1. **Multimodality imaging of the HER-kinase axis in cancer.**
   Cai W, Niu G, Chen X.
   The human epidermal growth factor receptor (HER) family of receptor tyrosine kinases controls critical pathways involved in epithelial cell differentiation, growth, division, and motility. Alterations and disruptions in the function of the HER-kinase axis can lead to malignancy. Many therapeutic agents targeting the HER-kinase axis are approved for clinical use or are in preclinical/clinical development. The ability to quantitatively image the HER-kinase axis in a noninvasive manner can aid in lesion detection, patient stratification, new drug development/validation, dose optimization, and treatment monitoring. This review summarizes the current status in multimodality imaging of the HER-kinase axis using PET, SPECT, optical, and MR imaging. The targeting ligands used include small-molecule tyrosine kinase inhibitors, peptides, proteins, antibodies, and engineered antibody fragments. EGFR and HER2 imaging have been well documented in the past, and imaging of HER3, HER4, HER heterodimers, and HER-kinase mutants deserves significant research effort in the future. Successful development of new HER-kinase-targeted imaging agents with optimal in vivo stability, targeting efficacy, and desirable pharmacokinetics for clinical translation will enable maximum benefit in cancer patient management.

2. **Automated live cell imaging of green fluorescent protein degradation in individual fibroblasts.**
   Halter M, Tona A, Bhadriraju K, et al.
   To accurately interpret the data from fluorescent proteins as reporters of gene activation within living cells, it is important to understand the kinetics of the degradation of the reporter proteins. We examined the degradation kinetics over a large number (>1,000) of single, living cells from a clonal population of NIH3T3 fibroblasts that were stably transfected with a destabilized, enhanced green fluorescent protein (eGFP) reporter driven by the tenascin-C promoter. Data collection and quantification of the fluorescence protein within a statistically significant number of individual cells over long times (14 h) by automated microscopy was facilitated by culturing cells on micropatterned arrays that confined their migration and allowed them to be segmented using phase contrast images. To measure GFP degradation rates unambiguously, protein synthesis was inhibited with cycloheximide. Results from automated live cell microscopy and image analysis indicated a wide range of cell-to-cell variability in the GFP fluorescence within individual cells. Degradation for this reporter was analyzed as a first order rate process with a degradation half-life of 2.8 h. We found that GFP degradation rates were independent of the initial intensity of GFP fluorescence within cells. This result indicates that higher GFP abundance in some cells is
likely due to higher rates of gene expression, because it is not due to systematically lower rates of protein degradation. The approach described in this study will assist the quantification and understanding of gene activity within live cells using fluorescent protein reporters. Published 2007 Wiley-Liss, Inc.

3. Functional imaging of cerebrovascular activities in small animals using high-resolution photoacoustic tomography.

Yang S, Xing D, Zhou Q, et al.

Photoacoustic imaging (PAI) is a noninvasive, nonionizing modality based on the differences in light absorption of various biological tissues. PAI utilizes the endogenous contrast characteristics of traditional optical imaging, while benefiting from high spatial resolution of the ultrasound imaging. A PAI system was developed to reconstruct the two-dimensional cross section image and to visualize the cerebrovascular activities of mouse in vivo. The spatial resolution of the PAI system was determined to be 0.110 mm by a two-point-source phantom with the Rayleigh criterion. The potential applications of the system were clearly demonstrated by successfully mapping a traumatic lesion in the mouse brain cerebral cortex, by its ability to monitor physiological changes in the brain due to carotid ligation and drug stimulation, and two-dimensional sliced images of a traumatic mouse brain at different depths were also provided. Our experimental results indicate that PAI has the potential for studying of traumatic brain injury and physiological functions of the brain.

4. Impact of tumor-specific targeting on the biodistribution and efficacy of siRNA nanoparticles measured by multimodality in vivo imaging.

Bartlett DW, Su H, Hildebrandt IJ, et al.

Targeted delivery represents a promising approach for the development of safer and more effective therapeutics for oncology applications. Although macromolecules accumulate nonspecifically in tumors through the enhanced permeability and retention (EPR) effect, previous studies using nanoparticles to deliver chemotherapeutics or siRNA demonstrated that attachment of cell-specific targeting ligands to the surface of nanoparticles leads to enhanced potency relative to nontargeted formulations. Here, we use positron emission tomography (PET) and bioluminescent imaging to quantify the in vivo biodistribution and function of nanoparticles formed with cyclodextrin-containing polycations and siRNA. Conjugation of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid to the 5’ end of the siRNA molecules allows labeling with (64)Cu for PET imaging. Bioluminescent imaging of mice bearing luciferase-expressing Neuro2A s.c. tumors before and after PET imaging enables correlation of functional efficacy with biodistribution data. Although both nontargeted and
transferrin-targeted siRNA nanoparticles exhibit similar biodistribution and tumor localization by PET, transferrin-targeted siRNA nanoparticles reduce tumor luciferase activity by approximately 50% relative to nontargeted siRNA nanoparticles 1 d after injection. Compartmental modeling is used to show that the primary advantage of targeted nanoparticles is associated with processes involved in cellular uptake in tumor cells rather than overall tumor localization. Optimization of internalization may therefore be key for the development of effective nanoparticle-based targeted therapeutics.

5. **Selective high-affinity ligand antibody mimics for cancer diagnosis and therapy: initial application to lymphoma/leukemia.**


   Balhorn R, Hok S, Burke PA, et al.


   **PURPOSE:** More than two decades of research and clinical trials have shown radioimmunotherapy to be a promising approach for treating various forms of cancer. Lym-1 antibody, which binds selectively to HLA-DR10 on malignant B-cell lymphocytes, has proved to be effective in delivering radionuclides to non-Hodgkin's lymphoma and leukemia. Using a new approach to create small synthetic molecules that mimic the targeting properties of the Lym-1 antibody, a prototype, selective high-affinity ligand (SHAL), has been developed to bind to a unique region located within the Lym-1 epitope on HLA-DR10. **EXPERIMENTAL DESIGN:** Computer docking methods were used to predict two sets of small molecules that bind to neighboring cavities on the beta subunit of HLA-DR10 surrounding a critical amino acid in the epitope, and the ligands were confirmed to bind to the protein by nuclear magnetic resonance spectroscopy. Pairs of these molecules were then chemically linked together to produce a series of bidentate and bisbidentate SHALs. **RESULTS:** These SHALs bind with nanomolar to picomolar K(d)'s only to cell lines expressing HLA-DR10. Analyses of biopsy sections obtained from patients also confirmed that SHAL bound to both small and large cell non-Hodgkin's lymphomas mimicking the selectivity of Lym-1. **CONCLUSIONS:** These results show that synthetic molecules less than 1/50th the mass of an antibody can be designed to exhibit strong binding to subtle structural features on cell surface proteins similar to those recognized by antibodies. This approach offers great potential for developing small molecule therapeutics that target other types of cancer and disease.

6. **Targeting and cellular trafficking of magnetic nanoparticles for prostate cancer imaging.**


   Antibody-conjugated iron oxide nanoparticles offer a specific and sensitive tool to enhance magnetic resonance (MR) images of both local and metastatic cancer.
Prostate-specific membrane antigen (PSMA) is predominantly expressed on the neovasculature of solid tumors and on the surface of prostate cells, with enhanced expression following androgen deprivation therapy. Biotinylated anti-PSMA antibody was conjugated to streptavidin-labeled iron oxide nanoparticles and used in MR imaging and confocal laser scanning microscopic imaging studies using LNCaP prostate cancer cells. Labeled iron oxide nanoparticles are internalized by receptor-mediated endocytosis, which involves the formation of clathrin-coated vesicles. Endocytosed particles are not targeted to the Golgi apparatus for recycling but instead accumulate within lysosomes. In T1-weighted MR images, the signal enhancement owing to the magnetic particles was greater for cells with magnetic particles bound to the cell surface than for cells that internalized the particles. However, the location of the particles (surface vs internal) did not significantly alter their effect on T2-weighted images. Our findings indicate that targeting prostate cancer cells using PSMA offers a specific and sensitive technique for enhancing MR images.

7. **Oxygen sensitivity of reporter genes: implications for preclinical imaging of tumor hypoxia.**
   
   
   Cecic I, Chan DA, Sutphin PD, et al.  
   
   Reporter gene techniques have been applied toward studying the physiologic phenomena associated with tumor hypoxia, a negative prognostic indicator. The purpose of this study was to assess the potential adverse effects of hypoxic conditions on the effectiveness of four commonly used reporter genes: Renilla luciferase, monomeric red fluorescent protein, thymidine kinase, and lacZ. Tumor-forming A375 cells expressing a trifusion reporter consisting of Renilla luciferase, monomeric red fluorescent protein, and thymidine kinase were subjected to decreasing oxygen tensions and assayed for reporter expression and activity. A375 cells expressing beta-galactosidase were similarly exposed to hypoxia, with activity of the reporter monitored by cleavage of the fluorescent substrate 7-hydroxy-9H-(1,3-dichloro-9,9-dimethylacridin-2-one)-beta-galactoside (DDAOG). Generation of signal in in vivo tumor models expressing bioluminescent or beta-galactosidase reporters were also examined over the course of hypoxic stresses, either by tumor clamping or the antivascular agent 5,6-dimethylxanthenone-4-acetic acid (DMXAA). Our findings indicate that bioluminescent and fluorescent reporter activity are decreased under hypoxia despite minimal variations in protein production, whereas beta-galactosidase reporter activity per unit protein was unchanged. These results demonstrate that combining beta-galactosidase with the DDAOG optical probe may be a robust reporter system for the in vivo study of tumor hypoxia.

8. **Preparations and Preliminary Evaluation of a Biotin-Targeted, Lectin-Targeted Dendrimer-Based Probe for Dual-Modality Magnetic Resonance and Fluorescence Imaging.**
A novel approach for the preparation of a biotinylated dendrimer-based MRI agent 5 is described, in which a unique disulfide bond in the core of the Gd(III)-1B4M-DTPA chelated G2 PAMAM dendrimer was reduced and then attached to a maleimide-functionalized biotin. The new MRI agent 5 features a well-defined dendron structure and a unique biotin functionality. Immobilization of up to four copies of biotinylated dendrimer 5 to fluorescently labeled avidin yields a supramolecular avidin-biotin-dendrimer-Gd(III) complex. Validation of the complex in mice bearing ovarian cancer tumors demonstrates that the avidin-biotin-dendrimer targeting system efficiently targets and delivers sufficient amounts of chelated Gd(III) and fluorophores (e.g., Rhodamine green) to ovarian tumors to produce visible changes in the tumors by both MRI and optical imaging, respectively. Thus, the avidin-biotin-dendrimer complex may be used as a tumor-targeted probe for dual-modality magnetic resonance and fluorescence imaging.

9. Improved tumor imaging and therapy via i.v. IgG-mediated time-sequential modulation of neonatal Fc receptor.

The long plasma half-life of IgG, while allowing for enhanced tumor uptake of tumor-targeted IgG conjugates, also results in increased background activity and normal-tissue toxicity. Therefore, successful therapeutic uses of conjugated antibodies have been limited to the highly sensitive and readily accessible hematopoietic tumors. We report a therapeutic strategy to beneficially alter the pharmacokinetics of IgG antibodies via pharmacological inhibition of the neonatal Fc receptor (FcRn) using high-dose IgG therapy. IgG-treated mice displayed enhanced blood and whole-body clearance of radioactivity, resulting in better tumor-to-blood image contrast and protection of normal tissue from radiation. Tumor uptake and the resultant therapeutic response was unaltered. Furthermore, we demonstrated the use of this approach for imaging of tumors in humans and discuss its potential applications in cancer imaging and therapy. The ability to reduce the serum persistence of conjugated IgG antibodies after their infusion can enhance their therapeutic index, resulting in improved therapeutic and diagnostic efficacy.

10. Enzyme-responsive PARACEST MRI contrast agents: a new biomedical imaging approach for studies of the proteasome.
Proteases are important biomarkers for many biological processes and are popular targets for therapeutics investigations. A protease can be detected by monitoring changes in the paramagnetic chemical exchange saturation transfer (PARACEST) effect of a MRI contrast agent that serves as a substrate for the protease. To translate this type of responsive PARACEST MRI contrast agent to in vivo applications, the sensitivity, timing, specificity and validation of the response of the agent must be evaluated. This report demonstrates that PARACEST MRI contrast agents can be used to detect nanomolar concentrations of proteases, can be designed to preferentially detect the protease caspase-3 relative to caspase-8, and can be detected within the 15 min time frame of typical MRI studies. The response can be validated using an unresponsive PARACEST MRI contrast agent as a control. A survey of the MEROPS database shows that this approach may also be applied to detect other proteases, and therefore may represent a new platform technology for studies of the proteasome.

BACKGROUND: The major hindrance to imaging the intact adult Drosophila is that the dark exoskeleton makes it impossible to image through the cuticle. We have overcome this obstacle and describe a method whereby the internal organs of adult Drosophila can be imaged in 3D by bleaching and clearing the adult and then imaging using a technique called optical projection tomography (OPT). The data is displayed as 2D optical sections and also in 3D to provide detail on the shape and structure of the adult anatomy. METHODOLOGY: We have used OPT to visualize in 2D and 3D the detailed internal anatomy of the intact adult Drosophila. In addition this clearing method used for OPT was tested for imaging with confocal microscopy. Using OPT we have visualized the size and shape of neurodegenerative vacuoles from within the head capsule of flies that suffer from age-related neurodegeneration due to a lack of ADAR mediated RNA-editing. In addition we have visualized tau-lacZ expression in 2D and 3D. This shows that the wholemount adult can be stained without any manipulation and that this stain penetrates well as we have mapped the localization pattern with respect to the internal anatomy. CONCLUSION: We show for the first time that the intact adult Drosophila can be imaged in 3D using OPT, also we show that this method of clearing is also suitable for confocal microscopy to image the brain from within the intact head. The major advantage of this is that organs can be represented in 3D in their natural surroundings. Furthermore optical sections are generated in each of the three planes and are not prone to the technical limitations that are associated with manual sectioning. OPT can be used to dissect mutant phenotypes and to globally map gene expression in both 2D and 3D.
12. A noninvasive multimodal technique to monitor brain tumor vascularization.


Saxena V, Gonzalez-Gomez I, Laug WE.


Determination of tumor oxygenation at the microvascular level will provide important insight into tumor growth, angiogenesis, necrosis and therapeutic response and will facilitate to develop protocols for studying tumor behavior. The non-ionizing near infrared spectroscopy (NIRS) technique has the potential to differentiate lesion and hemoglobin dynamics; however, it has a limited spatial resolution. On the other hand, magnetic resonance imaging (MRI) has achieved high spatial resolution with excellent tissue discrimination but is more susceptible to limited ability to monitor the hemoglobin dynamics. In the present work, the vascular status and the pathophysiological changes that occur during tumor vascularization are studied in an orthotopic brain tumor model. A noninvasive multimodal approach based on the NIRS technique, namely steady state diffuse optical spectroscopy (SSDOS) along with MRI, is applied for monitoring the concentrations of oxyhemoglobin, deoxyhemoglobin and water within tumor region. The concentrations of oxyhemoglobin, deoxyhemoglobin and water within tumor vasculature are extracted at 15 discrete wavelengths in a spectral window of 675-780 nm. We found a direct correlation between tumor size, intratumoral microvessel density and tumor oxygenation. The relative decrease in tumor oxygenation with growth indicates that though blood vessels infiltrate and proliferate the tumor region, a hypoxic trend is clearly present.


Zhang GJ, Chen TB, Bednar B, et al.


The in vivo hollow fiber assay, in which semipermeable hollow fibers filled with tumor cells, are implanted into animals, was originally developed to screen for anticancer compounds before assessment in more complex tumor models. To enhance screening and evaluation of anticancer drugs, we have applied optical imaging technology to this assay. To demonstrate that tumor cells inside hollow fibers can communicate with the host mice, we have used fluorescence imaging in vivo and CD31 immunostaining ex vivo to show that angiogenesis occurs around cell-filled hollow fibers by 2 weeks after subcutaneous implantation. Bioluminescence imaging has been used to follow the number of luciferase-expressing tumor cells within implanted hollow fibers; proliferation of those cells was found to be significantly inhibited by docetaxel or irinotecan. We also used bioluminescence imaging of hollow fibers to monitor the nuclear factor kappaB (NFkappaB) pathway in vivo; NFkappaB activation by lipopolysaccharide and tumor necrosis factor-alpha was evaluated in tumor cell lines genetically
engineered to express luciferase controlled by an NFkappaB-responsive element. These results demonstrate that optical imaging of hollow fibers containing reporter tumor cells can be used for the rapid and accurate evaluation of antitumor activities of anticancer drugs and for measurement of molecular pathways.

Briley-Saebo KC, Mulder WJ, Mani V, et al.  
The vulnerability or destabilization of atherosclerotic plaques has been directly linked to plaque composition. Imaging modalities, such as magnetic resonance (MR) imaging, that allow for evaluation of plaque composition at a cellular and molecular level, could further improve the detection of vulnerable plaque and may allow for monitoring the efficacy of antiatherosclerotic therapies. In this review we focus on MR imaging strategies for the detection and evaluation of atherosclerotic plaques and their composition. We highlight recent advancements in the development of MR pulse sequences, computer image analysis, and the use of commercially available MR contrast agents, such as gadopentic acid (Gd-DTPA), for plaque characterization. We also discuss molecular imaging strategies that are currently being used to design specific imaging probes targeted to biochemical and cellular markers of atherosclerotic plaque vulnerability.

15. Predicting melanoma metastatic potential by optical and magnetic resonance imaging.  
Accurate prediction of tumor metastatic potential would be helpful in treatment planning and in the design of agents that modify the tumor phenotype. We report that three methods that are potentially transferable to the clinic--dynamic contrast enhanced MRI (DCE MRI), T(1rho)-weighted imaging and low temperature fluorescence imaging (that could be performed on biopsy specimens)--distinguished between relatively indolent (A375P) and aggressive (C8161) metastatic human melanoma xenografts in nude mice, whereas T1 and T2 relaxation time measurements did not. DCE MRI data analyzed by the BOLus Enhanced Relaxation Overview (BOLERO) method in conjunction with concurrent measurements of the arterial input function yielded a blood transfer rate constant (Ktrans) which measures perfusion/permeability, that was significantly higher in the core of the indolent tumor than in the core of the aggressive tumor. Histological staining indicated that aggressive tumors had more blood vascular structure but fewer functional vascular structure than
Indolent tumors. Indolent tumors exhibited T(1rho), values that were significantly higher than those of aggressive tumors at spin-locking frequencies >500 Hz. The mitochondrial redox ratio, Fp/(Fp+NADH), where Fp and NADH are the fluorescence of oxidized flavoproteins and reduced pyridine nucleotides, respectively, of aggressive tumors was much higher (more oxidized) than that of indolent tumors and often showed a bimodal distribution with an oxidized core and a reduced rim. These differences observed between these two types of tumors, one indolent and one aggressive, if generalizable, would be very valuable in predicting human melanoma metastatic potential.

Gulec SA, Baum R.
The majority of Neuroendocrine tumors (NET) express somatostatin (SS) receptors, and thus can be successfully targeted with radiolabeled SS analogs in vivo. Somatostatin receptor scintigraphy (SRS) with (111)In-DTPA Octreotide is the main imaging technique for evaluation of NETs. Radio-guided surgery for NETs, therefore, primarily utilizes (111)In-DTPA Octreotide tumor localization mechanism and kinetics. Somatostatin analog radiopharmacy continues to evolve to include the development of more selective and higher affinity analogs and PET tracers. These changes are expected to open new venues for radio-guided surgery technology.

A novel lysine-based trifunctional chelate 3 was designed, synthesized, and characterized and bears both a chelating moiety (CHX-A') for sequestering radiometals (86Y or 111In) and the near-infrared dye Cy5.5 for dual modality PET (or SPECT) and fluorescence imaging, respectively. Successful conjugation of 3 to the monoclonal antibody trastuzumab (Herceptin) was achieved by efficient thiol-maleimide chemistry, thereby yielding immunoconjugate 2. Analysis of 2 by flow cytometry and competitive binding assay demonstrates that immunoconjugate 2 binds to SKOV3 tumor cells comparably to native trastuzumab and, thus, may be used as a tumor-targeted monoclonal antibody probe for multimodality imaging.