed quantitative pharmacokinetic analysis of the uptake of nanoparticles in the vasculature, brain, and glioma tissues.[31] Furthermore, addition of polyethylene glycol of varying sizes (0.6, 2, and 10 kDa) to the surface of the PAM nanoparticles resulted in an increase in plasma half-life and affected tumor uptake and retention of the nanoparticles as quantified by changes in tissue contrast using MRI. These systems could potentially be used to image resections of glioma brain tumors in real time and could correlate preoperative diagnostic images with intraoperative pathology at a cellular-level resolution.[61] The $\alpha_v\beta_3$ integrin is one the most specific biomarkers that can differentiate newly formed capillaries from their mature counterparts.[62] High affinity $\alpha_v\beta_3$ selective ligands, RGD Arg-Gly-Asp, have been identified by phage display studies. The cyclic form, with a conformationally constrained RGD, has a higher binding affinity than the linear form. Doxorubicin-loaded PEG nanoparticles conjugated to cyclic RGD and paclitaxel-cyclic RGD nanoparticles have also been reported recently.[63–65]

Tumor targeting by antibodies with engineered nanoparticles is one of the most attractive methods in drug delivery, yet, this research is in its infancy. The monoclonal antibody (mAb) BR96 (antisialyl Lewis Y antigen), conjugated with doxorubicin, has proven to be highly efficacious in tumor xenograft studies but has shown little or no efficacy in phase II trials for metastatic breast cancer and advanced gastric adenocarcinoma.[66] Moreover, dose-limiting gastrointestinal toxicities are observed in the breast cancer trial, because the immunoconjugate binds to antigen-positive normal cells in gastric mucosa, small intestine,

and pancreas. Calicheamicins and maytansinoids are the most extensively evaluated of many small-molecule toxins that are used for direct antibody conjugation.[67–69] However, utilization of antibody as a targeting agent has achieved better success.[70] For example, anti-ERBB2 immunoliposomes loaded with doxorubicin show greater antitumor activity than the free drug or the drug loaded in untargeted liposomes in several tumor xenograft models. Additionally, the systemic toxicity of the immunoliposome-targeted doxorubicin was much less than that of free doxorubicin.[71,72] Bispecific antibodies, which are unnatural antibodies with two different epitopes, have been used most widely for the delivery of immune effector cells and, to a lesser extent, for the delivery of radionuclides, drugs, and toxins to tumors.[73] Recently, in preclinical human xenograft models, iron oxide harboring EGFRvIII synthetic antibody-conjugated theranostic nanoparticles were evaluated for imaging and therapy of glioblastoma.[74] A significant increase in animal survival was found in animals treated with theranostic nanoparticles.[74] These authors used convection-enhanced delivery, a minimally invasive procedure that imparts a fluid convection by pressure gradient in the brain to allow passage of theranostic agents through the blood-brain barrier. Similarly, to specifically image 9L tumor xenografts, Sun et al. used chlorotoxin targeted superparamagnetic nanoparticles and showed a high target specificity and minimal systemic toxicity.[75,76] Chlorotoxin was also used to target iron oxide nanoparticles covalently linked to polyethylene glycol and near-infrared optical probes to image preferential uptake in glioma cells. These nanoparticles have the potential to be used in image-guided brain tumor surgery.[60] Employing targeted nanoparticles in imaging as a diagnostic agent or for cancer staging could provide a dramatically improved contrast enhancement specific for detection of the tumor extent and at an earlier stage than by current methods, with sensitivity sufficient for avoiding invasive biopsy. Since the specific molecular signature of one tumor type may be different from another, and cannot be differentiated based upon traditional anatomical imaging, the ability to diagnose tumors based on their genetic presentation in a targeted manner would be of great value.

## F3 Targeted Theranostic Nanoplatforms for Brain Cancer Therapy and Imaging

Our lab has been involved with the development and application of a modular theranostic nanoplatform, based on a polyacrylamide (PAA) nanoparticle core, with encapsulated components for synergistic cancer detection, diagnosis and treatment (Figure 2).[16,31,77,78] This platform combined magnetic resonance imaging contrast enhancement, photodynamic therapy and specific targeting to tumor sites using F3 peptide.[77] F3 peptide, a 31–amino acid fragment of a high mobility group protein, was shown to home to the vasculature of a number of tumor types by interacting directly with endothelial cells.[79, 80] It has two attractive features compared to the RGD containing peptide: (1) RGD peptide needs to be cyclized to be functional, which is not optimal for large scale studies, while F3 peptide does not have these requirements. (2) In some human cancers F3 peptide can interact directly with tumor cells, where it is specifically taken up at the cell surface, then internalized into the cell and transported to the nucleus.[79, 80] Cell surface nucleolin is a specific marker for angiogenic endothelial cells within tumor vasculature.[80] Further evidence for the possibility of using F3 to target nanoparticles was provided in a recent study wherein quantum dots (Qdots; <10 nm) were targeted to tumor blood vessels in human xenograft tumors.[81] We have recently shown that significant therapeutic benefit with photodynamic therapy was obtained when an F3-targeted polymeric nanoparticle formulation consisting of encapsulated imaging agent (iron oxide) and photosensitizer (Photofrin) was administered to glioma bearing rats. Using these multifunctional nanoparticles we showed that nanoparticles could be targeted to intracerebral rat 9L gliomas and detected using MRI (figure 3).[76] F3-targeted nanoparticles provided a significantly increased survival time over that of nontargeted

Photofrin encapsulated nanoparticles or Photofrin alone.[76] These results suggest that polymeric multifunctional nanoparticle formulations are a versatile drug delivery vehicle for the supply of imaging and therapeutic agents to a brain cancer milieu.

## $^{19}$-F Based Nanoparticles

$^{19}$F-MRI has been used to evaluate the potential of developing perfluorcarbon (PFC)-based nanoscale agents for multimodal molecular imaging and targeted therapeutics.[82] This approach involves the microfluidization production of PFC-nanoparticles which allows for opportunities to encapsulate large quantities of imaging or therapeutic agents which can be targeted to specific sites using antibodies or other targeting approaches. The versatility of this approach is highlighted by the ability to use $^{19}$F MRI to detect the PFC component of the nanoagent but also, through attachment or encapsulation of a wide variety of contrast agents, multimodal imaging, targeted/therapeutic agents can potentially be developed for a wide variety of clinical applications.

## Nuclear Imaging and CT-Based Nanoparticles

There are significiant opportunities to investigate the use of nuclear imaging techniques such as positron emission tomography (PET) and single-photon emission computed tomography (SPECT) for development of radiolabeled nanoparticles targeting cancer. The versatility, sensitivity and tomographic imaging capabilities of these imaging modalities will provide excellent opportunities for future development of targeted nanoparticle formulations. Isotopes available for attachment or encapsulation within nanoparticles include, for example, $^{18}$F, $^{11}$C, $^{13}$N, $^{64}$Cu, $^{68}$Ga, $^{124}$I, $^{82}$Rb, and $^{86}$Y. While investigations of nuclear imaging-based nanoparticle formulations are emerging, applications to cancer imaging are scarce as the focus has been primarily on the chemical design and characterization of these technologies along with limited *in vivo* evaluation.[83] Furthermore, there is significant interest in the development of iodinated nanomaterial formulations for application in X-ray imaging and computed tomography (CT) with the overall goal of producing an agent with higher localized iodine concentrations for improved contrast.[83] Finally, the use of gold nanomaterials is also an active area of investigation for improving X-ray contrast agent performance.[83] It is anticipated that further nanoparticle/nanomaterial research will lead to important advances in the areas of diagnostic imaging and treatment over the next decade.

## Regulatory Perspective

The National Institutes of Health has created the Nanotechnology Characterization Laboratory (NCL) to assist in the overall testing and evaluation of nanoparticle formulations. In brief, nanomaterials can be submitted to the NCL for characterization which includes a standardized analytical series of tests which evaluates the pre-clinical toxicology, pharmacology, and efficacy of nanoparticles and devices. Nanomaterials submitted to the NCL undergo characterization of physical attributes, *in vitro* biological properties and *in vivo* compatibility with the goal of providing the originator of the nanoparticle formulation with the documentation needed to file an Investigational New Drug (IND) or Investigational Device Exemption (IDE) application with the FDA. A recent international meeting of scientists and various agencies resulted in summary of how to best evaluate the immunotoxicity of nanoparticle formulations[84] including *in vitro* and *in vivo* testing methods considered needed to be applied for proper overall evaluation. Overall, nanoparticle-containing drugs are regulated internationally by the same criteria as conventional drugs and the development of new guidelines will require further scientific input.

## Summary

This article discusses the development and application of a polymeric nanoparticle-based platform that encompasses both imaging and therapeutic agents as well as cloaking and targeting to brain tumor. These theranostic nanoparticles are one of the most recent and attractive tools developed to combat cancer and have the potential to increase survival of cancer patients by imaging and reporting on the response of tumor to a treatment in real time. Although in its infancy, extensive developments available in polymer chemistry will likely lead to development of lower toxicity nanotheranostics for clinical use.

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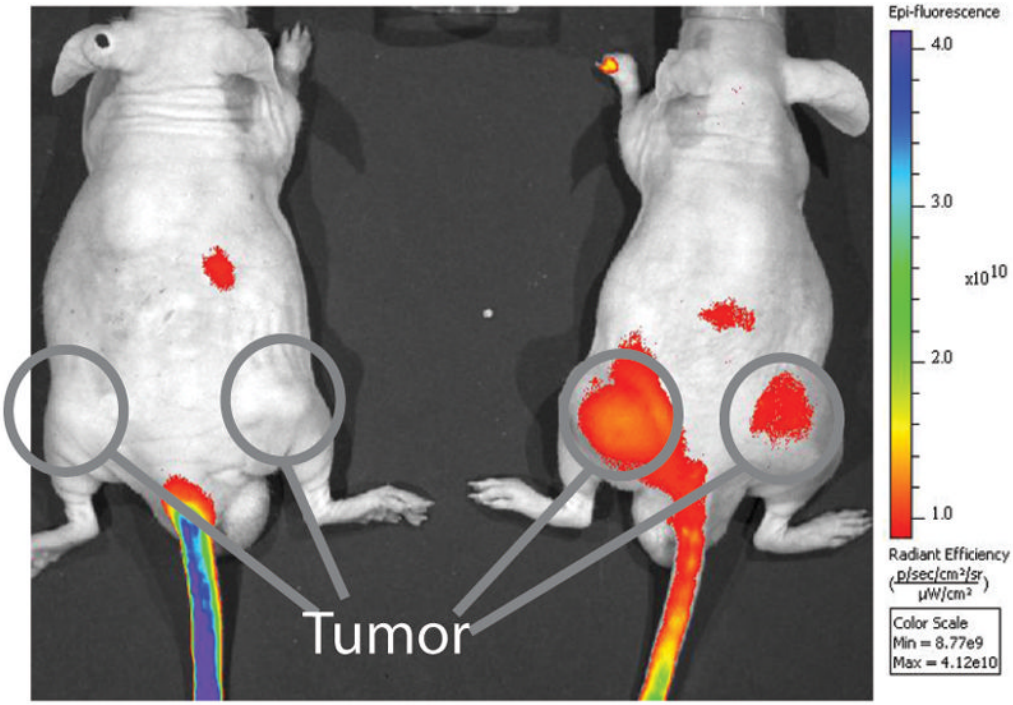


**Figure 1.**

Tumor targeting ability of F3 peptide. The ability of F3 peptide to carry a cargo to tumor cells was assessed in mouse xenografts of human glioblastoma cell line D54. The animal in the left panel received an 100 $\mu$g/ml amount of control peptide (AAVALLPAVLLALLAPESASGASADASVNFLC) conjugated to a near infrared fluorescent cargo while the animal in the right panel received an F3 peptide (KDEPQRRSARLSAKPAPPKPEPKPKKAPAKK) coupled to fluorescent cargo.

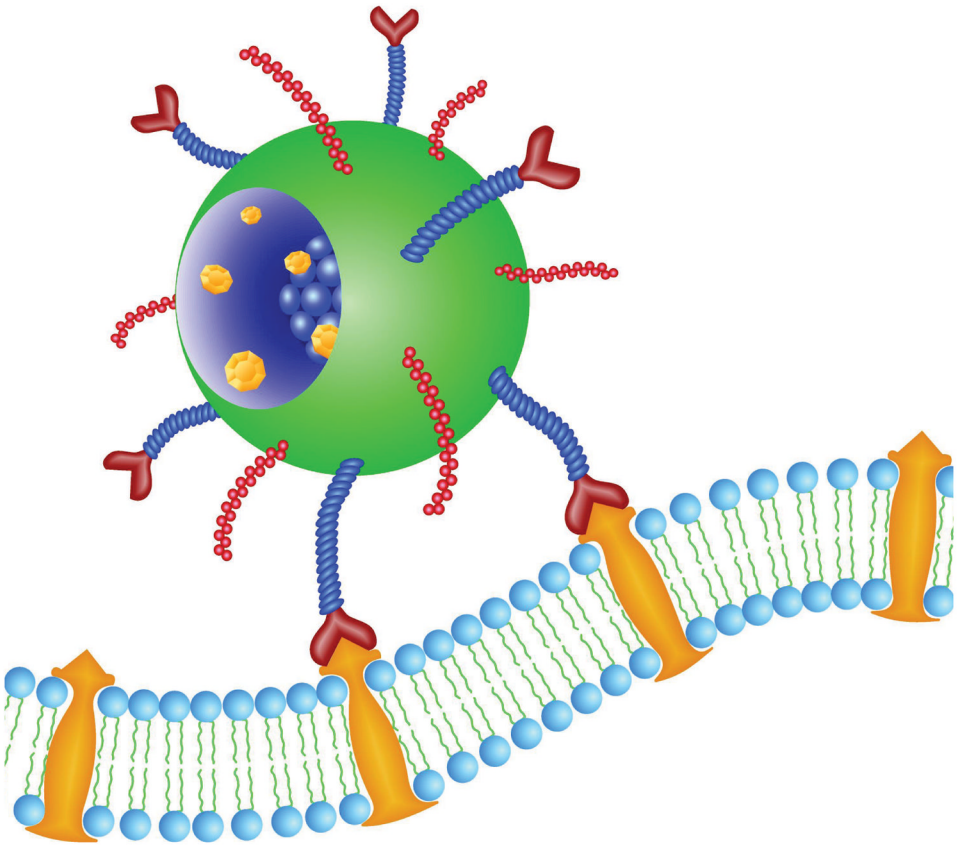

**Figure 2.**
Schematic diagram of a Theranostic nanoparticle. Multifunctional nanoparticles targeted to cancer cell membranes using a ligand to tumor cell specific surface receptor. The nanoparticles harbor an imaging (blue sphere within the core) and therapeutic agent (yellow structures with the core).

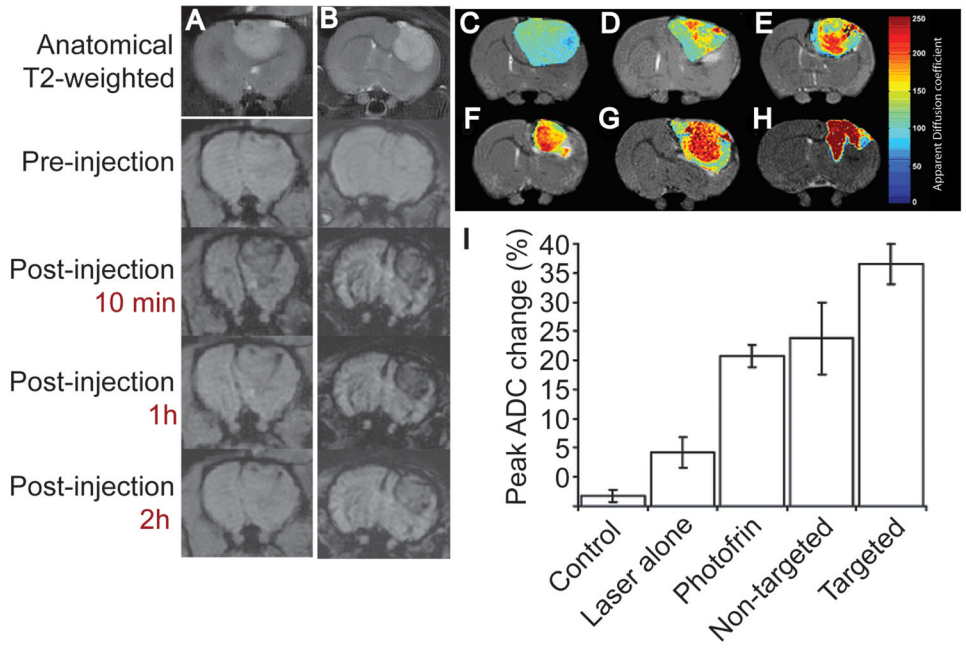


**Figure 3.**

Imaging and monitoring of therapeutic efficacy using multifunctional nanoparticles in 9L brain tumors. Shown in A and B are representative images from two animals. A images were collected from a rat following iv administration of nontargeted nanoparticles. Images shown were acquired at 0, 10, 60, and 120 min following injection, revealing that significant tumor contrast enhancement was achieved at 10 min. Results following administration of targeted nanoparticles are shown in B, which reveal that the contrast enhancement was increased in both magnitude and duration for this preparation. The F3-targeted nanoparticles had ~3-fold prolonged tumor transit time ($P < 0.001$). Moreover, the presence of the F3-targeting moiety also resulted in a significantly improved contrast-to-noise ratio of ~2-fold at 1 h ($P < 0.01$) and 2 h ($P < 0.008$) after contrast administration. Color diffusion maps overlaid on top of T2-weighted images represent the apparent diffusion coefficient (ADC) distribution in each tumor slice shown. MR images shown from day 8 post-treatment from (C) a representative control 9L tumor and tumors treated with (D) laser light only, (E) iv administration of Photofrin plus laser light, and (F) nontargeted nanoparticles containing Photofrin plus laser light. Treatment with F3-targeted Photofrin-encapsulated nanoparticles resulted in the most significant increase in mean tumor apparent diffusion coefficient values (G). (H) Tumor from the same tumor shown in (G), which was treated with the F3-targeted nanoparticle preparation but at day 40 after treatment revealed a high diffusion value indicative of a cystic cavity. (I) Mean peak percentage change in tumor apparent diffusion coefficient values for each of the experimental groups (bars ± SEM).