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High Field Small Animal Magnetic Resonance Oncology Studies

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Abstract

This review focuses on the applications of high magnetic field magnetic resonance imaging (MRI) and spectroscopy (MRS) to cancer studies in small animals. High field MRI can provide information about tumor physiology, the microenvironment, metabolism, vascularity and cellularity. Such studies are invaluable for understanding tumor growth and proliferation, response to treatment and drug development. The MR techniques reviewed here include ¹H, ³¹P, Chemical Exchange Saturation Transfer (CEST) imaging, and hyperpolarized ¹³C MR spectroscopy as well as diffusion-weighted, Blood Oxygen Level Dependent (BOLD) contrast imaging, and dynamic contrast-enhanced MR imaging. These methods have been proven effective in animal studies and are highly relevant to human clinical studies.

Keywords

Cancer; magnetic resonance imaging; magnetic resonance spectroscopy; metabolism; perfusion; diffusion; MRS; MRI; metastases; lactate

1. Introduction

The original magnetic resonance images published by Paul Lauterbur (Lauterbur, 1973) were of inanimate objects. Shortly thereafter, *in vivo* images of tumors in mice and patients were published (Damadian *et al.*, 1976a; Damadian *et al.*, 1977; Damadian *et al.*, 1978; Damadian *et al.*, 1976b). While *ex vivo* spectra had been obtained previously on muscle, attempts to obtain *in vivo* spectra awaited the development of localization techniques, primarily the development of surface coils which had a limited field of view (Ackerman *et*

et al., 1980). These developments led to the widespread application of magnetic resonance spectroscopic studies to studies of many organs (muscle, brain, kidney, etc), as well as tumor development, progression and response to treatment.

In vivo small animal magnetic resonance imaging and spectroscopic studies have been used extensively for tumor studies since the 1980's (Evanochko *et al.*, 1982; Griffiths *et al.*, 1981; Okunieff *et al.*, 1986). Early studies usually employed vertical high resolution wide bore magnets although some non-localized spectroscopic studies used narrow bore systems, which were readily available at that time. The development of surface coils (Ackerman *et al.*, 1980) led to the widespread use of this technology for the study of tumors, often investigating subcutaneously implanted tumors to enhance the sensitivity and to easily localize the signal. This manuscript will focus on the applications of magnetic resonance to small animal cancer studies. Minimal attention is paid to anatomical imaging but instead the review will concentrate on physiologic and metabolic studies. The techniques summarized include spectroscopic studies with ^1H , ^{31}P , hyperpolarized ^{13}C , dynamic contrast enhanced MRI (DCE-MRI), and diffusion weighted (DW) MRI. Space limitations preclude a broader review which could include ^{19}F , ^{23}Na , conventional ^{13}C MR studies, and other nuclei which are very interesting but are more likely further away from potentially important clinical studies.

2. ^1H MR Spectroscopy

2.1 ^1H Spectroscopic Localization Techniques

We focus initially on *in vivo* ^1H spectroscopy since it is readily translated to patients and numerous clinical studies are ongoing. To isolate a tissue volume from which to obtain an MR spectrum, proton spectroscopy protocols in animals commonly employ a volume localization technique such as Point Resolved Spectroscopy (PRESS) (Bottomley, 1984), STimulated Echo Acquisition Mode (STEAM) (Frahm *et al.*, 1989) or outer volume suppression (Shungu and Glickson, 1994) to eliminate signal from outside the volume of interest (tumor). PRESS and STEAM excite three orthogonal sections of tissue using RF excitation pulses with limited bandwidth combined with linear magnetic field gradients. The rectilinear volume formed by the intersection of the three orthogonal "slices" is termed the excitation volume or voxel. Only spins within this voxel give rise to the spin-echo (PRESS) or stimulated-echo (STEAM) from which the MR spectrum is generated. The accuracy of voxel localization is on the order of tenths of millimeters using these techniques. A large tumor or tissue voxel may be subdivided using gradient phase-encoding to generate a chemical shift image (Brown *et al.*, 1982) with volume elements on the order of tens of cubic millimeters.

2.2 Metabolites Detectable by Proton Spectroscopy

The proton-containing metabolites detectable by magnetic resonance spectroscopy vary according to the tissue being studied and the timing parameters of the pulse sequence used for obtaining the data. A ^1H MR spectrum from the mouse brain obtained at 7 Tesla with repetition time $\text{TR} = 2500$ ms and echo time $\text{TE} = 12.0$ ms is shown in Figure 1a. Large contributions are seen from viable-neuron-specific n-acetyl-aspartate (NAA) and the sum of

creatine and phosphocreatine which reflects precursor and product in the reversible creatine kinase reaction and whose sum is relatively constant in normal brain. Other detectable metabolites include myo-inositol and glutamine/glutamate. The neurotransmitter GABA may also be resolved at 7T when high field homogeneity is obtained. The Cho or total choline (tCho) peak contains contributions from free choline, phosphocholine (PC), and glycerophosphocholine (GPC) and neighboring taurine. Figure 1b demonstrates the potential improvement when a very high field (14.0 Tesla) spectrometer is used. Higher field strength increases the spectral dispersion, i.e. increasing the spacing between peaks and improving the precision of their measurement.

Outside the brain, other tissues/organs, show different metabolite profiles, and, in tumors, there is derangement of the metabolite profile with a very common finding of an elevation of the tCho peak. PC and GPC have been studied intensively in multiple tumor lines in an effort to determine the specific biological processes they reflect. Because PC is a precursor of the membrane phospholipid phosphatidylcholine (PtdCho), and GPC is a breakdown product of PtdCho, elevation of the tCho peak is presumed to represent cell membrane activity. An early MR study of intact Friend leukemia cells demonstrated an increase in PC with an increase in cell differentiation (Agris and Campbell, 1982). In a rat mammary tumor study, Smith et al. showed a positive correlation between PC content and cell proliferation measured by two *ex vivo* assays (Smith *et al.*, 1991). A comparison of human mammary epithelial cells and breast cancer cells proliferating at similar rates demonstrated an elevation in PC in the cancer cells indicating a relationship between PC and the malignant phenotype (Ting *et al.*, 1996; Aboagye and Bhujwalla, 1999). Thus, while PC may reflect proliferation, there are other contributing factors. Further studies have related malignant transformation of various types of cancer cells to phospholipid metabolism, in particular the levels of PC and GPC (Bhakoo *et al.*, 1996; Aboagye and Bhujwalla, 1999; Bhujwalla *et al.*, 1999; Ackerstaff *et al.*, 2001; Mori *et al.*, 2004). Studies aimed at determining the cause of PC elevation have revealed that the level and expression of choline kinase is upregulated in tumors, as well as the rate of choline transport into the cell and activity of phosphatidylcholine-specific phospholipases (Glunde *et al.*, 2006; Ramirez de Molina *et al.*, 2002; Katz-Brull and Degani, 1996; Noh *et al.*, 2000; Guthridge *et al.*, 1994; Iorio *et al.*, 2005). Thus the level and changes in the tCho peak in small animal studies may be prognostic of treatment response.

Lactic acid (lactate) may also be detected in tumors by spectral-editing techniques (Hetherington *et al.*, 1985; He *et al.*, 1995; Lee *et al.*, 2008; Lee *et al.*, 2010; Muruganandham *et al.*, 2004; Star-Lack *et al.*, 1998). These permit assessment of cell energetics and the well-known glycolytic phenotype demonstrated in many tumors (Warburg, 1956). There is currently much emphasis on the investigation of the purpose and implications of glycolytic upregulation in tumors. The potential advantages of this phenotype are well-summarized by Fantin et al. (Fantin *et al.*, 2006) and include the ability to thrive in the hypoxic environment of rapid proliferation, supplying metabolic intermediates for cell proliferation, protection from apoptosis, and reduced production of reactive oxygen species due to reduced oxidative phosphorylation. By opening a non-invasive window on tumor metabolism, *in vivo* proton spectroscopy on high-field systems

allows researchers to address many questions regarding tumor biology, metabolic changes with growth, and effects of treatment.

2.2 Technical Challenges in Proton Spectroscopy

Proton spectroscopy has the distinct advantage of fairly simple translation to clinical studies because it can be performed using standard hardware in most cases. However, there are still multiple challenges. Cellular metabolites which contain protons generally exist at concentrations in the millimolar range, approximately 10,000 times lower than the concentration of water. This low concentration leads to low sensitivity which in turn necessitates adjustments of acquisition parameters such as larger voxel sizes (i.e. coarser spatial resolution compared to imaging) and signal averaging (longer scan times) in order to achieve adequate signal-to-noise ratio (SNR). High-field small animal MR systems provide a distinct advantage in this regard since signal-to-noise ratio increases with field strength. Radiofrequency coils for signal transmission and reception on high-field magnets tend to be optimized in shape and size for the particular organ to be studied to further enhance SNR. This is obviously more feasible when studying small animals, particularly with subcutaneous tumors.

Because water is present in all tissues at high concentration, steps must be taken to ensure that the very high water peak in the proton spectrum does not contaminate the much smaller metabolite peaks. As in all MR spectroscopy experiments, field uniformity is crucial. Shimming of the magnet field is necessary to minimize the width of the peaks and reduce overlap among them. Even with good shimming, the base of the very large water peak can contaminate nearby regions and/or distort the baseline, thus leading to inaccurate measurements of metabolite peak areas. Water suppression techniques are commonly applied such as Chemical Shift Selective Suppression (CHESS) (Haase *et al.*, 1985) and Variable Pulse Power and Optimized Relaxation Delays (VAPOR) (Tkac *et al.*, 1999). The latter tends to be more useful in small animal applications because it is less sensitive to B_1 variations and works better with tailored surface coils. Water suppression also helps to avoid dynamic range limitations which can be caused by the extremely high water peak.

In addition to water, lipids can contribute large peaks to the proton spectrum at various locations corresponding to different proton moieties in triglycerides. Figure 2 contains a proton spectrum obtained at 7.0 Tesla from a mouse with fatty liver. Protons at specific locations in triglycerides give rise to peaks representing total unsaturated fatty acids, polyunsaturated fatty acids, methylene and methyl moieties and other moieties. Information about the relative content of saturated and unsaturated fatty acids can be derived from high-resolution lipid spectra such as these. However, lipid peaks may also mask peaks arising from lower concentration metabolites of interest. For example, the methylene (CH_2) protons from lipids (Fig. 2) at 1.3 ppm can mask the lactate methyl protons which co-resonate with the methylene peak and exist at a concentration that is several orders of magnitude lower than lipid protons in many tissues. In the brain, this is less problematic because lipids are contained in the subcutaneous region and can be excluded from the region of interest. In the rest of the body, lipids are present in essentially all organs. Because lactate is related to glycolysis and tumor cell bioenergetics, it is of high research interest. Spectral editing

techniques make use of the fact that protons on lactate J-coupled to make lactate detectable even in the presence of lipid. These techniques require modifying standard localization pulse sequences such as PRESS and therefore some degree of expertise is necessary, in order to implement them (He *et al.*, 1995; Smith *et al.*, 2008; Star-Lack *et al.*, 1998).

Due to the difference in chemical shift frequency between two or more peaks in the proton spectrum, the PRESS or STEAM voxel giving rise to one peak will be spatially shifted relative to the voxels giving rise to other peaks (Yablonskiy *et al.*, 1998). This occurs due to the finite bandwidth of the three excitation pulses and the limited gradient strength available and becomes more severe at higher field strengths. The greater the chemical shift difference between metabolites, the greater the relative voxel displacement. Furthermore, spectral editing techniques which utilize J-coupling modulation for the detection of lactate and other metabolites are degraded by this effect (Edden *et al.*, 2006; Kelley *et al.*, 1999; Slotboom *et al.*, 1994; Yablonskiy *et al.*, 1998). As this effect is generally seen near the edges of the voxels where there is no overlap of the excitation volumes, one solution is to prescribe a voxel which is larger than necessary and then use spatially selective saturation pulses to “trim” the voxel back to its originally desired size and eliminate signals from the edge regions (Edden *et al.*, 2006; Smith *et al.*, 2008).

2.3 Metabolite Quantitation

The area under a metabolite peak in the NMR spectrum is directly proportional to the concentration of that metabolite. Therefore measurement of the area under the peak and comparison to the peak area of a known reference standard permits quantitation of metabolites as long as differences in relaxation times, number of nuclei per molecule, sample volume and concentration of the reference standard are taken into account. Depending on whether the reference standard used is internal or external, correction factors for field sensitivity profile and RF coil loading may also be necessary. A simplified approach which does not give absolute quantity but eliminates the need for multiple correction factors is to calculate the ratio of two peak areas in the spectrum. For comparison to local data, this can be satisfactory. However, to compare to other studies in the literature, using metabolite ratios is flawed unless identical acquisition parameters have been used. To measure the area under a peak, there are two common options. The baseline can be artificially flattened and then simple numerical integration performed. A second option is to fit the peak using the theoretically expected lineshape (usually Lorentzian, Gaussian, or a combination of the two). There are multiple sophisticated software packages available including LCModel (Provencher, 1993) and jMRUI (Naressi *et al.*, 2001) which fit peaks in the time or frequency domains, incorporate prior knowledge including expected peak location and multiplet shape, and are capable of processing multivoxel chemical shift imaging (CSI) data. In post-acquisition data processing of CSI data, it is possible to select a single peak in the metabolite spectrum and generate a map of the area under that peak over all the voxels in the CSI field of view, i.e. a metabolite map. Figure 3 contains a total choline (tCho) map from a prostate tumor xenograft in a mouse. The map indicates higher tCho intensity around the rim of the tumor with reduced tCho in the more necrotic interior.

Spatial encoding for chemical shift imaging incorporates a point-spread function which results in less precise voxel definition (Brown *et al.*, 1982) than single-voxel studies; therefore, single-voxel techniques tend to be more accurate in terms of metabolite quantitation. In a group of four mice, a recent 7 T study reported normal brain metabolite concentrations of 7.5 ± 0.7 mM for tCr, 5.7 ± 1.1 mM for NAA, 1.6 ± 0.1 mM for tCho, and 7.6 ± 1.3 mM for taurine (Doblas *et al.*, 2012). Standard deviations of metabolite concentrations in tumors tend to be larger due to intra and inter-tumor heterogeneity. In CSI data, spatial filtering can be employed during the CSI acquisition (k-space weighting) (Hugg *et al.*, 1996) or in post-processing. This improves the point-spread function but changes the effective voxel size. In general, multivoxel metabolite maps provide a qualitative picture but do not provide high-precision metabolite concentrations.

2.5 Applications of Proton spectroscopy in High-field Animal Studies

The ability to detect relevant proton metabolites at near-millimeter resolution on high-field animal scanners has led to many studies in mouse models of cancer. The potential ease of translation to clinical scanners makes these studies highly relevant. While cancer detection and assessment of aggressiveness are worthy goals and are the focus of many human spectroscopy studies, small animal studies tend to focus on evaluating new anti-cancer treatments, early measurement of tumor response, and understanding the tumor microenvironment and the biology of treatment effects. As some new agents act cytostatically, and therefore do not result in changes in tumor volume, non-invasive analysis of tumor metabolism is important (Belouèche-Babari *et al.*, 2010). In the following discussion, several examples of proton spectroscopic investigations in small animal cancer are presented.

2.5.1 ^1H MRS for Monitoring the Effect of Radiation on Tumor Metabolism—

Dyke et al. used ^1H MR spectroscopic imaging (MRSI) on a 4.7 T MR scanner to examine the effect of a single 20 Gy dose of radiation in an androgen-dependent human tumor line implanted in nude mice (Dyke *et al.*, 2003). Two pertinent findings were noted. First, in untreated mice, the tCho/water ratio did not change with tumor growth, indicating that changes seen with treatment were not simply due to a change in tumor volume. Second, the tCho peak decreased at 24 hours after radiation and then recovered toward baseline values. This decrease occurred in the absence of any change in serum PSA value. The time course of tCho levels reveals the underlying proliferative changes caused by the radiation which most likely killed some cells and/or slowed proliferation but ultimately did not prevent regrowth. In the WSU-DLCL2 xenograft model of diffuse large B-cell lymphoma studied on a 9.4 T scanner, a single 15 Gy dose of radiation caused a reduction in tCho and an increase in lipids at 24 and 72 hours (Lee *et al.*, 2010). Concurrent lactate-edited spectroscopy also showed a decreased lactate-to-water ratio at these time points. Correlative histological staining at 2 hours after radiation showed decreased proliferation (Ki-67), increased apoptosis (terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)) and increased lipids (oil-red-0). These data indicate that ^1H MRSI of both tCho and lactate is valuable for non-invasively monitoring the effect of radiation on tumor cells in clinically relevant tumor models.

2.5.2 ^1H MRS for Monitoring the Effect of Targeted Anti-tumor Agents—Tumor chemotherapy development is moving toward agents that target specific cellular signaling pathways alone or in combination with other drugs. The elevation in phosphocholine levels in tumors and its relationship to cell membrane synthesis have led to an interest in inhibiting choline kinase (CK), the enzyme in the Kennedy pathway which facilitates the phosphorylation of free Cho to PC. Of note, because of its ability to resolve PC and GPC in high field animal studies, ^{31}P MR spectroscopy (discussed below) is often utilized in combination with ^1H spectroscopy. In human colon tumor xenografts in nude mice, the choline-kinase inhibitor MN58B was found to reduce tCho content at 4 days post-treatment (Al-Saffar *et al.*, 2006). In vitro experiments confirmed that the change in tCho was due to decreased PC. Previous studies showed that the decrease in the intracellular PC pool due to MN58B treatment resulted in G1 arrest. Therefore, the decrease in the *in vivo* tCho peak in this report appears to reflect cell cycle interruption. In work that spans multiple disciplines and MR modalities, Bhujwalla's group have studied a nanoplex molecule which delivers two tumor treatment agents linked to two imaging reporters for optical imaging and MRI (Li *et al.*, 2010). The treatment agents were cytosine deaminase which converts the prodrug 5FC to active 5FU, and small-interfering RNA for choline kinase. While the imaging agents were used to monitor delivery, the effect of treatment in a human breast tumor xenograft was monitored using *in vivo* ^1H spectroscopic imaging of tCho as well as ^{19}F MR spectroscopy of 5FU metabolites on a 9.4 T MR scanner. ^1H MRSI showed not only the distribution of tCho in the tumor, but a reduction in tCho due to si-RNA inhibition of choline kinase. The MRSI-monitored time course of the tCho change was then used to determine the optimal time for delivery of 5FC.

17-allylamino, 17-demethoxygeldanamycin (17-AAG) is an inhibitor of the heat shock protein hsp-90. HSP-90 chaperones and prevents the degradation of several proteins which are needed for tumor growth including androgen receptor, Akt and Her-2/neu. Proton MRSI on a 4.7 T scanner was used to study the effect of 17-AAG in androgen resistant and androgen sensitive CWR22 tumors in nude mice (Le *et al.*, 2009). Prior to treatment, the androgen-resistant tumors demonstrated higher levels of tCho. Treatment with a single bolus of 17-AAG resulted in significant reductions in tumor tCho in both tumor types; however, a significant change occurred more rapidly in the hormone-sensitive tumors than in the hormone resistant tumors. In both types, the changes in tCho concentration were observed before growth inhibition compared to controls was noted. The differences in tCho at baseline and the time courses of change provide a window on membrane phospholipid metabolism in the two tumor types and may give information on pathways affected by the development of hormone resistance.

The hypoxia-inducible factor, HIF1- α , is known to promote multiple oncogenic pathways and therefore is a potential target for therapy. Jordan *et al.* studied the effect of the HIF1- α inhibitor PX-478 on HT-29 human cancer xenografts using localized proton MR spectroscopy at 4.7 T (Jordan *et al.*, 2005). A significant reduction in the total choline peak was detected within the first 24 hours of treatment. High-resolution *ex vivo* and in vitro NMR studies revealed reductions in the components of the tCho peak including phosphorylcholine and glycerophosphorylcholine. This study, as well as those discussed

above, demonstrate that proton MR spectroscopy has the potential to be an early *in vivo* marker of treatment response to various targeted therapies in multiple tumor types.

2.5.3 ^1H MRS for Monitoring the Evolution of Cancer—Although lipids are often considered an obstacle in ^1H MRS, they can also contain valuable information. Griffiths et al. performed *in vivo*, high spectral resolution ^1H MRS to obtain the lipid profile in the livers of transgenic mice which develop hepatocellular carcinoma (Griffiths *et al.*, 2009). They found that the tumors had lower bis-allyl (lipid) protons at 2.8 ppm than normal liver. Using the ratio of lipid at 2.8 ppm to lipid at 5.3 ppm as a measure of lipid unsaturation, this group found that the lipids in the pre-neoplastic liver had a lower degree of unsaturation than the normal liver, suggesting that ^1H MRSI could detect metabolic changes related to carcinogenesis.

3.0 Hyperpolarized ^{13}C MR

Carbon is ubiquitous in all important biomolecules making it a natural probe for studying tumor biochemistry and metabolism. Unfortunately, ^{12}C , the naturally occurring isotope, is MR invisible. Another carbon isotope, ^{13}C , occurs only in approximately 1.1% natural abundance and also has intrinsically reduced MR sensitivity because of its lower gyromagnetic moment (~25% that of ^1H). This has limited its usefulness for *in vivo* studies, although many elegant metabolic studies have been performed after injection of different ^{13}C labeled compounds (Terpstra *et al.*, 1998; Shen *et al.*, 1999; Chase *et al.*, 2001). Recently, the development of methods to hyperpolarize ^{13}C nuclei and hereby drastically alter the Boltzmann distribution of spins has led to enhancement of ^{13}C sensitivity by 4–5 orders of magnitude and bring it into the realm of feasibility for cell and animal studies, with its use in patients currently undergoing clinical trials (Kurhanewicz et al; personal communication). A number of excellent review articles (Viale *et al.*, 2009; Golman *et al.*, 2003b; Mansson *et al.*, 2006) have appeared that summarize the principles of different methods of hyperpolarization and also the optimal characteristics of molecules for ^{13}C hyperpolarization studies and the reader is referred to these or some of the original papers (Ardenkjaer-Larsen *et al.*, 2003; Golman *et al.*, 2003a) of dynamic nuclear polarization (DNP) for more details.

3.1 Basic Principles

MR is a low sensitivity technology, based on the physics giving rise to the MR signal. The MR experiment is based on the fact that in a magnetic field, spins are polarized into different energy (spin) states. For convenience, we will refer only to nuclei that are polarized into two different energy or spin states. The difference in population of the two states, gives rise to net magnetization of the nuclei. The MR signal is proportional to this net magnetization, the number of spins in the sample, the gyromagnetic ratio of the nucleus under study, and coil sensitivity. Spin polarization is determined by the Boltzmann distribution of the spins between low and high energy states; at room temperature at equilibrium, these states are almost equally populated. In a field strength of about 1.5 T, there is a difference in population of approximately 1 in a million spins at room temperature, which explains the low signal strength of ^{13}C MR experiments. To increase the difference in spin state

populations (hyperpolarization) and thereby increase the signal strength, a number of techniques are available (Golman *et al.*, 2003b; Ardenkjaer-Larsen *et al.*, 2003; Golman *et al.*, 2001; Hovener *et al.*, 2009; Goldman *et al.*, 2005). The Boltzmann distribution depends on the temperature of the sample so the most straightforward method of hyperpolarizing spins would be to lower the temperature of the sample (~1–4K). Unfortunately, this neither yields adequate hyperpolarization for *in vivo* ^{13}C NMR, nor is it practical for studies of living material. Fortunately, recently other methods have been developed. Primarily, two methods of hyperpolarization have been explored primarily for ^{13}C MR studies.

Dynamic nuclear polarization—The principle behind dynamic nuclear polarization (DNP) is to induce polarization transfer in the solid state at low temperature, from high polarization electrons to nuclei by irradiating the sample at the electron resonance frequency which induces the polarization transfer. The medium supplying the electrons is usually a reagent containing a stable radical with unpaired electrons. The temperature of the sample is lowered to approximately 1.5 K and the sample exposed to microwave radiation at the frequency of the electron resonance frequency (Fig. 4). After the polarization transfer occurs, sample irradiation is stopped, the sample removed from the liquid helium chamber, and dissolved in a hot solution to rapidly raise the temperature of the sample. Since the longitudinal spin relaxation times (T_1) of most probe molecules are of the order of 10–30 seconds, the signal is lost relatively rapidly due to relaxation and therefore it is imperative to rapidly transfer the sample to the MR system for study with minimal loss of time. The hyperpolarized sample is rapidly injected into animals or a cell perfusion apparatus and data collected as rapidly as possible. This methodology is used by a commercial instrument to perform preclinical DNP (Ardenkjaer-Larsen *et al.*, 2003) and more recently an instrument for clinical use has been developed. Data acquisition is usually performed with one of several very rapid MR sequences such as low flip angle EPI-CSI (echo-planar imaging-chemical shift imaging) (Larson *et al.*, 2010; Svensson *et al.*, 2003), centric k-space encoding (Golman *et al.*, 2008), true-FISP (FISP = Fast Imaging with Steady Precession) (Svensson *et al.*, 2003), FID-CSI (FID=free induction decay) or single shot 2D-spectroscopy (Hurd *et al.*, 2010; Giraudeau *et al.*, 2009).

Parahydrogen—Molecular hydrogen exists either in the para (singlet state where the spins are anti-parallel) or ortho spin states (triplet spin state with parallel spins). Parahydrogen has no net spin and therefore is NMR invisible. Hydrogenation of an unsaturated chemical with a molecule of parahydrogen as a unit allows the hydrogen atoms to maintain their spin states in the product. The spin order of the parahydrogen molecule can induce ^{13}C polarization either by diabatic-adiabatic field cycling (Golman *et al.*, 2001; Johannesson *et al.*, 2004a) or by a radiofrequency pulse sequence such as that developed by Johannesson *et al.* (Johannesson *et al.*, 2004b). This hyperpolarization has been exploited for both metabolic (Bhattacharya *et al.*, 2007) and angiographic studies (Golman *et al.*, 2001). This review will focus on the DNP studies because they are easier to implement, given the commercial equipment that makes this technique a potentially straightforward tool.

3.2 Chemicals Appropriate for Hyperpolarized ^{13}C Studies

A major challenge with hyperpolarized studies is the necessity for a long spin lattice relaxation times T_1 because when the hyperpolarization decays rapidly, signal is lost and the sample requires a repetition of the hyperpolarization which typically takes hours. Thus, one has a maximum of a few minutes and usually much less, to extract the chemical from the polarizer, dissolve and raise the temperature of the chemical, deliver it to the organism/cells, ensure that the sample is in the magnet ready for data acquisition, have the organism undergo metabolic processes of interest, and perform the measurements. Though challenging, this has been shown to be quite feasible with DNP if appropriate probe molecules are selected. The longitudinal relaxation depends on multiple processes including dipole-dipole relaxation which is the dominant cause of relaxation. Dipole-dipole relaxation is minimal in molecules with quaternary carbons. Molecules such as urea, pyruvate, carboxyl groups, etc., have long T_1 and are appropriate for study. Choline has a quaternary nitrogen so ^{15}N labeled choline is a potential agent that has been suggested for study (Gabellieri *et al.*, 2008; Cudalbu *et al.*, 2010). Since the nuclei relax rapidly, the requirements for an “appropriate” metabolic probe for hyperpolarized studies are that in addition to having a long T_1 , they must undergo metabolic reactions quickly, have well separated peaks (parent and metabolic products), and the hyperpolarized compound must have high solubility.

3.3 In Vivo Biological Studies

3.3.1 Prostate Tumor Metabolism—Many of the initial studies focused on prostate tumor models. Previous *ex vivo* studies have indicated that lactate is elevated in prostate cancer, although such studies are subject to errors, because lactate concentration increases with tissue death during and after surgery and subsequent removal of the tissue. The conversion rates of pyruvate to lactate have been investigated using hyperpolarized ^{13}C MRSI. Golman *et al.* (Golman *et al.*, 2006) injected hyperpolarized ^{13}C -pyruvate into rats bearing P22 tumors. On a 1.5T magnet, they were able to obtain chemical shift images of pyruvate, lactate, and alanine. Tumors had higher lactate than normal tissue. Kohler *et al.* (Kohler *et al.*, 2007) demonstrated the feasibility of monitoring the conversion of hyperpolarized 1- ^{13}C -pyruvate to lactate, pyruvate-hydrate, alanine, and bicarbonate and obtained chemical shift images of each of the metabolites. Chen *et al.* (Chen *et al.*, 2007) studied the transgenic adenocarcinoma of mouse prostate (TRAMP) and found that, after infusion of ^{13}C labeled pyruvate, lactate synthesis could be detected in both primary and metastatic prostate tumors. Albers *et al.* (Albers *et al.*, 2008) observed that, after the injection of hyperpolarized ^{13}C labeled pyruvate, the lactate levels were about twice as high in high-grade compared with low-grade TRAMP tumors, wherein tumor grade was assessed by a histological index developed by the authors.

3.3.2 Markers of Response—Metabolic changes have been shown to be early markers of response using non-invasive *in vivo* ^1H and ^{31}P NMR spectroscopic studies (Evanochko *et al.*, 1983; Koutcher *et al.*, 1992; Koutcher *et al.*, 1987; Aboagye and Bhujwalla, 1999). Hyperpolarization has allowed these studies to be extended to ^{13}C metabolic studies at comparable or higher spatial resolution. Day *et al.* (Day *et al.*, 2007) studied mice bearing EL-4 lymphomas and found that they were able to measure the flux of pyruvate to lactate *in*

vivo which was reduced post chemotherapy with etoposide. This study was followed by an investigation of Kettunen et al. (Kettunen *et al.*, 2010) who demonstrated that the flux of label from pyruvate to lactate was due to exchange of the label. Using magnetization transfer methods, they were able to fit the data to a kinetic model with two site exchange. They also used this methodology to detect response in a glioma tumor models after treatment with radiation (Day *et al.*, 2011). Labeled pyruvate was found primarily in the brain tumor and minimally in the adjacent normal brain tissue. Treatment resulted in a reduction in the ratio of ^{13}C label in lactate:pyruvate 72 hours after radiation. Gallagher et al. (Gallagher *et al.*, 2009) and Witney et al. (Witney *et al.*, 2010) used 1,4- $^{13}\text{C}_2$ fumarate to study the effect of chemotherapy on treatment. In a lymphoma model, they noted 24 hours after treatment with etoposide, an increase in tumor necrosis and a 2.4 fold increase in malate production compared to control tumors and suggested it might be an *in vivo* marker of cell death. Treatment of MDA-MB-231 breast xenografts with doxorubicin caused an increase in fumarate conversion to malate, similar to the study in lymphomas. Witney et al. (Witney *et al.*, 2009) compared ^{18}F FDG positron emission tomography (PET) and hyperpolarized ^{13}C pyruvate utilization for detecting tumor response after chemotherapy treatment of lymphomas. These studies suggest the potential of this method to be used as an early marker of response. They will require validation and comparison with other markers such as Positron emission tomography (PET), ^1H MRS and MRSI.

3.3.3 Targeted Agents—A number of recent studies have focused on the metabolic effects of novel targeted agents. Ward et al. (Ward *et al.*, 2010) studied glioblastoma cells treated with LY294002 or Everolimus which inhibit phosphatidylinositol 3 kinase (PI3K) and found a decrease in lactate production through a decrease in lactate dehydrogenase A (LDH-A) activity. This decrease in LDH activity, LDHA mRNA, and LDH-A protein levels was associated with tumor growth inhibition. Dafni et al. (Dafni *et al.*, 2010) noted that treatment of PC-3MM2 tumors with imatinib to inhibit the platelet derived growth factor receptor, decreased lactate production from hyperpolarized 1- ^{13}C pyruvate and was accompanied by decreased vascular endothelial growth factor (VEGF) and glutaminase expression. They suggested that the etiology was due to decreased expression of HIF-1 and c-Myc and suggested that hyperpolarized ^{13}C MRSI could detect response to molecularly targeted agents. Seth et al. (Seth *et al.*, 2011) noted that inhibition of LDH-A was associated with an increase in reactive oxygen species and enhanced sensitivity to paclitaxel, and that activation of pyruvate dehydrogenase (PDH) by dichloroacetate inhibited glycolysis which could be monitored using hyperpolarized 1- ^{13}C -pyruvate MR studies. Gallagher et al. (Gallagher *et al.*, 2008) have studied 5- ^{13}C glutamine to investigate glutaminase activity. After hyperpolarization of the 5- ^{13}C -glutamine, they were able to detect its conversion to glutamate by glutaminase in cultured hepatoma cells. They inhibited glutaminase by adding 6-diazo-5-oxonorleucine (DON) and found a decrease in glutamate production and suggested this could be used for detecting changes in cell proliferation.

3.3.4 pH—While MR methods have been used for decades for *in vivo* pH monitoring (Hoult *et al.*, 1974), until recently most studies focused on intracellular pH methods, although more recently methods to measure extracellular pH (pH_e) have been proposed (Garcia-Martin *et al.*, 2006; Zhou *et al.*, 2008; Garcia-Martin *et al.*, 2001; Gillies *et al.*,

1994). If the ratio of HCO_3^- to CO_2 in a compartment or space is known, the pH can be calculated with the Henderson-Hasselbach equation. Gallagher et al. (Gallagher *et al.*, 2011) used hyperpolarized ^{13}C labeled HCO_3^- and measured the pH of phantoms and found good agreement between electrode and pH measurement by hyperpolarized ^{13}C MRS measurements. To confirm that the measurement was pH_e , they compared their measurements with another extracellular pH agent (3-aminopropylphosphonate (3APP)) with good agreement.

Much of the work with DNP ^{13}C hyperpolarization has focused on *in vivo* studies. An elegant cell study was performed by Harris et al. (Harris *et al.*, 2009) who studied perfused T47D cells and were able to measure initial rates of pyruvate to lactate conversion using both ^{31}P and ^{13}C MRS data. These studies revealed that transport was the rate limiting step for the production of lactate from extracellular pyruvate. Hypoxia enhanced lactate production and quercetin decreased the conversion rate. Quercetin is an inhibitor of MCT-1 that transports pyruvate and induced a decrease in the conversion of pyruvate to lactate which was in agreement with the model's conclusion that the transport is the rate limiting step.

As noted above, there has been a major focus on studying pyruvate since it is present in cells, as well as in animal and human tissues, has a long T_1 and other properties noted above, as necessary for hyperpolarization studies. Over the last few years, several reports have appeared on methodologies to synthesize and utilize other compounds for hyperpolarized ^{13}C MR studies.

Wilson et al. (Wilson *et al.*, 2010) demonstrated the feasibility of simultaneously injecting more than one agent at a time. They simultaneously hyperpolarized ^{13}C NaHCO_3 and 1- ^{13}C pyruvate as one sample. They achieved high polarization efficiency and the compounds also were found to have long T_1 values at 3T. The compounds were injected into mice bearing prostate tumors allowing the authors to monitor pH and metabolism simultaneously. They found no differences in their results whether the labeled agents were injected separately or concurrently. They were able to simultaneously polarize four ^{13}C labeled substrates (1- ^{13}C pyruvic acid, ^{13}C NaHCO_3 , 1,4- ^{13}C fumaric acid and ^{13}C urea) successfully without negatively impacting on polarization efficacy or T_1 . They also have developed methods of generating other novel ^{13}C labeled agents (Wilson *et al.*, 2009; Keshari *et al.*, 2009). Wilson et al (Wilson *et al.*, 2009) used 1,1, ^{13}C acetic anhydride to generate labeled amino acids and other biomolecules. Thus it is highly likely that a wide variety of agents can be developed to explore metabolism using ^{13}C hyperpolarization techniques.

4.0 Preclinical ^{31}P MR Spectroscopy of Solid Tumors

In vivo ^{31}P MRS permits non-invasive monitoring of phospholipid metabolism, high-energy phosphate metabolism, and pH regulation during tumor progression and in response to treatment. *In vivo* ^{31}P MRS studies have been reviewed previously in detail in experimental tumors (de Certaines *et al.*, 1993) and in living systems (Van Den Thillart and Van Waarde, 1996). This section reviews parts of the newer literature available published after 1993, focusing on recent advances and only referring to some of the earlier literature.

4.1 The *in vivo* ^{31}P MR Spectrum and Technical Developments

A ^{31}P MR spectrum inherently contains information about phospholipid catabolites and anabolites (phosphoethanolamine (PE), phosphocholine (PC), glycerophosphocholine (GPC), glycerophosphoethanolamine (GPE)), tumoral pH (via the chemical shift of inorganic phosphate (Pi)), phosphocreatine (PCr), nucleoside di- and triphosphates (NDP, NTP), NADP(H), and diphosphodiester (DPDE) (Fig. 5, (Ruiz-Cabello and Cohen, 1992; de Certaines *et al.*, 1993; Leach, 1996)). While sugar phosphates resonate in the same spectral region as PE and PC, perchloric acid extracts of solid tumors have shown that the signal in vertebrate tumors emanate predominantly from PE and PC (Evanochko *et al.*, 1984; de Certaines *et al.*, 1993). However, sugar phosphates have been shown to be present in the liver (Leach, 1996) as well as in other organisms (Van Den Thillart and Van Waarde, 1996). Although in patients ^{31}P MR spectra have been acquired typically with ^1H decoupling and nuclear Overhauser effect (NOE) to significantly improve SNR and enhance spectral resolution (Negendank *et al.*, 1996; Leach, 1996; Arias-Mendoza and Brown, 2003), pre-clinical ^{31}P MR spectra have been acquired with (Fig. 5, (Street *et al.*, 1997; Darpolor *et al.*, 2011a; Koutcher *et al.*, 2000), and without ^1H decoupling and significant NOE enhancement (Fig. 6, (Huang *et al.*, 2007)).

The increase in available magnetic field strength of horizontal-bore magnets, has led to substantial improvement of SNR permitting the measurement of smaller tumors and enhancing temporal, spectral, and MRSI resolution (de Certaines *et al.*, 1993; Arias-Mendoza and Brown, 2003). Volume selection and spectral localization methods, including 3-dimensional spectroscopic localization, have been improving continuously (de Certaines *et al.*, 1993; Arias-Mendoza and Brown, 2003), further advancing the applicability of localized ^{31}P MRS. Potential signal contamination due to signal contributions from skin and muscle/animal body can be reduced significantly by the application of spectroscopic localization techniques (Howe *et al.*, 1993).

Typically, changes in metabolite levels with tumor growth and in response to nutritional availability (Fig. 5B), environmental challenges, or treatments (Fig. 6), are quantified *in vivo* using signal ratios, such as PME/NTP (Al-Saffar *et al.*, 2006; Bezabeh *et al.*, 2004; Darpolor *et al.*, 2011a; Gade *et al.*, 2009; Chung *et al.*, 2008; Morse *et al.*, 2007; van Laarhoven *et al.*, 2006). However, efforts have been made to improve on data analysis routines and absolute metabolite quantification – clinically (Arias-Mendoza *et al.*, 2006) and preclinically (Zakian *et al.*, 2000; Stoyanova and Brown, 2002) – to better understand the underlying biology of *in vivo* metabolic changes associated with tumor growth and treatment response as well as to identify clinically useful *in vivo* biomarkers.

Additional developments, such as the building of probes with cryogenically cooled builtin first stage receivers and radiofrequency (rf) coils, reduce the thermal noise produced in the probes and, thus, improve SNR (Ginefri *et al.*, 2007). Currently, cryogenic coils are being implemented successfully for *in vivo* ^1H MR (Bosshard *et al.*, 2010; Hu *et al.*, 2012). Also, the implementation of more advanced pulse sequences (e.g. Refocused insensitive nucleus enhancement by polarization transfer (RINEPT) (Gonen *et al.*, 1997)) or acquisition schemes (e.g. dual interleaved acquisition of ^1H and ^{31}P CSI (Gonen *et al.*, 1994), may be

useful in increasing SNR and reducing measurement times. Recent studies focusing on applying compressed sensing (Candes *et al.*, 2006) to MRI (Lustig *et al.*, 2008) and MRS (von Morze *et al.*, 2012) are exciting and may improve temporal and spatial resolution significantly with the potential of making ^{31}P MRSI more clinically applicable in the future.

4.2 Measurement of tumoral pH by ^{31}P MRS

Although the Pi signal in solid tumors contains contributions from both extracellular and intracellular Pi, the predominant contribution is from intracellular Pi, due to the densely packed cell mass (Soto *et al.*, 1996). Thus, the tumoral pH determined from the chemical shift of the Pi signal represents primarily the intracellular tumoral pH (pHi) (Prichard *et al.*, 1983). To measure the extracellular pH (pHe), exogenous probes, such as 3-APP (Gillies *et al.*, 1994) (Bhujwala *et al.*, 1998), need to be administered (Fig. 7, (Raghuand, 2006)). The chemical shift δ of Pi and 3-APP (relative to α -NTP at -10.05 ppm) are related to pHi and pHe by their respective Henderson-Hasselbach equations:

$$\text{pHi} = 6.85 + \log_{10} \frac{\delta - 0.58}{3.14 - \delta} \quad [1A]$$

$$\text{pHe} = 6.91 + \log_{10} \frac{\delta - 21.10}{24.32 - \delta} \quad [1B]$$

The intensity axes of the Pi and 3-APP signal need to be corrected for the nonlinear relationship between pH and ppm, as the apparent center of the resonance depends, aside from the tissue pH distribution, also on its width and distance from the acid dissociation constant pKa (6.85 and 6.91 for Pi and 3-APP, respectively) (Graham *et al.*, 1994) (Raghuand, 2006). The pH distributions obtained after intensity corrections are the result of the tumoral pH heterogeneity, as well as signal broadening due to T_2 relaxation and, in the case of 3-APP, also due to its multiplet structure from ^1H coupling in ^{31}P MR spectra acquired without proton decoupling (Raghuand, 2006; Fig. 7B).

New pHe markers for MR are typically developed for ^1H MRS since it has about 15-fold higher relative sensitivity than ^{31}P MRS (Gadian, 1982), thus improving temporal and spatial resolution (see e.g. (Gillies *et al.*, 2004; Raghuand, 2006; Pacheco-Torres *et al.*, 2011)). As of today, 3-APP is the only reported tracer to measure pHe by ^{31}P MRS in small animals (Gillies *et al.*, 1994) (Fig. 7). The measurement of tumor pH (pHi and pHe) by a variety of MR methods and exogenous pHe markers has been described comprehensively by others (Gillies *et al.*, 2004; Raghuand, 2006; Pacheco-Torres *et al.*, 2011) and references therein) and will not be reviewed in detail here.

4.3 ^{31}P MRS – Tumor Growth and Treatment Response

In preclinical models of solid tumors, *in vivo* ^{31}P MRS – unlocalized and localized – has been used extensively to evaluate the effects of tumor growth and treatments (chemotherapy, radiotherapy, photodynamic therapy, etc.) on the high energy phosphate metabolism, phospholipid metabolism, and tumoral pH (Steen, 1989; Raghuand and Gillies, 2001; de

Certaines *et al.*, 1993). In this section we focus on untreated tumors and on two of the most common used anti-neoplastic treatment modalities, radiation and chemotherapy

Over the years, it has become more apparent that care has to be taken how the experimental conditions, such as anesthesia and temperature, may impact the study (de Certaines *et al.*, 1993), and are critical to obtaining meaningful data. When performing any *in vivo* MRS studies on small animals, it is recommended to select an anesthetic, such as isoflurane, that affects minimally the tumor physiology and use it at the lowest possible dose (Van Den Thillart and Van Waarde, 1996). Additionally, the core temperature of the animal can (and preferably should) be kept at normal body temperature by the use of a water bath (Street *et al.*, 1997), heating pad (Morse *et al.*, 2007; Chiche *et al.*, 2012), or warm air.

4.3.1 Untreated Tumors—As reviewed in detail previously (de Certaines *et al.*, 1993), the earliest studies investigated spectral changes in ^{31}P MR spectra associated with tumor growth in untreated tumors, focusing on the energy status, pH, and metabolite identification. Even though there is not a single constant feature describing the bioenergetics and spectral behavior of a growing tumor, there are some common trends (de Certaines *et al.*, 1993). Typically, NTPs and $\beta\text{-PCr}$, decrease with tumor growth, while Pi increases (de Certaines *et al.*, 1993; Vaupel, 1996). These changes appear to be related rather to tumor cells in a pre-lethal stage rather than actual tumor necrosis (dead cells do not produce a visible ^{31}P MRS signal). The necrotic fraction of preclinical tumors often increases with size, as their fast growth outpaces the blood supply, limiting oxygen and nutrient supply to the tumor (de Certaines *et al.*, 1993). Additionally, while the pH_i of tumors remains at or above (slightly alkaline) physiological levels, the pH_e often acidifies with tumor growth, especially in tumors with extensive aerobic glycolysis (Warburg effect) and poor perfusion (de Certaines *et al.*, 1993; Raghunand, 2006). The ^{31}P MRS signal intensities of PME and PDE are typically normalized to $\beta\text{-NTP}$, Pi, or total phosphate. Relative PME levels (normalized to $\beta\text{-NTP}$) frequently increase during rapid tumor growth (de Certaines *et al.*, 1993). However, it cannot be assessed from these ratios whether increasing PME or decreasing NTP or both contribute to these changes in the spectral phospholipid pattern (de Certaines *et al.*, 1993; Podo, 1999). One common feature though is that with the development of necrosis, PME and NTP signals decrease and Pi increases (de Certaines *et al.*, 1993).

Later studies investigated if phosphometabolites noninvasively detected by ^{31}P MRS could serve as a surrogate biomarker for tumor hypoxia, which has been recognized as an important factor in tumor progression and treatment response (de Certaines *et al.*, 1993; Vaupel, 1996). While the hypoxic fraction of a tumor cannot be directly and reproducibly determined from the tumor energy status as obtained from ^{31}P MRS (Nordsmark *et al.*, 1995; Saitoh *et al.*, 2002; Wendland *et al.*, 1992), breathing of different oxygen tensions of the anesthetized animal can affect the metabolite levels in an *in vivo* ^{31}P MR spectrum of a tumor, and are indicative of oxygenation changes in a tumor (Okunieff *et al.*, 1987). These changes may improve effectiveness of radiation therapy (Nordsmark *et al.*, 1995) or delivery (as well as cytotoxicity) of chemotherapeutic drugs (Rodrigues *et al.*, 2002).

Over the years, the significance of the PME and PDE resonances, most notably PC and GPC, in relation to malignant transformation, growth rates, and treatment response has been recognized through *in vitro* as well as *in vivo* preclinical and clinical studies (Podo, 1999; Negendank *et al.*, 1996; de Certaines *et al.*, 1993; Ronen and Leach, 2001; Arias-Mendoza and Brown, 2003). The elevation of PC and choline-containing metabolites has emerged as a common characteristic for malignant transformation and tumor aggressiveness in a variety of solid tumors (Negendank *et al.*, 1996; Podo, 1999). Thus, more recent research, including but not limited to ^{31}P MRS studies, focuses on (i) understanding the underlying biochemistry of tumoral phospholipid metabolism, (ii) assessment of treatment response based on associated PME and PDE changes, and (iii) use of the knowledge obtained about tumoral phospholipid metabolism to develop targeted treatment altering PME (and PDE levels) toward levels associated with a less aggressive tumor phenotype (Podo *et al.*, 2011).

4.3.2 Radiation Therapy—A common feature of tumor response to radiotherapy (RT) is an improved bioenergetic status, consistent with tumor reoxygenation secondary to increases in tumor blood perfusion/permeability after irradiation (reviewed in (Robinson *et al.*, 1997; de Certaines *et al.*, 1993)). Other possible explanations for the improved bioenergetic status after RT are that tumor cell kill by RT (i) increases the availability of oxygen and nutrients per cell for the surviving cancer cells (fewer viable cells competing for oxygen and nutrients) and/or (ii) induces an inflammatory response increasing the fraction of normal cells contributing to the tumor spectrum (de Certaines *et al.*, 1993). PME and PDE levels did not vary in tumors exposed to sublethal RT, and the PME/NTP ratio did not change significantly in responsive tumors while it was observed to increase in non-responsive tumors (de Certaines *et al.*, 1993). The investigation of radiation dose-dependent changes in tumor metabolism showed that PE/PC ratio increased strongly with radiation dose reaching a maximum for a range of doses (8–65 Gy) 7 days postirradiation (Mahmood *et al.*, 1994). The tumoral pH was not significantly affected by the single-dose RT, while the bioenergetic status improved subsequent to treatment with radiation doses from 8–65 Gy and worsened after 0 Gy and 4 Gy (Mahmood *et al.*, 1994).

Building on these earlier studies, a ^{31}P MR study by Jackel *et al.* on human hypopharynx carcinoma xenografts (Jackel *et al.*, 2000) found that pretreatment ratios of PME/total phosphate and PDE/total phosphate were indicative of tumor radiosensitivity and related to tumor growth delay for tumors treated with a single dose of 30 Gy but not in the 15 Gy group. After 30 Gy single-dose RT, the PME/total phosphate ratio decreased while the PDE/total phosphate ratio increased (Jackel *et al.*, 2000). Similarly, PME levels decreased, and GPC levels increased by $95 \pm 15\%$ in 4–7h, while $\beta\text{-ATP/Pi}$ decreased by $25 \pm 5\%$ in subcutaneous murine Ehrlich ascites tumors after a single-dose of 10 Gy (Sharma and Jain, 2001). However, while tumoral pH increased after 30 Gy in the human hypopharynx carcinoma xenografts (Jackel *et al.*, 2000), pH decreased after 10 Gy RT in the subcutaneous murine Ehrlich ascites tumors (Sharma and Jain, 2001).

In preclinical models, the decrease of bioenergetic status with increasing tumor volume and associated decrease in tumor mean oxygen tension has been correlated with increased radioresistance (Saitoh *et al.*, 2002). Radiosensitization of a breast cancer model with 6-aminonicotinamide (6-AN) induced complete regressions in 21% of tumors treated with 6-

AN plus RT which was significantly higher than for either treatment alone. While radiation alone did not produce any significant changes in the spectral pattern, 6-phosphogluconate was detected 10 h post administration of 6-AN, indicating the inhibition of the pentose phosphate pathway by 6-AN (Koutcher *et al.*, 1996).

4.3.3 Chemotherapy—Phosphorus MRS has been used successfully to follow tumoral drug uptake and elimination *in vivo* for chemotherapeutic agents containing a phosphorus group, such as ifosfamide or glucosylifosfamide mustard (Haberkorn *et al.*, 1998; Rodrigues *et al.*, 2002). Enhanced drug uptake of ifosfamide induced by the breathing of hypercapnic, hyperoxic gases could be assessed non-invasively by *in vivo* ^{31}P MRS (Rodrigues *et al.*, 2002). A short-term decrease of NTP/total phosphate observed by ^{31}P MRS at 30 min (and which reversed within 1–2 h) after ifosfamide administration was probably due to drug-induced hypotension and not a direct effect of the drug on tumor energetics (Rodrigues *et al.*, 2002).

In search of a biomarker that could assess efficacy non-invasively, either pre-treatment, or early after chemotherapeutic treatment, the effects of multiple chemotherapeutic drugs on the *in vivo* spectral pattern of ^{31}P MR data have been investigated, relative to tumor growth delay. In two preclinical colon cancer models, carbogen breathing enhanced uptake of 5-fluorouracil (5-FU) (as measured by ^{19}F MRS), was accompanied by a decrease of pHe and enhanced systemic toxicity, but did not alter the tumoral bioenergetic status and pH_i (van Laarhoven *et al.*, 2006). Interestingly in the control group of these tumor models, pHe was higher than pH_i (van Laarhoven *et al.*, 2006). In contrast, 5-FU administered to a fibrosarcoma model did not alter pHe; however a pH_i increase at 48 h post treatment related to decreased tumor size and improved energetic status (Bhujwalla *et al.*, 1998). Tumoral 5-FU uptake could also be increased by collagenase treatment of colorectal tumor xenografts, albeit cytotoxicity did not increase (Gade *et al.*, 2009; Bhujwalla *et al.*, 1998).

As detailed previously (Raghunand and Gillies, 2001), the effectiveness of chemotherapy may be affected significantly by a typically acidic tumoral pHe. An *in vivo*, acidic pHe may reduce the uptake of weakly basic chemotherapeutic drugs, such as anthracyclines and Vinca alkaloids, and therefore reduce their cytotoxic effect. It has been shown in MCF-7 xenografts, that raising the tumoral pHe by adding sodium bicarbonate to the drinking water of tumor-bearing mice significantly improved the therapeutic efficacy of doxorubicin (Raghunand and Gillies, 2001) and reduced the formation of metastases in a breast and prostate cancer model (Robey *et al.*, 2009). Thus, assessing tumoral pHe by ^{31}P MRS (or ^1H MR) has the potential to evaluate the likelihood of efficacy of a treatment regimen consisting of weakly basic drugs before the start of treatment and also assess the potential benefits of pHe-altering agents (Raghunand and Gillies, 2001).

The alteration of pH_i, assessed non-invasively by ^{31}P MRS, can also be beneficial in the context of assessing tumor treatment response. For example, treatment with the protein kinase C inhibitor bryostatin-1, which is known to enhance the cytotoxicity of a variety of chemotherapeutic agents, decreased pH_i and high energy phosphates in a preclinical tumor model (Koutcher *et al.*, 2000). Furthermore, cycloprodigosin hydrochloride is a drug that disturbs intracellular pH homeostasis through H^+/Cl^- symport activity, thus decreasing pH_i

in vivo and ultimately inducing apoptosis (Yamamoto *et al.*, 2002). While pHi and pHe can be acidified by glycolytic or non-glycolytic means in some tumor models (Spees *et al.*, 2005), in a melanoma xenograft model resistant to tumoral acidification by hyperglycemia, tumoral pHi and pHe could be lowered significantly by the administration of *m*-iodobenzylguanidine (MIBG, inhibitor of mitochondrial respiration) under hyperglycemic conditions, coinciding with a reduction in tumoral bioenergetic status (Zhou *et al.*, 2000). Also, the stimulation of glycolysis *in vivo* by the administration of MIBG alone decreased pHi in a 9L glioma model (Biaglow *et al.*, 1998). Supplementing the diet of mice bearing human colon adenocarcinoma tumors with creatine and cyclocreatine increased tumoral (PCr+phosphocyclocreatine)/NTP levels, lowered pHi and overall delayed tumor growth (Kristensen *et al.*, 1999).

The bioenergetics of tumors in response to treatment as assessed by ³¹P MRS has to be seen as the result of experimental method artifacts as well as direct and indirect treatment effects (de Certaines *et al.*, 1993). Excluding experimental method artifacts, the death of low energy cells, stimulation of oxidative phosphorylation, recruitment of quiescent cells, or non-cancerous cell infiltration, can increase the tumoral bioenergetic level, while lowered bioenergetic tumor status can result from vascular effects, or mitochondrial impairment (de Certaines *et al.*, 1993). Also, changes of tumoral PME and PDE levels do not follow a consistent pattern in response to a variety of anticancer treatments (de Certaines *et al.*, 1993). These varying response to treatments are also reflected in newer studies and the treatment response as manifested by *in vivo* metabolic changes can significantly vary over time following treatment. For example, increased bioenergetic status has been observed following treatment with tamoxifen (Degani *et al.*, 1994) and cyclophosphamide (96 h post treatment) (Street *et al.*, 1995). In contrast, tumoral bioenergetic status decreased within the first 24 h post treatment with cyclophosphamide (Street *et al.*, 1995), at 6 h and 12 h after treatment with a chemotherapeutic regimen of N-(phosphonacetyl)-L-aspartate (PALA) followed 17 h later by 6-methylmercaptapurine riboside (MMPR) and 6-aminonicotanamide (6AN) (Mahmood *et al.*, 1996), after treatment with the NAD⁺ inhibitor FK866 accompanied by tumor cell death (and tumor growth delay) (Muruganandham *et al.*, 2005), and in response to the histone deacetylase (HDAC) inhibitor LAQ824 (Chung *et al.*, 2008). Targeting phospholipid metabolism with the choline kinase inhibitor MN58b decreased PME signals *in vivo* while leaving pHi and bioenergetic levels unaffected (Al-Saffar *et al.*, 2006). Phosphomonoester levels have been observed to increase in response to (i) cyclophosphamide at 48–168 h post treatment (Street *et al.*, 1995), (ii) a multidrug regimen of N-(phosphonacetyl)-L-aspartate (PALA) followed 17 h later by 6-methylmercaptapurine riboside (MMPR) and 6-aminonicotanamide (6AN) coinciding with apparent pentose phosphate pathway inhibition (Mahmood *et al.*, 1996), (iii) the NAD⁺ inhibitor FK866 accompanied by lowered pHi (Muruganandham *et al.*, 2005), (iv) the histone deacetylase (HDAC) inhibitor LAQ824 along with a drop in pHi (Chung *et al.*, 2008). The PME/PDE ratio increased significantly after treatment with the heat shock protein 90 (HSP90) inhibitor 17-Allylamino,17-demethoxygeldanamycin (17AAG) in a colorectal cancer xenograft (Chung *et al.*, 2003). On the other hand, the PME/β-NTP ratio decreased significantly after 2 cycles of combination chemotherapy with cyclophosphamide, hydroxydoxorubicin, oncovin, prednisone, and bryostatin 1 (Fig. 6, (Huang *et al.*, 2007)),

while the combination chemotherapy consisting of cyclophosphamide, hydroxydoxorubicin, oncovin and prednisone did not alter PME/ β -NTP ratio significantly but decreased GPE levels in human diffuse large B-cell lymphoma xenografts (Lee *et al.*, 2008). The treatment of human breast cancer xenografts lowered PC levels significantly at 2–4 days post treatment (Morse *et al.*, 2007). Significantly delayed tumor growth after sequential administration of irinotecan followed by flavopiridol was associated with decreased PC and Pi levels in colon cancer xenografts (Darpolor *et al.*, 2011a). These data support (i) that specific changes of the tumoral ^{31}P MR spectral pattern vary by treatment and tumor type, and (ii) that assessing *in vivo* the tumoral metabolic response to treatment longitudinally is also of importance when evaluating treatment efficacy.

5.0 CEST

Introduction and Theory

Recently an MR contrast mechanism has been proposed by Ward and Balaban for signal enhancement and contrast in MRI (Ward *et al.*, 2000). Magnetic resonance in general suffers from lower signal sensitivity compared to optical, radioactive detection methods, and therefore requires higher concentration of contrast agents to be detectable. Chemical Exchange Saturation transfer (CEST) MRI allows the contrast agent presence to be detected through the surrounding water signal change, which is several orders of magnitude higher in concentration. This has opened up new areas of MR contrast agents and *in vivo* imaging methods.

CEST MRI measures water signal change after a long, low power RF irradiation pulse applied at a different resonance frequency from water. If the irradiated frequency overlaps with a proton resonance which is exchangeable with water, the irradiation will lead to a reduction of water proton resonance signal intensity due to proton-exchange induced magnetization exchange. The frequency variation of the normalized water saturation, S_{sat}/S_0 where S_{sat} is the saturated water signal intensity and S_0 is the intensity in unsaturated measurement, is the magnetization transfer (MT) spectrum or Z-spectrum. The magnetization transfer ratio (MTR) is defined as $\text{MTR} = 1 - S_{\text{sat}}/S_0$. In a simple two-pool exchange model with a small and large pool of protons and no back exchange, MTR can be expressed by the following expression (McMahon *et al.*, 2006; Zhou *et al.*, 2004):

$$\text{MTR} = x_s \alpha^* k_{\text{sw}} T_{1\text{water}} (1 - e^{-t_{\text{sat}}/T_{1\text{water}}}) \quad [2]$$

Where x_s is the solute proton concentration, α the saturation efficiency, k_{sw} the exchange rate, $T_{1\text{water}}$ is the water T_1 without saturation and t_{sat} the saturation time. Saturation efficiency can be expressed by:

$$\alpha \sim (\gamma B_1)^2 / [(\gamma B_1)^2 + (k_{\text{sw}})^2] \quad [3]$$

Therefore larger irradiation power will be needed for faster exchange, which may cause SAR concerns for *in vivo* measurements. Using amide proton as the exchange solute, whose

exchange rate is known to be approximately 29 Hz, a typical B_1 field of 1 μ T will result in transfer efficiency of 0.99.

In addition to endogenous CEST contrast agents, such as protein amide protons, a large class of molecules can be chosen and engineered to be CEST agents, for example glycogen, glycosaminoglycans, liposomes and polypeptides (Aime *et al.*, 2005; Ling *et al.*, 2008; van Zijl *et al.*, 2007). CEST sensitivity depends critically on various CEST contrast agents, their number of proton exchangeable sites, water-proton exchange rate. Liposomes offer extremely desirable high detection sensitivity due to very large number of proton exchangeable sites. Figure 8 shows a detectability plot for various CEST contrast agent function groups (van Zijl and Yadav, 2011).

CEST MR detection method is similar to those used in MT contrast experiments. However there are differences due to different exchange regime and less semi-solid like behavior for CEST contrast agents. The basic CEST pulse sequence consists of a long, low power saturation pulse before the phase encoding imaging section. The saturation pulse can be considered as a member of the magnetization transfer period or the more general label transfer frame (LTF), where the CEST agent magnetization can be manipulated to achieve frequency labeling, dephasing and magnetization transferred to water pool in various fashion (Friedman *et al.*, 2010). As magnetization buildup is a relatively slow process, many cycles of magnetization transfer module may be incorporated within the LTF. For example using an inversion recovery module, would double the magnetization transfer rate from the CEST agent to water proton. Frequency labeling allows Fourier transformation of the frequency labeling period and distinguishing multiple CEST agents in the extra frequency axis.

Signal processing and quantification uses a so-called asymmetry analysis whereby signals from both sides of the water resonance with equal frequency separation are used to calculate CEST agent saturation transfer (ST):

$$ST_{\text{asym}} = (S(-\Delta\omega) - S(\Delta\omega)) / S(\Delta\omega) \quad [4]$$

where S is the signal intensity and ω is the frequency difference with water. Saturation transfer signal includes contributions from the CEST agent, endogenous macromolecules, magnetization transfer from other sources and water direct saturation effect due to its non-zero spectral density at the irradiation frequency. ST_{asym} will in the first order remove the contribution from direct saturation.

Cancer Applications

CEST agents have been made to fulfill different imaging needs. A wide range of *in vivo* applications have been studied, which includes pH imaging, detection of metabolites, mobile proteins and lipids, liposome labeling, temperature imaging and CEST reporter gene etc (Gilad *et al.*, 2007; Li *et al.*, 2008; Liu *et al.*, 2012; Terreno *et al.*, 2008; Zhou *et al.*, 2008; Zhou *et al.*, 2003). CEST contrast imaging has been applied in clinical and preclinical cancer studies to assist in tumor staging, tumor metabolite imaging, tumor pH imaging, tumor reporter gene imaging etc (ref). Zhou et al used endogenous tissue protein and peptide as CEST contrast agents (endoCEST) in 9L glioma tumors in rat brain (8). In comparing

with other MR imaging protocol, CEST brain image has the clearest tumor contrast compared with T₂, T₁- weighted, diffusion weighted images. The superior contrast was attributed to a much elevated exchangeable amide proton density in the tumor, 140 ± 21.4 mM, versus 51.7 ± 16.3 mM in the peritumoral region. EndoCEST of tumor protein and peptide contents was shown also to be less sensitive to edema and had been applied to distinguish tumor necrosis and in tumor monitoring after radiation therapy (Zhou *et al.*, 2011).

pH

Amide protons in endogenous mobile proteins provide CEST contrast in many instances. The amide proton exchange process is pH dependent, which can be utilized for CEST based pH imaging *in vivo*. Zhou et al showed the endogenous protein and peptide CEST image is pH sensitive in rat 9L glioma tumors (Zhou *et al.*, 2003). Newer paramagnetic CEST (PARACEST) contrast agents proposed for pH imaging are lanthanide based to improve frequency separation between the CEST amide group and water resonance. Figure 9 shows two Yb(III)-DOTAM complexes synthesized by Pikkemaat et al, which is pH sensitive in aqueous solution (Pikkemaat *et al.*, 2007). The two agents have their maximum CEST effect at different pH values, 6.5 and 7.5 respectively, shown in two different rows in the figure. Liu et al synthesized a Yb based PARACEST agent with wider pH range and were able to measure tumor pH in a xenograft model, shown in Figure 10. Figure 10B is the initial pH map and figure 10C is the pH map with statistically significant amide and amine CEST effects.

Reporter Gene Detection

A novel application of CEST imaging is to design a report gene system to encode the expression of the class of CEST agent lysine rich-proteins (LRP), whereby LRP will be constitutively expressed in cells encoded with the LRP reporter gene. The 200-residue poly-lysine protein has rapidly exchanging amide protons detectable with CEST in the tens of μ molar concentration range (Gilad *et al.*, 2007). In their study eight synthetic oligonucleotides encoding LRP were cloned in an expression vector, along with enhanced green fluorescent protein (EGFP). The vector was transfected into rat 9L glioma cells. The *in vitro* detectability of constitutively expressed LRP is shown in Fig. 11. The expression in the LRP pool is clear visible from CEST. CEST was acquired at the frequencies $\omega = \pm 3.76$ ppm, LRP CEST is selectively enhanced with no detectable amide exchange from other macromolecular sources. The *in vivo* CEST imaging (Fig. 12) was done on a rat brain with the LRP expressing tumor cells and control tumor cells, transfected with an empty vector, implanted in opposite spheres of a mouse brain. Therefore CEST imaging has been successful in detecting the expression of LRP in the tumor is clearly detected with CEST imaging.

6.0 Dynamic Contrast-Enhanced MRI

6.1 Tumor Vasculature and Angiogenesis

Malignant tumors that attain a size of 1–2 mm in diameter cannot support their metabolic needs by diffusion of nutrients from surrounding tissues. Deprived of oxygen, tumor cells

become necrotic at a distance of about 160 μm or greater from the nearest blood vessel (Thomlinson and Gray, 1955). After undergoing the “angiogenic switch”, tumors establish their own vascular network, which supplies oxygen and nutrients and may also serve as a route for tumor spread. Angiogenesis, the process of formation of new blood vessels from existing ones, depends on the tumor phenotype and is a crucial factor in tumor proliferation and metastasis (Kerbel, 2008; Folkman, 1971; Carmeliet, 2005). The relationship between angiogenesis and tumor microenvironment is currently a subject of intense study.

Tumor vascularity is related to disease aggressiveness (van Dijke *et al.*, 1996), and numerous antiangiogenic and vascular disruptive agents have been developed to arrest the tumor growth by inhibiting its vasculature. Tumor vasculature can be characterized using serological markers, such as Vascular Endothelial Growth Factor (VEGF) levels and circulating endothelial cells, or biopsy. However, the former lack sensitivity and the latter is invasive and probes only a small area of the tumor. Alternatively, various imaging modalities, including MRI, can be used for assessing the tumor vasculature non-invasively and repeatedly, for example, to monitor the effects of treatment. Dynamic contrast-enhanced (DCE) MRI enables assessing the functionality of the vasculature through measurements of perfusion, vascular permeability and blood volume. Dynamic susceptibility contrast (DSC) imaging yields measurements of blood flow and blood volume, whereas vessel size imaging (VSI) provides estimates of the characteristic diameter and density of the tumor capillaries. Finally, MR angiography (MRA) can visualize the morphology and architecture of the tumor vascular network.

6.2 T1-weighted Dynamic Contrast-Enhanced MRI

6.2.1 Contrast agents—DCE MRI involves acquisition of images before and after administration of an exogenous contrast agent, usually gadolinium based, and capturing the tissue signal enhancement due to the shortening of the tissue T_1 relaxation time induced by the contrast agent. The predominant contrast agents used clinically are low-molecular-weight (LMW, molecular weight < 1000 Daltons) gadolinium chelates, such as gadopentetate dimeglumine (Gd-DTPA, molecular weight, 550 Da). LMW agents are rapidly cleared by renal filtration: in humans, the elimination half-life of Gd-DTPA is 1.6 hours, whereas in rats it is about 6–13 minutes (Weinmann *et al.*, 1984) (Bui *et al.*, 2010). The molecules of LMW contrast agents do not cross the intact blood-brain barrier, but in body tissues they rapidly extravasate through the endothelium of both normal and neoplastic vessels. Thus the LMW contrast agents are limited in their ability to depict the permeability of the leaky angiogenic tumor vessels, as the tissue enhancement with LMW contrasts reflects contributions of both flow and permeability.

In preclinical research, numerous studies have been performed with macro-molecular weight agents (MMW), which include dendrimers, such as Gadomer-17 (Bayer Schering Pharma AG, Berlin, Germany; molecular weight, 18 kDa; apparent weight, 32–35 kDa) (Misselwitz *et al.*, 2001), carboxymethyl-dextran (P717, Guerbet; 52 kDa) and gadomelitol (P792; Guerbet, Roissy, France, molecular weight 6.47 kDa), as well as gadolinium-labeled macromolecules, such as gadolinium-albumin (albumin-(Gd-DTPA)₃₀; 92 kDa, 6 nm diameter) (Ogan, 1988) and ultrasmall superparamagnetic iron oxide particles (USPIO).

MMW contrast agents remain in the bloodstream longer than LMW contrast media. For example, in rats, the half-life of Gadomer-17 is about 40 min (Misselwitz *et al.*, 2001), whereas the half-life of albumin-(Gd-DTPA)₃₀ is 2–3 h (Raatschen *et al.*, 2008; Bumb *et al.*, 2010). MMW contrast agents do not cross through normal endothelia and are therefore more suitable for probing the hyperpermeable tumor neovasculature. Simulations by Jaspers *et al.* (Jaspers *et al.*, 2009) showed that dynamic studies enhanced with MMW provide more reliable measures of perfusion/permeability and blood volume than LMW contrast agents at the same noise levels and temporal resolution of the DCE MRI experiment.

6.2.2. Acquisition Methods—The acquisition of dynamic series is performed before, during and after an injection of a bolus of a contrast agent usually followed by saline flush. The typical doses of the clinically approved gadolinium-based contrast agents (concentration 0.5 mM) are 0.1–0.2 mmol/kg. Serial images are typically acquired using spoiled gradient recalled echo (GRE) sequences with short repetition and echo time on the order of a few milliseconds and flip angles of about 30. The temporal resolution and duration of experiments depends on the rate of contrast agent extravasation. In studies with LMW agents, the temporal resolution is on the order of 3–20 s and the total duration of dynamic acquisition can range from 3 to 30 minutes. When slowly extravasating MMW agents are used, temporal resolution can be as low as 1–2 min, and the acquisition time is usually longer than in experiments with LMW agents. Prior to the dynamic series, T₁-mapping data are often acquired using either multiple flip-angle GRE images or inversion recovery techniques for subsequent quantification of the contrast agent concentration.

6.2.3 Quantification of DCE-MRI data—Tissue signal enhancement relative to the baseline signal intensity is converted into the change of the relaxation rate $R_1(t) = 1/T_1(t)$ or concentration (Heilmann *et al.*, 2006). When tissue water is assumed to be in the fast exchange limit, the concentration over time, $c(t)$, is proportional to the change in the relaxation rate $c(t) = (R_1(t) - R_{10})/r_1$, where $R_{10} = 1/T_{10}$ is the unenhanced relaxation rate and r_1 is the relaxivity of the contrast agent (Rohrer *et al.*, 2005; Pintaske *et al.*, 2006; Noebauer-Huhmann *et al.*, 2010)

A variety of methods for quantification of functional tissue parameters from DCE experiments with LMW (Tofts *et al.*, 1999) and MMW agents (Ewing *et al.*, 2006) are currently in use. The semi-quantitative measures include the maximum concentration, maximum initial upslope, time-to-peak (TTP), the initial area under the gadolinium concentration curve (IAUC) (Leach *et al.*, 2005) and the fraction of highly enhancing (or non-enhancing) voxels, which can be defined, for example, as the fraction of tumor voxels with IAUC above (or below) a given threshold value, such as the IAUC of the muscle (Bradley *et al.*, 2007; Evelhoch, 1999). The semi-quantitative measures are convenient and easy to calculate, yet they depend on the experimental situation, do not have a straightforward physiological meaning and can reflect a combination of tissue properties. For example, IAUC, has been shown to depend on tissue blood volume, blood flow/permeability and the leakage space volume (Walker-Samuel *et al.*, 2006).

Pharmacokinetic modeling is a more complex approach, but presumably less dependent on the experimental variables for deriving tissue parameters from DCE MRI data. The most

common model used today is the two-compartment Tofts-Kety model (Tofts *et al.*, 1999), which represents the tissue as a combination of two compartments, plasma compartment and extracellular extravascular space (EES), with fractional volumes of v_p and v_e , respectively. The contrast agent is delivered to the tissue by the arterial vasculature and passes into the tissue plasma compartment, from which it diffuses into the EES compartment and leaks back. If the vascular volume in the tissue is considered to be negligibly small, the Tofts-Kety model yields two parameters: the plasma-to-EES volume transport constant K^{trans} and v_e , or alternatively, the EES-to-plasma leakage rate constant $k_{ep} = K^{trans}/v_e$. The extended Tofts model (Fig. 13), which includes the plasma compartment, provides three parameters, K^{trans} , v_e and v_p . The model solution is given by the following expression for the concentration of contrast in tissue $C_t(t)$:

$$C_t(t) = v_p C_p(t) + K^{trans} \int_0^t C_p(t') \exp\left(-\frac{K^{trans}}{v_e}(t-t')\right) dt' \quad (5)$$

The meaning of K^{trans} depends on the perfusion/permeability regime and reflects the parameter that limits the delivery of the contrast to the tissue. For a freely diffusible tracer that extravasates during the first pass, the tissue enhancement primarily reflects perfusion, i.e., K^{trans} F(flow). If permeability and flow are comparable, K^{trans} contains contributions from both perfusion and permeability. When permeability is much smaller than perfusion, K^{trans} reflects mainly the permeability surface area product (PS) of the tissue, K^{trans} PS (Tofts *et al.*, 1999). In studies of MMW and macromolecular agents, a model with unidirectional plasma-to-EES flow of contrast (essentially the Patlak-Rutland model (Patlak *et al.*, 1983)) is frequently used with two parameters, fractional plasma volume (fPV) and K^{PS} . In this case the transfer constant is often denoted K^{PS} instead of K^{trans} , to emphasize the dominant role of permeability.

Measurements of the arterial input function (AIF), or more precisely, vascular input function (VIF), required by the Tofts-Kety model and other pharmacokinetic models, are even more challenging in small animals than in humans. Nevertheless, several groups have successfully measured individual and population-based AIF at temporal resolutions up to 0.5 s (Loveless *et al.*, 2012; Kim *et al.*, 2012a; Ragan *et al.*, 2010; McGrath *et al.*, 2009; Pickup *et al.*, 2003) (Fig. 14). The biexponential functional form, introduced by Tofts and Kermode in early human studies (Tofts and Kermode, 1991), has been shown to describe well the AIF in rats (McGrath *et al.*, 2009).

When a measured AIF is not available, an alternative approach is represented by the reference region (RR) models. In its basic form, the RR model determines the parameters of the tissue of interest (K^{trans} and v_e) based on the enhancement of a reference tissue, which is assumed to be supplied by the same feeding vessel and has well characterized parameters (Yankeelov *et al.*, 2005). The main benefit of the RR model is its ability to determine pharmacokinetic parameters without an AIF; however, the RR model tends to be less precise than AIF-based models and relies on fixed reference tissue parameters, although several methods have been proposed to improve this model's reliability (Fan *et al.*, 2010; Faranesh and Yankeelov, 2008; Yang *et al.*, 2007).

6.2.4 Validation of DCE MRI with Histology and Other Imaging Modalities—

Validating the findings of DCE MRI by comparison with histology is challenging because of tissue deformation during histological processing and the differences in slice thickness and orientation between MRI and histology. The most commonly used histological measure to be compared with the results of DCE MRI is the microvessel density (MVD) (Hlatky *et al.*, 2002), which is determined from staining with antibodies to endothelial cell markers, such as CD31 or CD34, and has been shown to be prognostic of outcome (Weidner, 1995). Serum and tissue levels of VEGF have also been used with variable success. Several human and animal studies have demonstrated a positive correlation between the initial upslope or K^{trans} and MVD and VEGF (Buckley *et al.*, 1997; Turetschek *et al.*, 2001) and a negative correlation with time to progression (TTP) (Tuncbilek *et al.*, 2004). However, the correlation between DCE MRI and histology remains poor (Su *et al.*, 2003; Atkin *et al.*, 2006), although it was shown to improve when histological sections were reconstructed in three dimensions to match the MR slice thickness. (Kiessling *et al.*, 2005). In animal studies, tissue perfusion can be visualized by injecting the animals with the Hoechst 33342 fluorescent marker shortly before sacrifice and evaluating the tumor sections for its distribution and intensity. The comparison of the Hoechst-stained sections and imaging-derived parameters helps to understand the functionality and spatial distribution of tumor perfusion (Kiessling *et al.*, 2004) (Cho *et al.*, 2009).

6.3 Applications

6.3.1 Characterizing Tumor Vasculature—Early studies have shown that the properties of the hyperpermeable tumor vasculature are better interrogated by using MMW contrast agents rather than conventional LMW agents. Van Dijke *et al.* (van Dijke *et al.*, 1996) showed that in slow- and fast-growing sublines R3230 mammary carcinoma grown in rats, permeability and plasma volume determined using albumin-(Gd-DTPA)₃₀ increased exponentially with increasing MVD, and fast growing tumors had a significantly higher microvascular characteristics than slow growing tumors.

Daldrup *et al.* (Daldrup *et al.*, 1998) studied a spectrum of chemically-induced mammary tumors in rats with DCE MRI enhanced with Gd-DTPA and albumin-(Gd-DTPA)₃₀. Data acquired with the macromolecular agent showed that in carcinomas, vascular permeability (K^{trans}) was nearly twice higher than in benign tumors and showed a strong positive correlation with histopathologic tumor grade. In contrast, the permeability determined from data acquired with the LMW agent (Gd-DTPA), which was much higher than the permeability to albumin-(Gd-DTPA)₃₀, could not differentiate benign and malignant tumors and did not correlate with the tumor grade. Similar observations in the same tumor model were made by Turetschek *et al.* (Turetschek, 2001 #3416) using MS-325 blood pool agent and albumin-(Gd-DTPA)₃₀. A subsequent study by Daldrup-Link *et al.* (Daldrup-Link *et al.*, 2000) showed a two-fold difference between the mean permeability and plasma volume in carcinomas and fibroadenomas when measured with Gadomer-17, although the overlap between the parameter values in benign and malignant groups was considerable and no correlations with the tumor grade were found. With gadopentetate dimeglumine, the permeability in both groups was similar, but the plasma volume and total leakage space were significantly higher in malignant tumors. Similarly, Su *et al.* (Su *et al.*, 1998) demonstrated

that permeability measured using macromolecular contrast agents was higher in a faster growing Walker 256 carcinosarcoma than in a slower growing R3230 AC mammary adenocarcinoma, whereas experiments performed with Gd-DTPA could not differentiate the tumors. Gossmann *et al.* (Gossmann *et al.*, 1999) showed that the low and high grade sublines of R3327 subcutaneous rat prostate tumors (MAT-LyLu and PAP) could be perfectly distinguished by their permeability values measured by DCE MRI with albumin-(Gd-DTPA)₃₀, whereas there was a wide overlap between K^{trans} values measured using Gd-DTPA.

In several studies, DCE MRI was used to probe tumor hypoxia. Cho *et al.* (Cho *et al.*, 2009) have demonstrated the complementarity of the highly enhancing areas on DCE MRI and areas of higher uptake of ¹⁸F-FMISO hypoxia PET tracer. The early parts of the DCE MRI contrast uptake and dynamic PET tracer uptake were similar, but the perfusion/permeability map derived from DCE MRI was complementary to the map of the late slope of the PET time-activity curve that characterizes the hypoxia-specific uptake. The comparison with histological hypoxia marker pimonidazole indicated the correspondence between the highly perfused rim, poorly perfused and non-perfused tumor parts on MRI and PET with viable tissue, hypoxic and necrotic parts, respectively.

Egeland *et al.* (Egeland *et al.*, 2012) performed Gd-DTPA DCE MRI studies in mice with eight human melanoma lines with different angiogenic activity (Fig. 15). They color coded their results to stratify their findings with regards to the values of v_e and K^{trans} . The imaging results were compared with the hypoxic tumor fractions determined histologically, from pimonidazole-stained sections, and radiobiologically, from the post-radiation measurements of cell survival. Because of the very high vascular permeability of the studied cell lines, their K^{trans} values mainly represented perfusion, which is usually complementary to hypoxia. After the necrotic areas were excluded, K^{trans} was shown to decrease exponentially and v_e to decrease linearly with increasing histological or radiobiological hypoxic fractions. This indicates that K^{trans} is more sensitive to hypoxia at lower necrotic fractions (<15% as measured on pimonidazole sections), while at larger necrotic fractions, v_e may be more useful.

6.3.2 Assessing Response to Treatment with Antiangiogenic therapy—The effects of antiangiogenic therapy on the tumor vasculature can be detected using DCE MRI as early as 24 h after initiation of treatment, which is well before any changes in tumor size become observable, and can be confirmed by the measurements of vessel density and perfused tumor fraction. Due to this ability, DCE MRI is widely used in drug development and testing of the efficacy of antiangiogenic agents.

Numerous preclinical DCE MRI studies using MMW contrast agents have demonstrated decreases in tumor permeability after antiangiogenic therapy. Brasch *et al.* (Brasch *et al.*, 1997) used DCE MRI with albumin-(Gd-DTPA)₃₀ to study the response of human breast cancer xenograft MDA-MB-435 in rats treated with bevacizumab. After a single dose of the anti-VEGF antibody, the vascular permeability-surface area decreased precipitously almost to zero at 24 h after treatment, whereas the fractional plasma volume showed no change. Turetschek *et al.* (Turetschek *et al.*, 2004) compared six contrast agents of different

molecular weights in a study of MD-MBA-435 breast cancer xenografts treated with bevacizumab. Significant decreases in K^{trans} and fractional plasma volume were observed in data obtained with contrast agents with molecular weights of 17 kDa (Gadomer-17) and higher, but not with Gd-DTPA (0.55 kDa) or ZK159560 (Bayer Schering, 8.7 kDa), which produced highly variable parameters. The tumor K^{trans} decreased exponentially with molecular weight (the correlation of $\ln(K^{\text{trans}})$ versus molecular weight was $r=-0.98$), and therefore K^{trans} determined from enhancement with USPIO and albumin-(Gd-DTPA)₃₀ was low and difficult to measure accurately, especially after treatment. The contrast agents, Gadomer-17 and ZK181 (25.9 kDa) delivered an optimum enhancement for reliable quantification of both pre- and post-treatment tumor contrast enhancement.

Bradley *et al.* (Bradley *et al.*, 2009) demonstrated a decrease of the initial upslope and amplitude of tumor contrast enhancement curves and about a 30% reductions in IAUC and K^{trans} in subcutaneous C6 and LoVo tumors in rats treated with cediranib for 48 h, while the change in v_e was non-significant. The same group (Bradley *et al.*, 2008) has shown dramatic drops of the maximum concentration, but only small changes in the upslope of the MMW agent gadomelitol (P792) after cediranib and vandetanib administered over 24 h to rats bearing subcutaneous SW620 colorectal xenografts. High temporal resolution sampling (0.5 s per image) enabled acquisition of the AIF in the left ventricle and model calculations of the blood volume, blood flow and permeability surface area product. Treatment with either agent led to 70–80% reductions in PSP, 45–70% reductions in blood volume, but no significant changes in blood flow. This observation indicates that the primary effect of acute treatment with cediranib and vandetanib is the inhibition of vascular permeability.

DCE MRI experiments with MMW contrast agents provide a more direct measure of vascular permeability. In DCE MRI experiments with albumin-(Gd-DTPA)₃₀, Marzola *et al.* (Marzola *et al.*, 2005) observed a 42% reduction of the vascular permeability ($p = 0.001$) in the viable tumor rim of subcutaneous HT29 colorectal tumors in mice treated with SU11248 (sunitinib) over 24 h. The IAUC determined from the Gd-DTPA-enhanced dynamic study decreased after treatment by 31% ($p < 0.05$). These changes were corroborated by a significant reduction of MVD by 30%. The same group has shown that the tumor response to sunitinib was spatially heterogeneous (Marzola *et al.*, 2004); in the tumor rim, the drop in permeability after injection of albumin-(Gd-DTPA)₃₀ was twice as high as in the core and the decrease of the blood volume was 70% larger than in the core.

It is also possible to detect the early changes in permeability after anti-angiogenic therapy using clinically approved LMW agents shown by other studies. The feasibility of using Gd-DTPA-enhanced MRI to monitor the effect of antiangiogenic therapy was demonstrated by Checkley *et al.* in prostate, lung and breast xenografts in mice treated with a VEGFR-2 inhibitor ZD4190 (vandetanib), for 1 day or 7 days of treatment (Checkley *et al.*, 2003b). In PC-3 prostate tumors, K^{trans} calculated using individual AIFs measured in the heart, showed significant dose-dependent reductions of up to 30% and 50% after 2 and 8 doses of 100 mg/kg vandetanib, respectively. In lung and breast tumors, the post-treatment reductions of K^{trans} were apparent on voxel histograms, but the statistical significance of these changes was lost when the mean tumor K^{trans} were compared. To account for heterogeneity, the authors used a multi-threshold analysis. In a related study of PC-3 tumors by the same group

(Checkley *et al.*, 2003a), they demonstrated that the post-treatment reductions of K^{trans} were greater for the 20% most enhancing rim voxels than for the less enhancing core voxels. Importantly, this study has also explored the reproducibility of the Tofts-Kety model parameters; the coefficient of variation within animal was found to be 16% for K^{trans} and 7% for v_e , although the variations among animals were larger (40% for K^{trans} and 18% for v_e).

The vascular changes in papillary renal cell carcinoma (RCC) orthotopic xenografts in mice treated with sunitinib were assessed using DCE MRI by Hillman *et al.* (Hillman *et al.*, 2009). The complex changes of kidney and tumor enhancement suggested that sunitinib at therapeutic dose of 40 mg/kg/day caused damage of the normal vasculature. In a subsequent study, Hillman *et al.* (Hillman *et al.*, 2010) used the same DCE MRI technique to design an optimal schedule for a combination therapy that consisted of sunitinib (20 mg/kg/day) and cytotoxic agent gemcitabine.

7. T_2^* -weighted Imaging and Related Techniques

In T_2^* -weighted imaging, the contrast mechanism is based on the magnetic susceptibility differences that create local field inhomogeneities, which lead to the variations of the transverse relaxation rate and the T_2^* -weighted signal loss. Contrast-enhanced (CE) T_2^* -weighted imaging is performed after administration of a paramagnetic contrast agent. Imaging based on the endogenous T_2^* -weighted contrast is discussed in a separate section of this review (Section 8, *BOLD Imaging*). Gradient echo and spin echo imaging enable determination of the ratio R_2^*/R_2 , which is related to the characteristic vessel size and forms the basis of the vessel size imaging technique, described in the next subsection. In studies of small animal cancer models, T_2^* -weighted CE imaging has several important applications, which include characterization of tumor vasculature, assessment of metastasis and monitoring response to treatment.

7.1 Gadolinium-enhanced T_2^* -Weighted Imaging

Gadolinium-based LMW contrast agents readily leak through the vessel walls into the interstitium, and therefore achieving intravascular contrast concentrations high enough to produce reliable T_2^* contrast requires injecting high doses of contrast material. This problem is exacerbated in small animals because of their high heart rate and short circulation times. As a result, T_2^* -weighted imaging enhanced with gadolinium-based contrast agents is performed mainly in the brain, where an intact or partially intact blood-brain barrier (BBB) limits the leakage of the contrast material into the EES.

Dynamic T_2^* -weighted imaging, also referred to as the dynamic susceptibility-contrast (DSC) imaging or perfusion-weighted imaging, is performed by measuring a transient loss of gradient recalled echo signal at multiple time points during the first pass of contrast material through the tissue. The tissue contrast concentration $C(t)$ is assumed to be proportional to R_2^* , which in turn can be derived from the pre- and post-contrast signal intensities, S_0 and S , respectively: $C(t) = k R_2^*(t) = -(k/TE)\ln(S(t)/S_0)$, where k is the proportionality constant dependent on the contrast agent and acquisition sequence (Ostergaard, 2005). In the absence of recirculation and leakage, the blood volume is

proportional to the area under the first-pass peak of $C(t)$ curve. Blood flow, F , can be found using the principles of the indicator dilution theory, according to which the tissue concentration $C(t)$ is represented by a convolution of the AIF, $C_A(t)$, and the residue function, $R(t)$: $C(t) = FC_A(t) \otimes R(t)$ (Ostergaard, 2005). The residue function can be obtained by a variety of methods. For a single, well-mixed vascular compartment, the residue function can be modeled as an exponential: $R(t) = \exp(-t/MTT)$, where MTT is the mean transit time (Larson *et al.*, 1994). If absolute measurements are unfeasible, blood flow and blood volume are expressed in relation to their respective values in the normal tissue, e.g., normal white matter in case of brain tumors, and the relative parameters are denoted rCBF (relative cerebral blood flow) and rCBV (relative cerebral blood volume).

Tumor blood volume and blood flow measured from DSC MRI were shown to correlate with tumor grade (Lee *et al.*, 2001; Shin *et al.*, 2002) and other markers of tumor aggressiveness (Aronen *et al.*, 2000). In cases of considerable breakdown of BBB, this correlation is preserved if the calculation of rCBV is corrected for the contrast agent leakage (Boxerman *et al.*, 2006). MR-derived values of rCBV also strongly correlate with the fractional vascular volume measured on histology, although they underestimated the histological measure (Pathak *et al.*, 2001).

DSC MRI has been used to study the evolution of tumor vasculature over time, which can help to understand the enhancement patterns of human tumors and develop quantitative methods for their assessment (Cha *et al.*, 2003). In GL261 mouse glioma studied at 7 T with gadolinium-enhanced T_2^* -imaging, both rCBV and the histological microvessel density (MVD) increased from week 1 after tumor implantation to a maximum at week 3 and decreased by week 4. These changes are in agreement with the well-known 4-week progression history of this tumor model, in which angiogenesis usually develops only during the third week. Interestingly, the tumor enhancement on T_1 -weighted images was observed during weeks 1 and 2, which suggests that MRI depicts not only angiogenic vessels, but other tumor vasculature as well.

DSC MRI enhanced with triple-dose of Omniscan (0.3 mmol/kg) was used by Chen *et al.* as a part of a imaging characterization of a hepatic tumor model, rhabdomyosarcoma implanted in rat liver (Chen *et al.*, 2006). Dynamic curves indicated that the contrast arrives in the tumor slightly earlier than in the normal liver, whereas maps of the relative blood flow, relative blood volume and vascular permeability showed significantly higher values of these parameters in the tumor compared to the normal parenchyma, in agreement with the hypervascularity of the tumor observed using *ex vivo* methods. In combination with T_1 -weighted DCE and DW MRI, DSC MRI is a valuable component in comprehensive functional evaluation of tumor models in translational studies (Chen *et al.*, 2011).

7.2 SPIO-enhanced T_2^* -weighted imaging

Alternatively, T_2^* -weighted imaging can be enhanced with superparamagnetic iron oxide particles (SPIO), which possess high r_2 relaxivity and long plasma half-life and thus enable imaging with high SNR and spatial resolution. SPIO-based imaging has been the subject of several comprehensive reviews (Peng *et al.*, 2008; Thorek *et al.*, 2006). In studies of animal models of cancer, SPIO-enhanced T_2^* -weighted MRI has been widely used for tumor

detection and localization, studies of tumor vasculature, lymph node metastases and inflammation (Wu *et al.*, 2004).

SPIO particles consist of a nanometer-sized iron oxide core surrounded by a hydrophilic surface coating. Their magnetic characteristics can be controlled by manipulating their size and coating. Their surface can be conjugated to ligands and therapeutic agents targeting various receptors on the tumor cells in order to achieve efficient tumor-specific accumulation of SPIO. In the body, SPIO of 100–250 nm size are rapidly taken up by the reticuloendothelial system in the liver and spleen. Ultra Small Paramagnetic Iron Oxide (USPIO) (<50 nm) particles remain in the circulation longer, extravasate into the interstitium, travel through the lymphatic vessels and eventually accumulate in the lymph nodes and bone marrow. SPIO are therefore suitable for studies of metastasis and inflammation and can be detected *ex vivo* by the immunohistochemical staining for iron and macrophages (Moore *et al.*, 2000). Two SPIO contrast agents have been approved for human imaging: ferumoxides (AMI-25, distributed as Endorem in Europe, Feridex in the US) and ferucarbotran (SHU555, or Resovist) (Wang, 2011). Additionally, ferumoxytol (Feraheme, AMAG Pharmaceuticals), an ultrasmall SPIO (USPIO) compound developed for treatment of iron deficiency in patients with chronic kidney disease, has been successfully used as an MR contrast agent in research protocols (Stabi and Bendz, 2011; Hamilton *et al.*, 2011).

Similarly to gadolinium-based contrast agents, SPIO contrast agents were used for characterization of the vasculature of brain tumors. Measurements of blood flow and blood volume derived from DSC MRI and vascular permeability obtained from T_1 -weighted DCE MRI demonstrate the differences in the vasculature of various mouse brain tumors as well as the heterogeneity of these parameters within each tumor (Gambarota *et al.*, 2008). The SPIO-enhanced T_2^* -weighted gradient echo images also provided the best tumor delineation among all sequences.

In liver imaging, SPIO are used to differentiate the normal liver tissue, in which SPIO are taken up by Kupffer cells in the reticuloendothelial system, from hepatic tumors, which lack Kupffer cells (Ni *et al.*, 2009). SPIO can also increase the conspicuity of splenic malignancies (Weissleder *et al.*, 1987).

USPIO compounds can help to distinguish lymph nodes invaded by the tumor from normal or reactive nodes. In normal and reactive nodes, the accumulation of USPIO leads to a decrease of the T_2^* -weighted signal, whereas metastatic nodes do not show the contrast agent uptake and show no signal loss. In MR lymphography experiments enhanced with USPIO agent AMI-227, popliteal metastases in rats showed a decrease of contrast-to-noise between the node and adjacent muscle, whereas lymphadenitis showed either stable or increased contrast-to-noise values (Vassallo *et al.*, 1994). Differences in contrast uptake by reactive and invaded lymph nodes were also observed in rabbits bearing VX2 liver tumors after an interstitial injection of an SPIO compound (Taupitz *et al.*, 1993) or an intravascular gadolinium-based agent (Herborn *et al.*, 2002). Zhang *et al.* demonstrated the feasibility of differentiation of metastatic lymph nodes in mice using DW MRI or SPIO-enhanced

imaging, although neither technique could distinguish the nodes at an early stage (Zhang *et al.*, 2013).

SPIO-enhanced imaging has been successfully applied to assess and predict the tumor response to treatment. In HT-1080 fibrosarcoma mouse tumors treated with a thrombogenic vascular targeting agent, the post-treatment steady-state R_2^* values and the vascular volume fraction were significantly lower than in controls and were negatively correlated with the grade of thrombosis and necrosis (Persigehl *et al.*, 2007a). Measurements of tumor perfusion with microbeads were in agreement with the MR results. Subsequent experiments in the same tumor model showed that the post-treatment decrease in R_2^* was much larger in tumors that responded to treatment than in non-responsive tumors, if response was determined by the histologic grading for thrombosis and necrosis (Persigehl *et al.*, 2007b).

Transient decreases in relative Cerebral Blood Volume (rCBV) were observed in 9L gliosarcoma tumor in rats treated with corticosteroid dexamethasone, whereas rCBV increased in control animals over the same time interval; *ex vivo* micro-CT analysis confirmed that hypervascularity was indeed reduced in the drug-treated rats (Darpolor *et al.*, 2011b).

With the introduction of ferumoxytol, a USPIO compound that can be administered as a bolus, it has become possible to perform the USPIO-enhanced dynamic T_2^* -weighted imaging (Varallyay *et al.*, 2002). Dual-contrast dynamic MRI enhanced by gadodiamide and ferumoxytol demonstrated larger decreases in vascular permeability and perfusion in U87 rat glioma model treated with an antiangiogenic compound (bevacizumab) than after treatment with corticosteroids (Varallyay *et al.*, 2009). Staining for iron demonstrated a concentration of ferumoxytol at the tumor periphery and in necrotic areas, and only a minimal ferumoxytol uptake was observed inside the tumor, which was attributed to the presence of the inflammatory components, activity of the reticuloendothelial system, and the BBB defects in the tumor periphery.

SPIO and USPIO are valuable tools in studies of macrophages and models of inflammation. Tumor-associated macrophages (TAMs) were studied in a model of breast cancer, in which the presence of an inflammatory component is associated with a poor outcome (Daldrup-Link *et al.*, 2011). MR imaging enhanced with USPIO agents, including ferumoxytol, at 24 h after contrast administration in mice with mammary carcinoma; the tumor T_2^* signal decreased because of the accumulation of USPIO. Depletion of TAM using an antibody has led to a significant decrease of USPIO accumulation, which was confirmed by histology. These data suggest that at late time points, the USPIO are localized in the TAM and not in the regular tumor cells.

Imaging of cells labeled with various markers, including SPIO, referred to as cell tracking or cell trafficking, has been actively studied in models of cancer and metastasis. The results of cell tracking obtained in clinical and pre-clinical imaging have been reviewed elsewhere (Bulte, 2009; Ottobriini *et al.*, 2011). T_2^* -mapping after systemic administration of SPIO-labeled breast cancer cells allows to perform longitudinal studies of cell migration and development of brain metastases (Song *et al.*, 2011). Subcutaneously injected labeled tumor

cells enable mapping of the tumor spread through the lymphatic network (Zhang *et al.*, 2012). Migration of hematopoietic progenitor cells labeled with ferumoxides in mice was visualized as the drop of the T_2^* signal in the liver, spleen and bone marrow (Daldrop-Link *et al.*, 2005).

7.3 Vessel-Size Imaging (VSI)

VSI enables probing the distribution of microvessels by exploiting the differences in behavior of their transverse relaxation rates R_2^* and R_2 induced by the administration of a paramagnetic contrast agent, such as USPIO, or a change in blood oxygenation level. In an external magnetic field, the contrast agent within the blood vessels generates an inhomogeneous local field that enhances the dephasing of spins. In spin echo experiments, the dephasing due to the local inhomogeneous magnetic field is refocused and, as a result, the dephasing due to the diffusion effects becomes pronounced. In gradient echo experiments, the refocusing of spins dephased by the local inhomogeneities does not occur. In regions near large vessels, a large increase in R_2^* and a small increase in R_2 are expected, whereas changes of R_2^* and R_2 are of approximately the same magnitude in the vicinity of small vessels, where the water diffusion length is comparable with the length scale of the local susceptibility gradient. As a result, R_2^* and R_2 have different dependences on the contrast concentration. The ratio R_2^*/R_2 is related to the average vessel radius, or vessel index (VI), R_v (Jensen and Chandra, 2000; Tropres *et al.*, 2001; Tropres *et al.*, 2004b)

$$R_v = 0.424 \left(\frac{D}{\gamma \Delta \chi B_0} \right)^{1/2} \left(\frac{\Delta R_2^*}{\Delta R_2} \right)^{3/2} \quad [6]$$

where D is the apparent diffusion coefficient (ADC) of extravascular water protons, γ is the gyromagnetic ratio, and B_0 is the external magnetic field. The dependence of R_v on the contrast agent concentration is established through the change in the susceptibility of blood, $\chi = \chi - \chi_0$, where χ and χ_0 are susceptibilities of blood with and without the contrast agent, respectively. For fully deoxygenated blood, $\chi_0 = 8 \cdot 10^{-8}$ (in CGS units) and χ_0 is zero for fully oxygenated blood. Eq. [5] is valid in the limit of high contrast concentrations, where $\chi \gg \chi_0$.

VSI also yields the fractional blood volume (BV), referred to as f :

$$f = \frac{3}{4\pi} \frac{\Delta R_2^*}{\gamma \Delta \chi B_0} \quad [7]$$

Jensen and Chandra (Jensen and Chandra, 2000) introduced the quantity Q , which at high contrast concentrations is independent of the concentration and reflects only the intrinsic properties of the tissue:

$$Q = \frac{\Delta R}{(\Delta R_2^*)^{2/3}} \quad [8]$$

For typical ADC values in human tissues $D \sim 1.0 \cdot 10^{-3} \text{ mm}^2/\text{s}$, the histological microvessel density (MVD) is related to Q : $N \sim Q^3 \cdot 10^3 \text{ s/mm}^2$, and thus VSI can provide noninvasive means for assessment of MVD (Ungersma *et al.*, 2011).

VSI has been successfully used to characterize the vasculature in normal tissues and tumors and evaluate the response to antiangiogenic and hormonal treatment. The majority of the VSI studies used a USPIO contrast agent (ferumoxtran-10; Sinerem/Combindex, Guerbet, France). The ratio of R_2^*/R_2 was shown to be related to the vessel size and was found to be about twice higher in rat glioma than in the normal brain tissue, in good agreement with Monte Carlo simulations and histological vessel size (Dennie *et al.*, 1998). The characteristic vessel size in glioma models was subsequently found to be much higher in the tumor (20 μm) than in the peritumoral region (7.5 μm) and nearly five times higher than in the contralateral cortical area (4.5 μm) (Tropres *et al.*, 2004b). The relative blood volume was twice lower in the tumor (2.0%) than in the peritumoral area (4.0%) or the contralateral cortex (4.3%). It has also been demonstrated that VI decreases with increasing contrast agent concentration (Tropres *et al.*, 2004a). USPIO-enhanced VSI was combined with gadolinium-enhanced DCE MRI to perform detailed characterization of the tumor vasculature in two rat glioma models, which showed similar leakiness in DCE MRI, yet different blood volumes and VSI, in agreement with histological analysis (Beaumont *et al.*, 2009).

The MRI-based vessel size measurements tend to overestimate the vessel size using reference imaging techniques or histology (Ungersma *et al.*, 2011; Douma *et al.*, 2010). In mice bearing colorectal adenocarcinoma, both MRI and the three-dimensional two-photon laser scanning microscopy (TPLSM) showed heterogeneous distribution of the tumor vessel sizes (Douma *et al.*, 2010). MR VSI strongly overestimated VI (muscle/tumor rim/tumor core, 11.9/38.0/34.3 μm) and underestimated the blood volume in the tumor (rim/core, 1.8%/1.2%) compared to TPLSM (vessel size muscle/rim/core 2.2/6.0/4.3 μm ; blood volume rim/core, 8.9%/4.6%); respectively). Interestingly, the two techniques yielded similar blood volume estimates in the muscle (MRI, 1.2%; TPLSM, 1.5%) (Douma *et al.*, 2010). A reasonable agreement was obtained between MRI and micro-CT estimates of VSI and blood volume, but both imaging techniques overestimated the histological VSI and underestimated the histological vessel density (Ungersma *et al.*, 2011). The discrepancy between VSI results obtained using MRI and other techniques may stem from the partial-volume effects in MR VSI, limitations of the theoretical VSI model, native paramagnetism of venous blood, or heterogeneity of the tumor perfusion. These results suggest that MR VSI results are strongly dependent on the experimental setup, and therefore VSI is more suitable for assessing changes due to treatment rather than absolute measurements.

VSI has been applied to assessing the effects of treatment. VSI studies of the hormone-dependent subcutaneous Shionogi tumors in castrated and intact mice found that tumors that regressed after castration had much a lower VI ($R_v = 15.1 \mu\text{m}$) than the progressing tumors ($R_v = 35.2 \mu\text{m}$) (Wade and Kozlowski, 2007). Decreases in viable tumor growth rate, blood volume and MVD-related quantity Q were shown in rats treated with an antiangiogenic drug, but the VSI increased in both treated and control animals (Ungersma *et al.*, 2011).

After short-term treatment of GH3 prolactinomas with a vascular disrupting agent DMXAA (5,6-dimethylxanthenone-4-acetic acid), both the VI and BV were significantly reduced, mainly due to the increase of the number of voxels with the lowest values of R_v and blood volume (Howe *et al.*, 2008). Areas of low blood volume ($BV < 1\%$) increased on average five-fold after treatment and correlated well with histologically measured necrosis, although MRI showed a tendency to overestimate necrotic areas relative to histology. These changes were interpreted as the vascular collapse and the reduction of the number of the large vessels due to the effect of the VDA.

VSI and DCE MRI were used to monitor vessel remodeling in bone metastases in rats bearing mammary carcinoma treated with zoledronic acid, sunitinib malate or their combination (Bauerle *et al.*, 2010). Treatment with all three protocols lead to significantly increased VI and decreased BV values compared to controls, which was corroborated by histological measurements of MVD and vessel size. Tumor ADC increases were observed after treatment, which underscores the importance of measuring ADC for VI quantification.

8.0 BOLD (Blood Oxygen Dependent) Imaging

8.1 Introduction

It was realized over 20 years ago that due to the paramagnetic nature of hemoglobin, tissue proton T_2^* is sensitive to blood deoxyhemoglobin or blood oxygen saturation level (Ogawa *et al.*, 1990). Based on this sensitivity Blood Oxygen Level Dependent (BOLD) MRI has been used successfully to non-invasively measure associated neural activity in brain, opening up an entire field of functional MRI (fMRI) (Hockel and Vaupel, 2001). There has been intense interest of applying BOLD MRI to non-invasively measure tumor oxygen level. Tumor hypoxia state is known to be an important factor in tumor resistance to radiation and chemotherapy (Zhao *et al.*, 2009). Clinical non-invasive assessment of tumor hypoxic state will be a valuable tool for effective treatment planning and monitoring. Many studies have been carried out to investigate tumor BOLD MRI measurement, both clinically and pre-clinically. Primarily the method relies on measuring tissue water proton T_2^* or R_2^* ($=1/T_2^*$) map of the tumor, and often comparing the R_2^* value changes under oxygen challenge.

8.2 Method and theory

Tissue proton T_2^* map is typically measured by GRE (Gradient Recall Echo) sequence with variable TE values but EPI and steady-state free precession (SSFP) sequences have also been successfully used for BOLD measurements (Remmele *et al.*, 2010; Zhao *et al.*, 2009). Tissue water proton T_2^* has contributions from T_2 and local field inhomogeneity component T_{inhom} .

$$1/T_2^* = 1/T_2 + 1/T_{inhom} \quad [9]$$

The contribution of blood volume, blood vessel susceptibility dependence on tissue proton T_2^* can be described by the following formula (Yablonskiy and Haacke, 1994):

$$1/T_{2blood} = k_i b v_i Hct_i (1 - Y_i) \Delta\chi \quad [10]$$

Where Hct is the hematocrit, Y is the fraction of oxygenated blood, b_v the fraction of tissue occupied by blood vessels, χ the absolute susceptibility difference between fully oxygenated and deoxygenated blood, and k a term dependent on blood vessel morphology and magnetic field strength; and the contribution summed over arterial, capillary and venular vascular network within the tissue. Tumor blood flow and hematocrit are not directly measurable from MRI, but can be inferred from results through other methods.

In quantitative BOLD (qBOLD), a MRI approach is developed to measure tissue T_2 , relevant blood volume parameters for the tissue, and derive tissue oxygen fraction from equations 9 and 10 directly. Christen et al used iron nanoparticle ultra-small paramagnetic iron particles (USPIO) as blood pool contrast agent to measure CBV and cerebral blood flow (CBF) in mouse brains with tumor. They showed in their brain tumor model the tumor oxygen level is not lower than the normal brain areas based on qBOLD measurement (Christen *et al.*, 2012)(7). The qBOLD method has the potential to realistically provide a non-invasively clinically viable way for tissue oxygen analysis (Christen *et al.*, 2012; He and Yablonskiy, 2007).

Paramagnetic deoxyhemoglobin (dHb) will also alter the tissue water proton T_1 relaxation rate. O'Connor et al proposed using tissue T_1 map change as a marker for changing tissue dHb level and therefore oxygen fraction instead of using R_2^* map (O'Connor *et al.*, 2009). They applied this method to ten patients with either locally recurrent cervical and hepatocellular carcinoma or metastases from ovarian and colorectal carcinoma. Upon change of breathing gas from air to oxygen, they were able to observe significant T_1 change in tumors, which partially correlated with tumor perfusion. Deviations from tumor perfusion pattern within the heterogeneous tumors were observed indicating other factors contributing to tumor T_1 change.

Under various gas, vascular or metabolically challenged regimes, in an ideal situation where blood volume in tumor and hematocrit remain the same pre- and post- challenge, the difference in measured R_2^* is directly proportional to $(1 - Y)$ from equation 10. But often there are changes in tumor vasculature, blood hematocrit, blood volume and blood flow, which makes the interpretation of R_2^* less straight forward on its own. Hyperoxia by breathing carbogen, oxygen with 5% CO_2 , is the most common method for gas challenge (Fan *et al.*, 2002; Rakow-Penner *et al.*, 2010). In several xenograft tumor models, tumor

R_2^* map revealed significant and heterogeneous changes of R_2^* . The R_2^* map does not fully correlate with MR Gd perfusion maps, although areas of high perfusion do show higher R_2^* changes. Robinson et al used a vascular targeting agent and observed a significant R_2^* change upon treatment (Robinson *et al.*, 2005). Metabolic altering agents which may speed up or inhibit tumor metabolism could alter local oxygen consumption and induce related blood flow change which may also induce changes in R_2^* value (Jordan *et al.*, 2006).

8.3 Applications

In vivo BOLD MRI measurements of tumor oxygen have been compared in a number of studies to other invasive and non-invasive tissue pO_2 measurement methods. Neeman et al applied R_2^* map, *in vivo* tissue hemoglobin absorption spectra from fiber optic spectrometer LESA (Biospec, Russia), and tissue oxygenation from Oxylite and Oxyflo probes (Oxford

Optonics, Oxford, UK) to study tumor vascular response under hypercapnia and hyperoxia in a subcutaneous tumor model (Neeman *et al.*, 2001). They showed that under gas challenge, MRI signal intensity significantly correlated with time matched oxygen saturation (sO_2). To address the question of the tumor oxygen partial pressure (pO_2) and BOLD MRI signal intensity relationship, Baudelet et al investigated the FSa fibrosarcoma inoculated into the mouse thigh. Tumor R_2^* signal intensity varied with pO_2 . However the relationship is not proportional. In different mice, similar pO_2 values were not associated with comparable local R_2^* signal intensities. The change in R_2^* at < 30 mm Hg pO_2 had a stronger dependence on pO_2 than at higher tissue pO_2 level (Baudelet and Gallez, 2002). Chopra et al used an Eppendorf Oxylite oxygen meter in nine prostate cancer patients to measure tumor pO_2 level. Although a direct correlation between R_2^* map and Oxylite oxygen level was not observed, a trend towards negative correlation between R_2^* and pO_2 ($r = -0.77$, $p = 0.07$) was shown. However, the R_2^* map and HP5 map (hypoxic fraction < 5 mm Hg) have a positive correlation ($r = 0.76$, $p = 0.02$). This study however has a serious limitation of a median delay of up to 4 days between the MR and needle oxygen measurement (Chopra *et al.*, 2009).

Electron Paramagnetic Resonance (EPR) can measure probe molecule EPR resonance linewidth change due to oxygen level variation in solution and tissues. EPR is another promising non-invasive method for tumor pO_2 measurement. Elas et al injected a trityl spin probe OX031 (Nycomed Innovations, Malmo, Sweden) i.v. in FSa tumor bearing mice. They were able to compare pO_2 measured from EPRI to BOLD MRI. Low BOLD MRI contrast area upon carbogen has similar area to EPRI hypoxic region. There appeared to be good correlation between the regional variations in the BOLD and EPR images of these tumors (Elas *et al.*, 2003). In manipulating tumor pO_2 by modifying hemoglobin-oxygen binding through a hemoglobin allosteric modifier RSR13, Hou et al measured maximum pO_2 changes to be around 8–12 mm Hg by EPR. However no R_2^* change was observed, which was attributed to lower detection sensitivity by BOLD MRI (Hou *et al.*, 2004).

Perfluorocarbons (PFC) have ^{19}F atoms whose T_1 relaxation is sensitive to surrounding oxygen concentration. It has been shown that PFC MR relaxometry is able to measure tissue pO_2 via injected or perfused PFC in tissues. Fan et al found in their study only 46% of tumor had accumulated PFC. In tumor regions where sufficient PFC had accumulated they reported good agreement between R_2^* and PFC results upon carbogen breathing in mice. The disagreement is suggested to result from insufficient proton SNR in T_2^* -weighted images. Good agreement also occurred in areas where pO_2 has large change or no change (10). In a separate study by Zhao et al comparing PFC pO_2 measurement and BOLD MRI, significant pO_2 and BOLD response upon air-to-oxygen breathing change was found, while there was poor correlation between baseline pO_2 and R_2^* (Zhao *et al.*, 2009).

8.4 Future

Tumor BOLD MRI has been used in prostate, breast, brain and other cancer patients clinically to assist staging and treatment selection (Atkin *et al.*, 2006; Carpenter *et al.*, 2010; Chopra *et al.*, 2009; Christen *et al.*, 2012; Diergarten *et al.*, 2005; Griffiths *et al.*, 1997; Hallac *et al.*, 2012; Hoskin *et al.*, 2007; Rijpkema *et al.*, 2002). In preclinical rodent tumor

models, BOLD MRI results have been shown to be a combination of factors from T_2 , perfusion, blood flow, blood vessel network, hematocrit and hypoxia. It therefore complements tumor perfusion studies of hypoxia. Due to the complexity of interpreting tissue pO_2 and hypoxia state from BOLD MR susceptibility measurement, there is currently no standard protocol for BOLD MRI measurements (Griffiths *et al.*, 1997; He and Yablonskiy, 2007; O'Connor *et al.*, 2009). Single R_2^* map measurement offers the measurement simplicity, but it usually is not sufficient to interpret tissue pO_2 level or hypoxia. The qBOLD method offers the potential of direct in-vivo tissue oxygen fraction measurement. Despite promising results, more correlation and validation is needed for blood flow imaging and tumor oxygen level with other methods. R_2^* change can be reliably measured by susceptibility weighted MR imaging before and after a gas challenge to the tumor. This method has been the most commonly used in preclinical tumor BOLD MRI. Clinically carbogen toxicity can result in hyperventilation in patient, which limits its usefulness for clinical application. The observed change in R_2^* has contributions from pO_2 change and other tumor vascular components, making its interpretation not straight forward.

Tumor BOLD MRI is an attractive method to non-invasively probe tumor oxygen state. Studies have demonstrated it provides complementary information in addition to other perfusion, hypoxia measurement. Further development of this method will bring it closer to clinical application.

9.0 Diffusion-weighted MRI (DWI)

9.1 Introduction

Diffusion-weighted (DW) MRI is sensitive to the Brownian motion of water molecules and enables probing the organization of the tissue on a microscopic level. In contrast to the free diffusion in bulk fluid, the mobility of water molecules in tissue can be impeded by the presence of cellular microstructural barriers, such as cell membranes, and the interactions with macromolecules. As a result, water diffusion is affected by tissue cellularity, membrane permeability as well as the volume and tortuosity of the extracellular space. The basic diffusion-sensitive pulse sequence was described by Stejskal and Tanner (Stejskal and Tanner, 1965) and represents a T_2 -weighted spin echo sequence with a pair of diffusion-sensitizing gradients inserted on both sides of the refocusing 180 pulse. Stationary protons that acquire phase from the first diffusion gradient are rephased by the second gradient without significant losses, but moving protons are not fully rephased and as a result contribute less to the signal. The signal decreases with the product of the diffusion weight factor (b-factor) ($b = \gamma^2 G^2 \delta^2 (-\delta/3)$, s/mm²), which represents a combination of the gyromagnetic ratio (γ), the diffusion gradient strength (G), duration (δ), and time separation of the diffusion gradients (δ). In homogeneous tissues, the DW signal versus b-value decreases exponentially with the rate described by the apparent diffusion coefficient (ADC, mm²/s):

$$S(b) = S_0 \exp(-b \cdot \text{ADC}) \quad [11]$$

where $S(b)$ is the DW signal at a given b-value and $S_0 = S(b = 0)$ is the signal without diffusion weighting. By measuring the DW signal at two or more different b-values, ADC

can be calculated using Eq. [1]. ADC is higher in areas with few obstacles and is usually lower in areas where the motion of water molecules is restricted by barriers. As a result, ADC values of malignant tumors, which often consist of tightly packed cells, tend to be lower than the ADCs of benign tissues, in which cells are packed more loosely.

In human imaging (1.5–3 T), DW is usually performed using spin-echo sequences with EPI readout, chosen for its high speed, which helps to reduce motion artifacts. In animal imaging, motion artifacts are of secondary importance, because animals are usually anesthetized. At the typical field strengths of magnets used in animal studies (4.7–11 T), increased field inhomogeneities (and shorter T_2^* values) become a priority. In this case, the EPI readout, which is sensitive to field inhomogeneities, is not advantageous; and a fast spin-echo method, which refocuses the phase dispersion caused by magnetic field inhomogeneities, is preferable.

In the majority of DWI animal studies published to date, the DW signal was assumed to follow the monoexponential dependence described by Eq. [1], but deviations of DW signal from this behavior are currently being actively studied, especially in clinical MRI. At low b-values, the intravoxel incoherent motion (IVIM) effect due to the microscopic flow of blood in the capillary network creates a pseudo-diffusion contribution to the DW signal with the diffusivity that is much higher than the ADC of true diffusion (Le Bihan *et al.*, 1988). In the presence of IVIM (intravoxel incoherent motion) effects, the DW signal is described by the bi-exponential function (Le Bihan *et al.*, 1986):

$$S(b) = S_0 [f_p \cdot \exp(-b \cdot D_p) + (1 - f_p) \cdot \exp(-b \cdot D)] \quad [12]$$

where D_p and D are the perfusion and true diffusion coefficients, respectively, and f_p is the perfusion fraction. The IVIM effect has been observed in some highly vascularized animal tumors (Wang *et al.*, 2001; Kim *et al.*, 2012b) and there are indications that D_p and D show different behavior after therapy (Thoeny *et al.*, 2005b; Wang *et al.*, 2001).

At higher b-values, the non-monoexponential behavior of DW signal may also be caused by the presence of diffusion barriers or tissue compartments with different ADCs (Jensen *et al.*, 2005; Yablonskiy *et al.*, 2003; Sukstanskii *et al.*, 2003; Bennett *et al.*, 2004), or by contributions from different pools of hydrogen atoms (intra vs extracellular water). This is well summarized in a previous article by Jensen and Helpern (Jensen and Helpern, 2010). The deviation of the DW signal from the monoexponential form leads to the dependence of the measured ADC on the choice of b-values (Zhang *et al.*, 2010). The number and range of b-values used in the literature vary considerably. Although the problem of b-value optimization has been extensively studied, there is no agreement regarding the optimal choice of b-values. Some studies use only two b-values, while others use multiple b-values. Studies of treatment response sometimes determine two separate diffusivities from low and high b-value ranges, e.g., ADC_{low} derived from $0 < b < 100$ s/mm² and ADC_{high} at $b > 500$ s/mm², respectively, to elucidate the differences in the behavior of perfusion-related and diffusion components of diffusivity. The minimum b-value is sometimes taken to be 0 s/mm², although sometimes $b > 0$ s/mm² is used, and the maximum b-value range can be as high as 4000 s/mm². The diffusivities determined at the lowest and highest b-values tend to

have low precision and therefore it remains unclear whether such measurements have any practical purpose (Hoff *et al.*, 2010).

9.2 Applications

Clinical DWI is often used for lesion detection and localization based on the differences between the ADC values in tumors and normal tissues. In studies of animal tumor models, detection and localization are usually less important, and therefore DW MRI is more often used for characterization of the tumor microenvironment and for prediction or assessment of the tumor response to treatment (Zhao *et al.*, 1996; Chenevert *et al.*, 1997; Chenevert *et al.*, 2000). Comparison of DW MRI images and ADC maps with histological sections enables characterizing the microstructure of the tumor tissue.

9.2.1 ADC for Assessment of Tumor Heterogeneity—In subcutaneous animal tumors, the mean ADC values were found to vary in the range of $(0.4 - 1.3) \cdot 10^{-3} \text{ mm}^2/\text{s}$ (Thoeny *et al.*, 2005a). In viable tumor tissue, ADC is usually lower than in the normal tissues, mainly because of the high cellular density of malignant tissue. Lesions with high fat content also tend to have low ADCs. On the other hand, tumors with low cellular density, such as myxoid tumors, have high ADCs that are close to ADCs typically seen in normal tissues ($\text{ADC } 2.0 \cdot 10^{-3} \text{ mm}^2/\text{s}$) (Maeda *et al.*, 2007). In necrotic areas and cysts, ADC can approach the diffusivity in bulk fluid. Several studies have shown that in some tumors ADC was negatively correlated with cell density (Lyng *et al.*, 2000; Gibbs *et al.*, 2009; Gupta *et al.*, 1999; Sugahara *et al.*, 1999). In tumors with a viable rim and necrotic center, the periphery of the tumor often shows lower ADC than the tumor core. Lyng *et al.* (Lyng *et al.*, 2000) have also demonstrated that in tumors in which necrosis was present as larger connected areas, tumor ADC was correlated with the histological necrotic fraction, but in tumors that contained multiple small necrotic regions, this correlation was not observed, possibly because partial volume effects made visualizing necrotic areas difficult.

ADC maps were shown useful in delineation of necrotic areas. Such segmentation can be performed using an empirically chosen threshold ADC value separating viable from necrotic voxels (Vogel-Claussen *et al.*, 2007) or, alternatively, machine learning and automatic clustering approaches can be employed. Carano *et al.* (Carano *et al.*, 2004) used k-means clustering of a three-dimensional feature vector comprised of ADC, T_2 , and proton density for differentiation of the tumor tissue populations in colon xenograft tumors in mice. Three clusters were identified: viable tumor tissue with low ADC ($\text{ADC} < 0.50 \cdot 10^{-3} \text{ mm}^2/\text{s}$) and two necrotic clusters with higher ADCs ($\text{ADC}_1 = 1.26 \cdot 10^{-3} \text{ mm}^2/\text{s}$ and $\text{ADC}_2 = 1.61 \cdot 10^{-3} \text{ mm}^2/\text{s}$). Histological analysis showed that the regions of the necrotic cluster with lower ADC contained red blood cells and evidence of tissue repair response, but regions belonging to the high-ADC cluster were generally acellular. The volumes of the viable and necrotic tissues determined by clustering of ADC agreed well with the corresponding volumes determined from histology.

9.2.2 Response to Treatment

9.2.2.1 Chemotherapy and Radiation therapy: Cytotoxic therapy may affect the diffusivity of tissue water by altering cellular density, the relative volumes of the

intracellular and extracellular compartments due to edema, or by affecting the permeability or integrity of the cell membranes. By detecting these changes, DWI may help to determine the efficacy of treatment soon after the initiation of therapy, before changes in the tumor volume or histology become apparent. Numerous studies have shown that after cytotoxic treatment, tumor ADC undergoes time-dependent and spatially heterogeneous changes. A dose-dependent increase in ADC in response to chemotherapy was first reported by Zhao *et al.* (Zhao *et al.*, 1996). Transient dose-dependent increases of ADC after therapy were also observed in mouse gliomas after administration of 1,3-bis(2-chloroethyl)-1-nitrosourea (Chenevert *et al.*, 1997), prostate tumors treated with docetaxel (Jennings *et al.*, 2002), breast tumors treated with paclitaxel (Galons *et al.*, 1999) and RIF-1 tumors treated with cyclophosphamide and 5-fluorouracil (Babsky *et al.*, 2007; Babsky *et al.*, 2005), and in a mouse model of Ewing sarcoma treated with treosulfan (Reichardt *et al.*, 2009). Tumor ADC values were found to increase within the first 24 to 72 hours after treatment and plateau or decrease at later times. Voxel histograms indicated that the rise of the mean tumor ADC is due to the redistribution of voxels towards higher ADC values (Jennings *et al.*, 2002). The increase in ADC was associated with the subsequent decrease of the tumor volume and was thus interpreted as an indicator of response to therapy (Galons *et al.*, 1999; Jennings *et al.*, 2002). The changes in ADC after apoptosis-inducing therapy were attributed to the cell shrinkage and the decrease of cellular density followed by the development of necrosis (Moffat *et al.*, 2005).

Several studies have used ADC as a *predictive marker* of response to therapy. The relationship between the ADC values among the responders and non-responders to treatment depend on the tumor cell line and therapeutic agent. In studies of mammary and colon cancer models, tumors with low baseline ADC values were found to respond to chemotherapy (Lemaire *et al.*, 1999; Roth *et al.*, 2004), whereas in a rat model of hepatocellular carcinoma treated with an apoptotic agent, tumors that responded to therapy had 50% higher pre-treatment ADC values than the non-responding tumors (Babsky *et al.*, 2011). The non-monoexponential signal shape was observed in rat gliomas imaged with six b-values in the range of 120–4000 s/mm² before and after chemotherapy (Hoff *et al.*, 2010); however, the parameters of the biexponential (Le Bihan *et al.*, 1986) and stretched exponential models (Bennett *et al.*, 2004) did not provide advantages in describing the response to treatment over conventional ADC calculated from data at two b-values.

Post-treatment increases in ADC were also observed after radiation therapy. Larocque *et al.* studied changes in tumor ADC and T₂ values at 9.4 T in subcutaneous glioma xenografts in nude mice after single-dose and fractionated radiotherapy (Larocque *et al.*, 2009, 2010b; Larocque *et al.*, 2010a). Both T₂ and ADC increased within the first two to three days after a single dose of 800 cGy, but by day 14, T₂ decreased slightly below its baseline value, whereas ADC continued to increase and reached about 20% above its pre-treatment value. Smaller ADC and T₂ changes were observed after lower radiation doses. The relative ADC increase on day 3 was negatively correlated with the post-treatment tumor growth rate and was thus an indicator of the treatment effectiveness. Similar ADC increases were observed after fractionated therapy. The maximum ADC was reached at 7 days after treatment for all treatment schedules, although the ADC changes after fractionated therapy were not strongly

dependent on the dose per fraction, which suggests that the total dose may be the main factor determining the size of the response. The post-treatment ADC changes were diminished in hypoxic tumors, in which ischemia was induced by applying a ligature to the tumor-bearing limb. In ischemic tumors, the ADC increased after treatment by about 10% after 800 cGy and the tumor growth was less effectively inhibited than in non-ischemic tumors, in which the ADC increased by 22%. Thus the post-treatment changes of ADC may also serve as an indicator of the tumor radiosensitivity.

The efficacy of chemoradiotherapy was studied by Seierstad *et al.* (Seierstad *et al.*, 2007) in human colorectal HT29 tumors in mice treated with fractionated radiation ($2 \text{ Gy} \times 10$) combined with either capecitabine or oxaliplatin alone or both drugs. The largest ADC increases ($\sim 25\%$ of the baseline value) were found on day 11 after treatment with radiation and both drugs. Radiation alone or radiation combined with capecitabine also resulted in higher ADC (by 12–15%). For these treatment regimens, the ADC returned to its baseline value after about 30 days after treatment and then began to increase again. The relative increase in ADC at day 11 was strongly and positively correlated with tumor growth delay ($r = 0.92$) and a weaker relationship was observed for the ADC increases on day 4 ($r = 0.77$) (Fig. 16). These results suggest that measurements of ADC may be used for tailoring individual treatment schedules early after the initiation of chemoradiotherapy.

The spatial heterogeneity of the post-therapy tumor ADC changes has been explored by Kim *et al.* (Kim *et al.*, 2008) in a mouse breast cancer model treated by an apoptosis-inducing antibody TRA-8. On day 3 after treatment, tumor ADC increased in proportion to the dose. Importantly, the ADC of the treated tumors increased mainly due to the changes in the tumor periphery, as indicated by the 30% ADC growth in the outer 1-mm-wide tumor region on day 3 at the highest dose. The increase of ADC in the viable rim of the tumor was positively correlated with the apoptotic cell density and negatively correlated with the density of proliferating cells determined from histology. In contrast, the ADC of untreated tumors increased steadily by about 8% on day 6. This increase was predominantly due to the development of the central necrosis, while the ADC of the tumor rim remained essentially unchanged. Other studies have shown that ADC decreases in untreated control tumors, presumably due to the increasing cellularity (Reichardt *et al.*, 2009).

Clustering-based segmentation of MRI-derived parameters can provide insight into the changes induced by radiation therapy in different tumor subpopulations. Henning *et al.* (Henning *et al.*, 2007) applied k-means clustering to the ADC and T_2 data of RIF-1 mouse tumors treated with single-dose radiotherapy (1000 cGy). Four clusters were identified: two viable clusters (oxygenated and hypoxic) and two necrotic clusters (cellular and acellular). The viable clusters had lower ADCs than the necrotic clusters and also the acellular necrotic cluster had a considerably longer T_2 than all other clusters. Within two days after treatment, the mean tumor ADC increased and plateaued until day 8. The increase was mainly seen in the viable oxygenated tissue, while the ADC of the hypoxic and necrotic tissue changed little. Higher pre-treatment volume of viable oxygenated tissue was associated with lower tumor growth delay (TGD) and lower log cell kill. In contrast, larger decreases in the volume of this tissue were correlated with greater TGD and cell kill. These results agree

with the findings of Kim et al. (Kim *et al.*, 2008) and suggest that spatial heterogeneity of ADC is a useful factor in predicting and assessing the tumor response to treatment.

9.2.2.2 Antivascular and Antiangiogenic Therapy: DWI is increasingly used in combination with DCE MRI to characterize the effects of agents that target tumor vasculature. These drugs include vascular-disrupting agents (VDA), which cause the tumor vasculature to collapse, and antiangiogenic drugs, which prevent the formation of new tumor vessels. Administration of VDAs often leads to a rapid onset of necrosis, and therefore, the ADC is expected first to decrease within hours after VDA treatment due to cell swelling (associated with a decrease in extracellular space) and then increase within one to three days, during which the tumor tissue becomes necrotic. Unlike VDA, which induce vascular shutdown, antiangiogenic drugs inhibit the formation of new tumor blood vessels, and therefore are not expected to produce large and rapid changes in ADC, except when antiangiogenic therapy reduces vessel permeability and relieves edema.

Measurements of ADC in conjunction with DCE MRI were used to evaluate the effect of the vascular targeting agent combretastatin A4 phosphate. Although an initial study did not find significant changes in ADC at ~ 2 hours after treatment (Beauregard *et al.*, 1998), subsequently, Thoeny *et al.* (Thoeny *et al.*, 2005b) observed a rich pattern of intratumoral changes in rat rhabdomyosarcomas studied at multiple time points after administration of Combretastatin. The mean tumor ADC decreased slightly, but significantly, at 1 and 6 hours post-treatment (by 6% and 14%, respectively), then increased by 40% at two days post treatment, and started to decline at 9 days after treatment. Histology showed that the early decreases of ADC were due to the diminished extracellular space, seen initially at 1 hour post-treatment subsequent to vascular collapse and crowding of endothelial cells in the tumor periphery, and at 6 h due to pronounced edema in the tumor rim. On DCE MRI, the thickness of the enhancing tumor rim first decreased dramatically at 1 and 6 hours and recovered at later time points. Imaging and histology at 2 and 9 days post-treatment showed the expansion of the central tumor necrotic region and a regrowth of the viable, enhancing tumor rim. Importantly, this study also demonstrated that the ADCs calculated at low and high b-values followed different courses over time, showing different behaviors of the perfusion-related and true diffusion components of diffusion signal.

Wang *et al.* (Wang *et al.*, 2010) observed an initial decrease followed by an increase in ADC in rats with liver rhabdomyosarcomas treated by vascular-disrupting agent ZD6126 (AstraZeneca, Cheshire, UK). In agreement with Thoeny *et al.* (Thoeny *et al.*, 2005b), the largest changes were observed in the fast component of ADC derived from low b-values (0, 50, and 100 s/mm²), which first dropped by 50% at 1 h and recovered afterwards. These changes were accompanied by a drop of K^{trans} at 1 h and started recovering afterwards, and the measurements of SUV_{max} from ¹⁸F-FDG micro-PET decreased significantly at 24 h compared to baseline. Similar observations were reported by Vogel-Claussen *et al.* (Vogel-Claussen *et al.*, 2007) in a study of ZD6126 in treating human prostate tumors in mice. Treatment initially induced a decrease (at 24 h) followed by a two-fold increase (at 48 h) of the fraction of tumor area with $ADC > 0.9 \times 10^{-3} \text{ mm}^2/\text{s}$, which was considered to be necrotic. Treatment led to decreases in vascular volume and permeability surface area product measured using albumin-Gd-DTPA DCE MRI. The initial decrease of ADC was

attributed to edema and swelling of acutely ischemic tumor cells and influx of water into the intracellular space, similar to the diffusion restriction in acute brain ischemia (“tumor stroke”).

After necrosis-inducing therapy, ADC first shows a small drop within hours after treatment followed by a large increase within 1–3 days. These changes may be attributed to the initial cell swelling and subsequent disintegration of cells during the development of necrosis. With apoptosis-inducing treatment, the ADC increases due to cell shrinkage and collapse. The course of ADC beyond the first 1–3 days after treatment varies, but in most tumors ADC decreases towards its baseline value within 1–2 weeks after treatment. The post-treatment increases in ADC are mainly attributed to the changes occurring in the viable tissue in the periphery of the tumor, whereas hypoxic or necrotic tissues in the center of the tumor show smaller ADC changes, if any at all. The different tissue subpopulations can be segmented using a variety of clustering approaches. The relative increases of ADC are dose-dependent and positively correlated with the treatment efficacy, expressed as tumor growth delay. Tumors with larger pre-treatment viable volumes show smaller growth delays. Post-treatment ADC changes may also be smaller in hypoxic tumors. Thus, measurements of ADC can serve as predictive factors of response to treatment and measures of treatment effectiveness.

10 Summary

High-field tumor MRS and MRSI have been used for more than 30 years. These studies provide valuable information, both to determine what studies may be useful clinically, and also to model unexplained results of clinical studies. In preclinical studies, high-field usually refers to fields of 4.7–9.4 T although field strengths of 14.6 T and above have been used. In comparison, common clinical “high” field strengths are 1.5 T and 3 T, although some studies are done at 7 T and even 9.4 T. Many of the advantages of the preclinical studies relate to the presence of the tumor in a more advantageous location and the higher field strength which are major factors in improving signal to noise. The use of high-field magnets will continue to be a valuable addition to magnetic resonance studies. New developments such as better data acquisition methods, multimodality imaging, new methods for hyperpolarization, the use of clinically relevant models, portend exciting new findings in the coming years.

From a clinical perspective, it remains unclear how well the high field preclinical studies have translated to the clinic. There are many reasons for this, including whether the preclinical tumor models are adequate models for human disease. Preclinical tumor models usually grow much faster than patient tumors and are usually significantly larger, relative to the size/weight of the host. Tumor heterogeneity is likely to be more of an issue in patients since most of the preclinical tumors that have been studied to date are transplanted (as opposed to spontaneous tumors) and therefore have less intra and inter-tumor heterogeneity. Thus there is likely to be less heterogeneity in the preclinical studies, mitigating the effect of volume averaging, which would be more important in clinical studies. The development of newer genetically engineered tumor models is likely to make

the use of high field MR more relevant to clinical studies since the study of an accurate model that recapitulates the problems of clinical oncology is paramount.

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Table of Metabolite Abbreviations

Cho	free choline
tCho	total choline
PC	phosphocholine
GPC	glycerophosphocholine
Eth	ethanolamine
PE	phosphoethanolamine
GPE	glycerophosphoethanolamine
Cr	creatine
PCr	phosphocreatine
ATP	adenosine triphosphate
NAA	n-acetyl-aspartate
PtdCho	phosphatidylcholine
PtdEth	phosphatidylethanolamine
Lac	lactic acid (lactate)
Pi	inorganic phosphate
NDP	nucleoside diphosphate
NTP	nucleoside triphosphate
DPDE	diphosphodiester
NAD⁺	NADH, nicotinamide adenine dinucleotide
NADP⁺	NADPH, nicotinamide adenine dinucleotide phosphate
PME	phosphomonoesters
PDE	phosphodiester

References

- Aboagye EO, Bhujwala ZM. Malignant transformation alters membrane choline phospholipid metabolism of human mammary epithelial cells. *Cancer Res.* 1999; 59:80–4. [PubMed: 9892190]
- Ackerman JJ, Grove TH, Wong GG, Gadian DG, Radda GK. Mapping of metabolites in whole animals by 31P NMR using surface coils. *Nature.* 1980; 283:167–70. [PubMed: 7350541]

- Ackerstaff E, Pflug BR, Nelson JB, Bhujwala ZM. Detection of increased choline compounds with proton nuclear magnetic resonance spectroscopy subsequent to malignant transformation of human prostatic epithelial cells. *Cancer Res.* 2001; 61:3599–603. [PubMed: 11325827]
- Agris PF, Campbell ID. Proton nuclear magnetic resonance of intact Friend leukemia cells: phosphorylcholine increase during differentiation. *Science.* 1982; 216:1325–7. [PubMed: 7079765]
- Aime S, Delli Castelli D, Terreno E. Highly sensitive MRI chemical exchange saturation transfer agents using liposomes. *Angewandte Chemie.* 2005; 44:5513–5. [PubMed: 16052647]
- Al-Saffar NM, Troy H, Ramirez de Molina A, Jackson LE, Madhu B, Griffiths JR, Leach MO, Workman P, Lacal JC, Judson IR, Chung YL. Noninvasive magnetic resonance spectroscopic pharmacodynamic markers of the choline kinase inhibitor MN58b in human carcinoma models. *Cancer Res.* 2006; 66:427–34. [PubMed: 16397258]
- Albers MJ, Bok R, Chen AP, Cunningham CH, Zierhut ML, Zhang VY, Kohler SJ, Tropp J, Hurd RE, Yen YF, Nelson SJ, Vigneron DB, Kurhanewicz J. Hyperpolarized ^{13}C lactate, pyruvate, and alanine: noninvasive biomarkers for prostate cancer detection and grading. *Cancer Res.* 2008; 68:8607–15. [PubMed: 18922937]
- Ardenkjaer-Larsen JH, Fridlund B, Gram A, Hansson G, Hansson L, Lerche MH, Servin R, Thaning M, Golman K. Increase in signal-to-noise ratio of > 10,000 times in liquid-state NMR. *Proc Natl Acad Sci U S A.* 2003; 100:10158–63. [PubMed: 12930897]
- Arias-Mendoza F, Brown TR. In vivo measurement of phosphorous markers of disease. *Dis Markers.* 2003; 19:49–68. [PubMed: 15096705]
- Arias-Mendoza F, Payne GS, Zakian KL, Schwarz AJ, Stubbs M, Stoyanova R, Ballon D, Howe FA, Koutcher JA, Leach MO, Griffiths JR, Heerschap A, Glickson JD, Nelson SJ, Evelhoch JL, Charles HC, Brown TR. In vivo ^{31}P MR spectral patterns and reproducibility in cancer patients studied in a multi-institutional trial. *NMR in biomedicine.* 2006; 19:504–12. [PubMed: 16763965]
- Aronen HJ, Pardo FS, Kennedy DN, Belliveau JW, Packard SD, Hsu DW, Hochberg FH, Fischman AJ, Rosen BR. High microvascular blood volume is associated with high glucose uptake and tumor angiogenesis in human gliomas. *Clin Cancer Res.* 2000; 6:2189–200. [PubMed: 10873068]
- Atkin G, Taylor NJ, Daley FM, Stirling JJ, Richman P, Glynn-Jones R, d'Arcy JA, Collins DJ, Padhani AR. Dynamic contrast-enhanced magnetic resonance imaging is a poor measure of rectal cancer angiogenesis. *Br J Surg.* 2006; 93:992–1000. [PubMed: 16673354]
- Babsky AM, Hekmatyar SK, Zhang H, Solomon JL, Bansal N. Application of ^{23}Na MRI to monitor chemotherapeutic response in RIF-1 tumors. *Neoplasia.* 2005; 7:658–66. [PubMed: 16026645]
- Babsky AM, Ju S, George B, Bennett S, Huang M, Jayaram HN, McLennan G, Bansal N. Predicting response to benzamide riboside chemotherapy in hepatocellular carcinoma using apparent diffusion coefficient of water. *Anticancer Res.* 2011; 31:2045–51. [PubMed: 21737621]
- Babsky AM, Zhang H, Hekmatyar SK, Hutchins GD, Bansal N. Monitoring chemotherapeutic response in RIF-1 tumors by single-quantum and triple-quantum-filtered (^{23}Na) MRI, (^1H) diffusion-weighted MRI and PET imaging. *Magn Reson Imaging.* 2007; 25:1015–23. [PubMed: 17707164]
- Baudelet C, Gallez B. How does blood oxygen level-dependent (BOLD) contrast correlate with oxygen partial pressure (pO_2) inside tumors? *Magn Reson Med.* 2002; 48:980–6. [PubMed: 12465107]
- Bauerle T, Merz M, Komljenovic D, Zwick S, Semmler W. Drug-induced vessel remodeling in bone metastases as assessed by dynamic contrast enhanced magnetic resonance imaging and vessel size imaging: a longitudinal in vivo study. *Clin Cancer Res.* 2010; 16:3215–25. [PubMed: 20530698]
- Beaumont M, Lemasson B, Farion R, Segebarth C, Remy C, Barbier EL. Characterization of tumor angiogenesis in rat brain using iron-based vessel size index MRI in combination with gadolinium-based dynamic contrast-enhanced MRI. *J Cereb Blood Flow Metab.* 2009; 29:1714–26. [PubMed: 19584891]
- Beauregard DA, Thelwall PE, Chaplin DJ, Hill SA, Adams GE, Brindle KM. Magnetic resonance imaging and spectroscopy of combretastatin A4 prodrug-induced disruption of tumour perfusion and energetic status. *British journal of cancer.* 1998; 77:1761–7. [PubMed: 9667644]

- Beloueche-Babari M, Chung YL, Al-Saffar NM, Falck-Miniotis M, Leach MO. Metabolic assessment of the action of targeted cancer therapeutics using magnetic resonance spectroscopy. *British journal of cancer*. 2010; 102:1–7. [PubMed: 19935796]
- Bennett KM, Hyde JS, Rand SD, Bennett R, Krouwer HG, Rebro KJ, Schmainda KM. Intravoxel distribution of DWI decay rates reveals C6 glioma invasion in rat brain. *Magn Reson Med*. 2004; 52:994–1004. [PubMed: 15508160]
- Bezabeh T, Evelhoch JL, Thompson P, Sloop DJ, Ackerman JJ. Therapeutic efficacy as predicted by quantitative assessment of murine RIF-1 tumour pH and phosphorous metabolite response during hyperthermia: an *in vivo* ^{31}P NMR study. *Int J Hyperthermia*. 2004; 20:335–57. [PubMed: 15204517]
- Bhakoo KK, Williams SR, Florian CL, Land H, Noble MD. Immortalization and transformation are associated with specific alterations in choline metabolism. *Cancer Res*. 1996; 56:4630–5. [PubMed: 8840976]
- Bhattacharya P, Chekmenev EY, Perman WH, Harris KC, Lin AP, Norton VA, Tan CT, Ross BD, Weitekamp DP. Towards hyperpolarized (13)C-succinate imaging of brain cancer. *J Magn Reson*. 2007; 186:150–5. [PubMed: 17303454]
- Bhujwala ZM, Aboagye EO, Gillies RJ, Chacko VP, Mendola CE, Backer JM. Nm23-transfected MDA-MB-435 human breast carcinoma cells form tumors with altered phospholipid metabolism and pH: a ^{31}P nuclear magnetic resonance study in vivo and in vitro. *Magn Reson Med*. 1999; 41:897–903. [PubMed: 10332871]
- Bhujwala ZM, McCoy CL, Glickson JD, Gillies RJ, Stubbs M. Estimations of intra- and extracellular volume and pH by ^{31}P magnetic resonance spectroscopy: effect of therapy on RIF-1 tumours. *British journal of cancer*. 1998; 78:606–11. [PubMed: 9744499]
- Biaglow JE, Manevich Y, Leeper D, Chance B, Dewhirst MW, Jenkins WT, Tuttle SW, Wroblewski K, Glickson JD, Stevens C, Evans SM. MIBG inhibits respiration: potential for radio- and hyperthermic sensitization. *Int J Radiat Oncol Biol Phys*. 1998; 42:871–6. [PubMed: 9845113]
- Bosshard SC, Baltes C, Wyss MT, Mueggler T, Weber B, Rudin M. Assessment of brain responses to innocuous and noxious electrical forepaw stimulation in mice using BOLD fMRI. *Pain*. 2010; 151:655–63. [PubMed: 20851520]
- Bottomley, P. Selective volume method for performing localized NMR spectroscopy. USA patent. 4,480,228. 1984.
- Boxerman JL, Schmainda KM, Weisskoff RM. Relative cerebral blood volume maps corrected for contrast agent extravasation significantly correlate with glioma tumor grade, whereas uncorrected maps do not. *AJNR Am J Neuroradiol*. 2006; 27:859–67. [PubMed: 16611779]
- Bradley DP, Tessier JJ, Ashton SE, Waterton JC, Wilson Z, Worthington PL, Ryan AJ. Correlation of MRI biomarkers with tumor necrosis in Hras5 tumor xenograft in athymic rats. *Neoplasia*. 2007; 9:382–91. [PubMed: 17534443]
- Bradley DP, Tessier JJ, Lacey T, Scott M, Jurgensmeier JM, Odedra R, Mills J, Kilburn L, Wedge SR. Examining the acute effects of cediranib (RECENTIN, AZD2171) treatment in tumor models: a dynamic contrast-enhanced MRI study using gadopentate. *Magn Reson Imaging*. 2009; 27:377–84. [PubMed: 18814988]
- Bradley DP, Tessier JL, Checkley D, Kuribayashi H, Waterton JC, Kendrew J, Wedge SR. Effects of AZD2171 and vandetanib (ZD6474, Zactima) on haemodynamic variables in an SW620 human colon tumour model: an investigation using dynamic contrast-enhanced MRI and the rapid clearance blood pool contrast agent, P792 (gadolmitol). *NMR Biomed*. 2008; 21:42–52. [PubMed: 17458919]
- Brasch R, Pham C, Shames D, Roberts T, van Dijke K, van Bruggen N, Mann J, Ostrowitzki S, Melnyk O. Assessing tumor angiogenesis using macromolecular MR imaging contrast media. *Journal of magnetic resonance imaging : JMRI*. 1997; 7:68–74. [PubMed: 9039595]
- Brown TR, Kincaid BM, Ugurbil K. NMR chemical shift imaging in three dimensions. *Proc Natl Acad Sci U S A*. 1982; 79:3523–6. [PubMed: 6954498]
- Buckley DL, Drew PJ, Mussurakis S, Monson JR, Horsman A. Microvessel density of invasive breast cancer assessed by dynamic Gd-DTPA enhanced MRI. *Journal of magnetic resonance imaging : JMRI*. 1997; 7:461–4. [PubMed: 9170027]

- Bui T, Stevenson J, Hoekman J, Zhang S, Maravilla K, Ho RJ. Novel Gd nanoparticles enhance vascular contrast for high-resolution magnetic resonance imaging. *PLoS One*. 2010;5.
- Bulte JW. In vivo MRI cell tracking: clinical studies. *AJR Am J Roentgenol*. 2009; 193:314–25. [PubMed: 19620426]
- Bumb A, Brechbiel MW, Choyke P. Macromolecular and dendrimer-based magnetic resonance contrast agents. *Acta Radiol*. 2010; 51:751–67. [PubMed: 20590365]
- Candes EJ, Romberg JK, Tao T. Stable signal recovery from incomplete and inaccurate measurements. *Commun Pur Appl Math*. 2006; 59:1207–23.
- Carano RA, Ross AL, Ross J, Williams SP, Koeppen H, Schwall RH, Van Bruggen N. Quantification of tumor tissue populations by multispectral analysis. *Magn Reson Med*. 2004; 51:542–51. [PubMed: 15004796]
- Carmeliet P. Angiogenesis in life, disease and medicine. *Nature*. 2005; 438:932–6. [PubMed: 16355210]
- Carpenter CM, Rakow-Penner R, Jiang S, Daniel BL, Pogue BW, Glover GH, Paulsen KD. Inspired gas-induced vascular change in tumors with magnetic-resonance-guided near-infrared imaging: human breast pilot study. *Journal of biomedical optics*. 2010; 15:036026. [PubMed: 20615028]
- Cha S, Johnson G, Wadghiri YZ, Jin O, Babb J, Zagzag D, Turnbull DH. Dynamic, contrast-enhanced perfusion MRI in mouse gliomas: correlation with histopathology. *Magn Reson Med*. 2003; 49:848–55. [PubMed: 12704767]
- Chase JR, Rothman DL, Shulman RG. Flux control in the rat gastrocnemius glycogen synthesis pathway by in vivo ¹³C/³¹P NMR spectroscopy. *Am J Physiol Endocrinol Metab*. 2001; 280:E598–607. [PubMed: 11254467]
- Checkley D, Tessier JJ, Kendrew J, Waterton JC, Wedge SR. Use of dynamic contrast-enhanced MRI to evaluate acute treatment with ZD6474, a VEGF signalling inhibitor, in PC-3 prostate tumours. *British journal of cancer*. 2003a; 89:1889–95. [PubMed: 14612898]
- Checkley D, Tessier JJ, Wedge SR, Dukes M, Kendrew J, Curry B, Middleton B, Waterton JC. Dynamic contrast-enhanced MRI of vascular changes induced by the VEGF-signalling inhibitor ZD4190 in human tumour xenografts. *Magn Reson Imaging*. 2003b; 21:475–82. [PubMed: 12878256]
- Chen AP, Albers MJ, Cunningham CH, Kohler SJ, Yen YF, Hurd RE, Tropp J, Bok R, Pauly JM, Nelson SJ, Kurhanewicz J, Vigneron DB. Hyperpolarized C-13 spectroscopic imaging of the TRAMP mouse at 3T-initial experience. *Magn Reson Med*. 2007; 58:1099–106. [PubMed: 17969006]
- Chen F, De Keyzer F, Ni Y. Cancer models-multiparametric applications of clinical MRI in rodent hepatic tumor model. *Methods Mol Biol*. 2011; 771:489–507. [PubMed: 21874495]
- Chen F, Sun X, De Keyzer F, Yu J, Peeters R, Coudyzer W, Vandecaveye V, Landuyt W, Bosmans H, Van Hecke P, Marchal G, Ni Y. Liver tumor model with implanted rhabdomyosarcoma in rats: MR imaging, microangiography, and histopathologic analysis. *Radiology*. 2006; 239:554–62. [PubMed: 16543589]
- Chenevert TL, McKeever PE, Ross BD. Monitoring early response of experimental brain tumors to therapy using diffusion magnetic resonance imaging. *Clin Cancer Res*. 1997; 3:1457–66. [PubMed: 9815831]
- Chenevert TL, Stegman LD, Taylor JM, Robertson PL, Greenberg HS, Rehemtulla A, Ross BD. Diffusion magnetic resonance imaging: an early surrogate marker of therapeutic efficacy in brain tumors. *J Natl Cancer Inst*. 2000; 92:2029–36. [PubMed: 11121466]
- Chiche J, Le Fur Y, Vilmen C, Frassinetti F, Daniel L, Halestrap AP, Cozzzone PJ, Pouyssegur J, Lutz NW. In vivo pH in metabolic-defective Ras-transformed fibroblast tumors: key role of the monocarboxylate transporter, MCT4, for inducing an alkaline intracellular pH. *Int J Cancer*. 2012; 130:1511–20. [PubMed: 21484790]
- Cho H, Ackerstaff E, Carlin S, Lupu ME, Wang Y, Rizwan A, O'Donoghue J, Ling CC, Humm JL, Zanzonico PB, Koutcher JA. Noninvasive multimodality imaging of the tumor microenvironment: registered dynamic magnetic resonance imaging and positron emission tomography studies of a preclinical tumor model of tumor hypoxia. *Neoplasia*. 2009; 11:247–59. 2p following 59. [PubMed: 19242606]

- Chopra S, Foltz WD, Milosevic MF, Toi A, Bristow RG, Menard C, Haider MA. Comparing oxygen-sensitive MRI (BOLD R2*) with oxygen electrode measurements: a pilot study in men with prostate cancer. *Int J Radiat Biol.* 2009; 85:805–13. [PubMed: 19728195]
- Christen T, Lemasson B, Pannetier N, Farion R, Remy C, Zaharchuk G, Barbier EL. Is T2* enough to assess oxygenation? Quantitative blood oxygen level-dependent analysis in brain tumor. *Radiology.* 2012; 262:495–502. [PubMed: 22156990]
- Chung YL, Troy H, Banerji U, Jackson LE, Walton MI, Stubbs M, Griffiths JR, Judson IR, Leach MO, Workman P, Ronen SM. Magnetic resonance spectroscopic pharmacodynamic markers of the heat shock protein 90 inhibitor 17-allylamino,17-demethoxygeldanamycin (17AAG) in human colon cancer models. *J Natl Cancer Inst.* 2003; 95:1624–33. [PubMed: 14600095]
- Chung YL, Troy H, Kristeleit R, Aherne W, Jackson LE, Atadja P, Griffiths JR, Judson IR, Workman P, Leach MO, Belouche-Babari M. Noninvasive magnetic resonance spectroscopic pharmacodynamic markers of a novel histone deacetylase inhibitor, LAQ824, in human colon carcinoma cells and xenografts. *Neoplasia.* 2008; 10:303–13. [PubMed: 18392140]
- Cudalbu C, Comment A, Kurdzesau F, van Heeswijk RB, Uffmann K, Jannin S, Denisov V, Kirik D, Gruetter R. Feasibility of in vivo 15N MRS detection of hyperpolarized 15N labeled choline in rats. *Phys Chem Chem Phys.* 2010; 12:5818–23. [PubMed: 20461252]
- Dafni H, Larson PE, Hu S, Yoshihara HA, Ward CS, Venkatesh HS, Wang C, Zhang X, Vigneron DB, Ronen SM. Hyperpolarized 13C spectroscopic imaging informs on hypoxia-inducible factor-1 and myc activity downstream of platelet-derived growth factor receptor. *Cancer Res.* 2010; 70:7400–10. [PubMed: 20858719]
- Daldrup-Link HE, Golovko D, Ruffell B, Denardo DG, Castaneda R, Ansari C, Rao J, Tikhomirov GA, Wendland MF, Corot C, Coussens LM. MRI of tumor-associated macrophages with clinically applicable iron oxide nanoparticles. *Clin Cancer Res.* 2011; 17:5695–704. [PubMed: 21791632]
- Daldrup-Link HE, Rudelius M, Piontek G, Metz S, Brauer R, Debus G, Corot C, Schlegel J, Link TM, Peschel C, Rummeny EJ, Oostendorp RA. Migration of iron oxide-labeled human hematopoietic progenitor cells in a mouse model: in vivo monitoring with 1.5-T MR imaging equipment. *Radiology.* 2005; 234:197–205. [PubMed: 15618382]
- Daldrup-Link HE, Shames DM, Wendland M, Muhler A, Gossmann A, Rosenau W, Brasch RC. Comparison of Gadomer-17 and gadopentetate dimeglumine for differentiation of benign from malignant breast tumors with MR imaging. *Acad Radiol.* 2000; 7:934–44. [PubMed: 11089696]
- Daldrup H, Shames DM, Wendland M, Okuhata Y, Link TM, Rosenau W, Lu Y, Brasch RC. Correlation of dynamic contrast-enhanced MR imaging with histologic tumor grade: comparison of macromolecular and small-molecular contrast media. *AJR Am J Roentgenol.* 1998; 171:941–9. [PubMed: 9762973]
- Damadian R, Goldsmith M, Minkoff L. NMR in cancer: XVI. FONAR image of the live human body. *Physiological chemistry and physics.* 1977; 9:97–100. 8. [PubMed: 909957]
- Damadian R, Minkoff L, Goldsmith M, Koutcher J. Field focusing NMR(FONAR): Formation of chemical scans in man. *Naturwissenschaften.* 1978; 65:250–2. [PubMed: 662021]
- Damadian R, Minkoff L, Goldsmith M, Stanford M, Koutcher J. Field focusing nuclear magnetic resonance (FONAR): visualization of a tumor in a live animal. *Science.* 1976a; 194:1430–2. [PubMed: 1006309]
- Damadian R, Minkoff L, Goldsmith M, Stanford M, Koutcher J. Tumor imaging in a live animal by focusing NMR (FONAR). *Physiol Chem Phys.* 1976b; 8:61–5. [PubMed: 959335]
- Darpolor MM, Kennealey PT, Le HC, Zakian KL, Ackerstaff E, Rizwan A, Chen JH, Sambol EB, Schwartz GK, Singer S, Koutcher JA. Preclinical study of treatment response in HCT-116 cells and xenografts with (1) H-decoupled (31) P MRS. *NMR Biomed.* 2011a; 24:1159–68. [PubMed: 21994185]
- Darpolor MM, Molthen RC, Schmainda KM. Multimodality imaging of abnormal vascular perfusion and morphology in preclinical 9L gliosarcoma model. *PLoS One.* 2011b; 6:e16621. [PubMed: 21305001]
- Day SE, Kettunen MI, Cherukuri MK, Mitchell JB, Lizak MJ, Morris HD, Matsumoto S, Koretsky AP, Brindle KM. Detecting response of rat C6 glioma tumors to radiotherapy using hyperpolarized

- [1-13C]pyruvate and 13C magnetic resonance spectroscopic imaging. *Magn Reson Med*. 2011; 65:557–63. [PubMed: 21264939]
- Day SE, Kettunen MI, Gallagher FA, Hu DE, Lerche M, Wolber J, Golman K, Ardenkjaer-Larsen JH, Brindle KM. Detecting tumor response to treatment using hyperpolarized 13C magnetic resonance imaging and spectroscopy. *Nat Med*. 2007; 13:1382–7. [PubMed: 17965722]
- de Certaines JD, Larsen VA, Podo F, Carpinelli G, Briot O, Henriksen O. In vivo 31P MRS of experimental tumours. *NMR Biomed*. 1993; 6:345–65. [PubMed: 8148230]
- Degani H, Furman E, Fields S. Magnetic resonance imaging and spectroscopy of MCF7 human breast cancer: pathophysiology and monitoring of treatment. *Clin Chim Acta*. 1994; 228:19–33. [PubMed: 7955426]
- Dennie J, Mandeville JB, Boxerman JL, Packard SD, Rosen BR, Weisskoff RM. NMR imaging of changes in vascular morphology due to tumor angiogenesis. *Magn Reson Med*. 1998; 40:793–9. [PubMed: 9840821]
- Diergarten T, Martirosian P, Kottke R, Vogel U, Stenzl A, Claussen CD, Schlemmer HP. Functional characterization of prostate cancer by integrated magnetic resonance imaging and oxygenation changes during carbogen breathing. *Investigative radiology*. 2005; 40:102–9. [PubMed: 15654255]
- Doblas S, He T, Saunders D, Hoyle J, Smith N, Pye Q, Lerner M, Jensen RL, Towner RA. In vivo characterization of several rodent glioma models by 1H MRS. *NMR in biomedicine*. 2012; 25:685–94. [PubMed: 21954105]
- Douma K, Oostendorp M, Slaaf DW, Post MJ, Backes WH, van Zandvoort MA. Evaluation of magnetic resonance vessel size imaging by two-photon laser scanning microscopy. *Magn Reson Med*. 2010; 63:930–9. [PubMed: 20373394]
- Dyke JP, Zakian KL, Spees WM, Matei C, Chen Y, Mao X, Shungu DC, Koutcher JA. Metabolic response of the CWR22 prostate tumor xenograft after 20 Gy of radiation studied by 1H spectroscopic imaging. *Clin Cancer Res*. 2003; 9:4529–36. [PubMed: 14555527]
- Edden RA, Schar M, Hillis AE, Barker PB. Optimized detection of lactate at high fields using inner volume saturation. *Magn Reson Med*. 2006; 56:912–7. [PubMed: 16964614]
- Egeland TA, Gulliksrud K, Gaustad JV, Mathiesen B, Rofstad EK. Dynamic contrast-enhanced-MRI of tumor hypoxia. *Magn Reson Med*. 2012; 67:519–30. [PubMed: 21661044]
- Elas M, Williams BB, Parasca A, Mailer C, Pelizzari CA, Lewis MA, River JN, Karczmar GS, Barth ED, Halpern HJ. Quantitative tumor oxymetric images from 4D electron paramagnetic resonance imaging (EPRI): methodology and comparison with blood oxygen level-dependent (BOLD) MRI. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine/Society of Magnetic Resonance in Medicine*. 2003; 49:682–91.
- Evanochko WT, Ng TC, Glickson JD, Durant JR, Corbett TH. Human tumors as examined by in vivo 31P NMR in athymic mice. *Biochemical and biophysical research communications*. 1982; 109:1346–52. [PubMed: 7168767]
- Evanochko WT, Ng TC, Lilly MB, Lawson AJ, Corbett TH, Durant JR, Glickson JD. In vivo 31P NMR study of the metabolism of murine mammary 16/C adenocarcinoma and its response to chemotherapy, x-radiation, and hyperthermia. *Proc Natl Acad Sci U S A*. 1983; 80:334–8. [PubMed: 6572896]
- Evanochko WT, Sakai TT, Ng TC, Krishna NR, Kim HD, Zeidler RB, Ghanta VK, Brockman RW, Schiffer LM, Braunschweiger PG, et al. NMR study of in vivo RIF-1 tumors. Analysis of perchloric acid extracts and identification of 1H, 31P and 13C resonances. *Biochim Biophys Acta*. 1984; 805:104–16. [PubMed: 6477969]
- Evelhoch JL. Key factors in the acquisition of contrast kinetic data for oncology. *Journal of magnetic resonance imaging : JMRI*. 1999; 10:254–9. [PubMed: 10508284]
- Ewing JR, Brown SL, Lu M, Panda S, Ding G, Knight RA, Cao Y, Jiang Q, Nagaraja TN, Churchman JL, Fenstermacher JD. Model selection in magnetic resonance imaging measurements of vascular permeability: Gadomer in a 9L model of rat cerebral tumor. *J Cereb Blood Flow Metab*. 2006; 26:310–20. [PubMed: 16079791]
- Fan X, Haney CR, Mustafi D, Yang C, Zamora M, Markiewicz EJ, Karczmar GS. Use of a reference tissue and blood vessel to measure the arterial input function in DCEMRI. *Magn Reson Med*. 2010; 64:1821–6. [PubMed: 20665893]

- Fan X, River JN, Zamora M, Al-Hallaq HA, Karczmar GS. Effect of carbogen on tumor oxygenation: combined fluorine-19 and proton MRI measurements. *International journal of radiation oncology, biology, physics*. 2002; 54:1202–9.
- Fantin VR, St-Pierre J, Leder P. Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance. *Cancer Cell*. 2006; 9:425–34. [PubMed: 16766262]
- Faranesh AZ, Yankeelov TE. Incorporating a vascular term into a reference region model for the analysis of DCE-MRI data: a simulation study. *Phys Med Biol*. 2008; 53:2617–31. [PubMed: 18441417]
- Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med*. 1971; 285:1182–6. [PubMed: 4938153]
- Frahm J, Bruhn H, Gyngell ML, Merboldt KD, Hanicke W, Sauter R. Localized high-resolution proton NMR spectroscopy using stimulated echoes: initial applications to human brain in vivo. *Magn Reson Med*. 1989; 9:79–93. [PubMed: 2540396]
- Friedman JJ, McMahon MT, Stivers JT, Van Zijl PC. Indirect detection of labile solute proton spectra via the water signal using frequency-labeled exchange (FLEX) transfer. *Journal of the American Chemical Society*. 2010; 132:1813–5. [PubMed: 20095603]
- Gabellieri C, Reynolds S, Lavie A, Payne GS, Leach MO, Eykyn TR. Therapeutic target metabolism observed using hyperpolarized ¹⁵N choline. *J Am Chem Soc*. 2008; 130:4598–9. [PubMed: 18345678]
- Gade TP, Buchanan IM, Motley MW, Mazaheri Y, Spees WM, Koutcher JA. Imaging intratumoral convection: pressure-dependent enhancement in chemotherapeutic delivery to solid tumors. *Clin Cancer Res*. 2009; 15:247–55. [PubMed: 19118052]
- Gadian, DG. Nuclear magnetic resonance and its applications to living systems. Oxford, New York: Clarendon Press; Oxford University Press; 1982.
- Gallagher FA, Kettunen MI, Brindle KM. Imaging pH with hyperpolarized ¹³C. *NMR in biomedicine*. 2011; 24:1006–15. [PubMed: 21812047]
- Gallagher FA, Kettunen MI, Day SE, Lerche M, Brindle KM. ¹³C MR spectroscopy measurements of glutaminase activity in human hepatocellular carcinoma cells using hyperpolarized ¹³C-labeled glutamine. *Magn Reson Med*. 2008; 60:253–7. [PubMed: 18666104]
- Gallagher FA, Kettunen MI, Hu DE, Jensen PR, Zandt RI, Karlsson M, Gisselsson A, Nelson SK, Witney TH, Bohndiek SE, Hansson G, Peitersen T, Lerche MH, Brindle KM. Production of hyperpolarized [1,4-¹³C₂]malate from [1,4-¹³C₂]fumarate is a marker of cell necrosis and treatment response in tumors. *Proc Natl Acad Sci U S A*. 2009; 106:19801–6. [PubMed: 19903889]
- Galons JP, Altbach MI, Paine-Murrieta GD, Taylor CW, Gillies RJ. Early increases in breast tumor xenograft water mobility in response to paclitaxel therapy detected by non-invasive diffusion magnetic resonance imaging. *Neoplasia*. 1999; 1:113–7. [PubMed: 10933044]
- Gambarota G, Leenders W, Maass C, Wesseling P, van der Kogel B, van Tellingen O, Heerschap A. Characterisation of tumour vasculature in mouse brain by USPIO contrast-enhanced MRI. *Br J Cancer*. 2008; 98:1784–9. [PubMed: 18506183]
- Garcia-Martin ML, Herigault G, Remy C, Farion R, Ballesteros P, Coles JA, Cerdan S, Ziegler A. Mapping extracellular pH in rat brain gliomas in vivo by ¹H magnetic resonance spectroscopic imaging: comparison with maps of metabolites. *Cancer Res*. 2001; 61:6524–31. [PubMed: 11522650]
- Garcia-Martin ML, Martinez GV, Raghunand N, Sherry AD, Zhang S, Gillies RJ. High resolution pH(e) imaging of rat glioma using pH-dependent relaxivity. *Magn Reson Med*. 2006; 55:309–15. [PubMed: 16402385]
- Gibbs P, Liney GP, Pickles MD, Zelhof B, Rodrigues G, Turnbull LW. Correlation of ADC and T2 measurements with cell density in prostate cancer at 3.0 Tesla. *Investigative radiology*. 2009; 44:572–6. [PubMed: 19692841]
- Gilad AA, McMahon MT, Walczak P, Winnard PT Jr, Raman V, van Laarhoven HW, Skoglund CM, Bulte JW, van Zijl PC. Artificial reporter gene providing MRI contrast based on proton exchange. *Nature biotechnology*. 2007; 25:217–9.

- Gillies RJ, Liu Z, Bhujwalla Z. ^{31}P -MRS measurements of extracellular pH of tumors using 3-aminopropylphosphonate. *Am J Physiol.* 1994; 267:C195–203. [PubMed: 8048479]
- Gillies RJ, Raghunand N, Garcia-Martin ML, Gatenby RA. pH imaging. A review of pH measurement methods and applications in cancers. *IEEE engineering in medicine and biology magazine : the quarterly magazine of the Engineering in Medicine & Biology Society.* 2004; 23:57–64.
- Ginefri JC, Poirier-Quinot M, Girard O, Darrasse L. Technical aspects: Development, manufacture and installation of a cryo-cooled HTS coil system for high-resolution in-vivo imaging of the mouse at 1.5 T. *Methods.* 2007; 43:54–67. [PubMed: 17720564]
- Giraudeau P, Shrot Y, Frydman L. Multiple ultrafast, broadband 2D NMR spectra of hyperpolarized natural products. *J Am Chem Soc.* 2009; 131:13902–3. [PubMed: 19743849]
- Glunde K, Ackerstaff E, Mori N, Jacobs MA, Bhujwalla ZM. Choline phospholipid metabolism in cancer: consequences for molecular pharmaceutical interventions. *Molecular pharmaceuticals.* 2006; 3:496–506. [PubMed: 17009848]
- Goldman M, Johannesson H, Axelsson O, Karlsson M. Hyperpolarization of ^{13}C through order transfer from parahydrogen: a new contrast agent for MRI. *Magn Reson Imaging.* 2005; 23:153–7. [PubMed: 15833606]
- Golman K, Ardenkjaer-Larsen JH, Petersson JS, Mansson S, Leunbach I. Molecular imaging with endogenous substances. *Proc Natl Acad Sci U S A.* 2003a; 100:10435–9. [PubMed: 12930896]
- Golman K, Axelsson O, Johannesson H, Mansson S, Olofsson C, Petersson JS. Parahydrogen-induced polarization in imaging: subsecond (^{13}C) angiography. *Magn Reson Med.* 2001; 46:1–5. [PubMed: 11443703]
- Golman K, Olsson LE, Axelsson O, Mansson S, Karlsson M, Petersson JS. Molecular imaging using hyperpolarized ^{13}C . *Br J Radiol.* 2003b; 76(Spec No 2):S118–27. [PubMed: 15572334]
- Golman K, Petersson JS, Magnusson P, Johansson E, Akeson P, Chai CM, Hansson G, Mansson S. Cardiac metabolism measured noninvasively by hyperpolarized ^{13}C MRI. *Magn Reson Med.* 2008; 59:1005–13. [PubMed: 18429038]
- Golman K, Zandt RI, Lerche M, Pehrson R, Ardenkjaer-Larsen JH. Metabolic imaging by hyperpolarized ^{13}C magnetic resonance imaging for in vivo tumor diagnosis. *Cancer Res.* 2006; 66:10855–60. [PubMed: 17108122]
- Gonen O, Hu J, Murphy-Boesch J, Stoyanova R, Brown TR. Dual interleaved ^1H and proton-decoupled- ^{31}P *in vivo* chemical shift imaging of human brain. *Magn Reson Med.* 1994; 32:104–9. [PubMed: 8084223]
- Gonen O, Mohebbi A, Stoyanova R, Brown TR. In vivo phosphorus polarization transfer and decoupling from protons in three-dimensional localized nuclear magnetic resonance spectroscopy of human brain. *Magn Reson Med.* 1997; 37:301–6. [PubMed: 9001156]
- Gossmann A, Okuhata Y, Shames DM, Helbich TH, Roberts TP, Wendland MF, Huber S, Brasch RC. Prostate cancer tumor grade differentiation with dynamic contrast-enhanced MR imaging in the rat: comparison of macromolecular and small-molecular contrast media--preliminary experience. *Radiology.* 1999; 213:265–72. [PubMed: 10540670]
- Graham RA, Taylor AH, Brown TR. A method for calculating the distribution of pH in tissues and a new source of pH error from the ^{31}P -NMR spectrum. *Am J Physiol.* 1994; 266:R638–45. [PubMed: 8141425]
- Griffiths JR, Stevens AN, Iles RA, Gordon RE, Shaw D. ^{31}P -NMR investigation of solid tumours in the living rat. *Bioscience reports.* 1981; 1:319–25. [PubMed: 7295895]
- Griffiths JR, Taylor NJ, Howe FA, Saunders MI, Robinson SP, Hoskin PJ, Powell ME, Thoumine M, Caine LA, Baddeley H. The response of human tumors to carbogen breathing, monitored by Gradient-Recalled Echo Magnetic Resonance Imaging. *International journal of radiation oncology, biology, physics.* 1997; 39:697–701.
- Griffitts J, Tesiram Y, Reid GE, Saunders D, Floyd RA, Towner RA. In vivo MRS assessment of altered fatty acyl unsaturation in liver tumor formation of a TGF α /c-myc transgenic mouse model. *Journal of lipid research.* 2009; 50:611–22. [PubMed: 19065002]
- Gupta RK, Sinha U, Cloughesy TF, Alger JR. Inverse correlation between choline magnetic resonance spectroscopy signal intensity and the apparent diffusion coefficient in human glioma. *Magn Reson Med.* 1999; 41:2–7. [PubMed: 10025604]

- Guthridge CJ, Stampfer MR, Clark MA, Steiner MR. Phospholipases A2 in ras-transformed and immortalized human mammary epithelial cells. *Cancer letters*. 1994; 86:11–21. [PubMed: 7954346]
- Haase A, Frahm J, Hanicke W, Matthaei D. 1H NMR chemical shift selective (CHESS) imaging. *Phys Med Biol*. 1985; 30:341–4. [PubMed: 4001160]
- Haberkorn U, Krems B, Gerlach L, Bachert P, Morri I, Wiessler M, van Kaick G. Assessment of glucosylifosfamide mustard biodistribution in rats with prostate adenocarcinomas by means of *in vivo* ^{31}P NMR and *in vitro* uptake experiments. *Magn Reson Med*. 1998; 39:754–61. [PubMed: 9581607]
- Hallac RR, Ding Y, Yuan Q, McColl RW, Lea J, Sims RD, Weatherall PT, Mason RP. Oxygenation in cervical cancer and normal uterine cervix assessed using blood oxygenation level-dependent (BOLD) MRI at 3T. *NMR in biomedicine*. 2012; 25:1321–30. [PubMed: 22619091]
- Hamilton BE, Nesbit GM, Dosa E, Gahramanov S, Rooney B, Nesbit EG, Raines J, Neuwelt EA. Comparative analysis of ferumoxytol and gadoteridol enhancement using T1- and T2-weighted MRI in neuroimaging. *AJR Am J Roentgenol*. 2011; 197:981–8. [PubMed: 21940589]
- Harris T, Eliyahu G, Frydman L, Degani H. Kinetics of hyperpolarized ^{13}C 1-pyruvate transport and metabolism in living human breast cancer cells. *Proc Natl Acad Sci U S A*. 2009; 106:18131–6. [PubMed: 19826085]
- He Q, Shungu DC, van Zijl PC, Bhujwalla ZM, Glickson JD. Single-scan *in vivo* lactate editing with complete lipid and water suppression by selective multiple-quantum-coherence transfer (Sel-MQC) with application to tumors. *J Magn Reson B*. 1995; 106:203–11. [PubMed: 7719620]
- He X, Yablonskiy DA. Quantitative BOLD: mapping of human cerebral deoxygenated blood volume and oxygen extraction fraction: default state. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine/Society of Magnetic Resonance in Medicine*. 2007; 57:115–26.
- Heilmann M, Kiessling F, Enderlin M, Schad LR. Determination of pharmacokinetic parameters in DCE MRI: Consequence of nonlinearity between contrast agent concentration and signal intensity. *Investigative radiology*. 2006; 41:536–43. [PubMed: 16763473]
- Henning EC, Azuma C, Sotak CH, Helmer KG. Multispectral tissue characterization in a RIF-1 tumor model: monitoring the ADC and T2 responses to single-dose radiotherapy. Part II. *Magn Reson Med*. 2007; 57:513–9. [PubMed: 17326182]
- Herborn CU, Lauenstein TC, Vogt FM, Lauffer RB, Debatin JF, Ruehm SG. Interstitial MR lymphography with MS-325: characterization of normal and tumor-invaded lymph nodes in a rabbit model. *AJR Am J Roentgenol*. 2002; 179:1567–72. [PubMed: 12438057]
- Hetherington HP, Avison MJ, Shulman RG. 1H homonuclear editing of rat brain using semiselective pulses. *Proc Natl Acad Sci U S A*. 1985; 82:3115–8. [PubMed: 2987910]
- Hillman GG, Singh-Gupta V, Al-Bashir AK, Zhang H, Yunker CK, Patel AD, Sethi S, Abrams J, Haacke EM. Dynamic contrast-enhanced magnetic resonance imaging of sunitinib-induced vascular changes to schedule chemotherapy in renal cell carcinoma xenograft tumors. *Translational oncology*. 2010; 3:293–306.
- Hillman GG, Singh-Gupta V, Zhang H, Al-Bashir AK, Katkuri Y, Li M, Yunker CK, Patel AD, Abrams J, Haacke EM. Dynamic contrast-enhanced magnetic resonance imaging of vascular changes induced by sunitinib in papillary renal cell carcinoma xenograft tumors. *Neoplasia*. 2009; 11:910–20. [PubMed: 19724685]
- Hlatky L, Hahnfeldt P, Folkman J. Clinical application of antiangiogenic therapy: microvessel density, what it does and doesn't tell us. *J Natl Cancer Inst*. 2002; 94:883–93. [PubMed: 12072542]
- Hockel M, Vaupel P. Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. *Journal of the National Cancer Institute*. 2001; 93:266–76. [PubMed: 11181773]
- Hoff BA, Chenevert TL, Bhojani MS, Kwee TC, Rehemtulla A, Le Bihan D, Ross BD, Galban CJ. Assessment of multiexponential diffusion features as MRI cancer therapy response metrics. *Magn Reson Med*. 2010; 64:1499–509. [PubMed: 20860004]
- Hoskin PJ, Carnell DM, Taylor NJ, Smith RE, Stirling JJ, Daley FM, Saunders MI, Bentzen SM, Collins DJ, d'Arcy JA, Padhani AP. Hypoxia in prostate cancer: correlation of BOLD-MRI with

pimonidazole immunohistochemistry-initial observations. *Int J Radiat Oncol Biol Phys.* 2007; 68:1065–71. [PubMed: 17637389]

Hou H, Khan N, O'Hara JA, Grinberg OY, Dunn JF, Abajian MA, Wilmot CM, Makki M, Demidenko E, Lu S, Steffen RP, Swartz HM. Effect of RSR13, an allosteric hemoglobin modifier, on oxygenation in murine tumors: an in vivo electron paramagnetic resonance oximetry and bold MRI study. *International journal of radiation oncology, biology, physics.* 2004; 59:834–43.

Hoult DI, Busby SJ, Gadian DG, Radda GK, Richards RE, Seeley PJ. Observation of tissue metabolites using ^{31}P nuclear magnetic resonance. *Nature.* 1974; 252:285–7. [PubMed: 4431445]

Hovener JB, Chekmenev EY, Harris KC, Perman WH, Robertson LW, Ross BD, Bhattacharya P. PASADENA hyperpolarization of ^{13}C biomolecules: equipment design and installation. *MAGMA.* 2009; 22:111–21. [PubMed: 19067008]

Howe FA, McPhail LD, Griffiths JR, McIntyre DJ, Robinson SP. Vessel size index magnetic resonance imaging to monitor the effect of antivasular treatment in a rodent tumor model. *Int J Radiat Oncol Biol Phys.* 2008; 71:1470–6. [PubMed: 18538948]

Howe FA, Stubbs M, Rodrigues LM, Griffiths JR. An assessment of artefacts in localized and non-localized ^{31}P MRS studies of phosphate metabolites and pH in rat tumours. *NMR Biomed.* 1993; 6:43–52. [PubMed: 8457426]

Hu BB, Varma G, Randell C, Keevil SF, Schaeffter T, Glover P. A Novel Receive-Only Liquid Nitrogen ($\text{LN}(2)$)-Cooled RF Coil for High-Resolution In Vivo Imaging on a 3-Tesla Whole-Body Scanner. *Ieee T Instrum Meas.* 2012; 61:129–39.

Huang MQ, Nelson DS, Pickup S, Qiao H, Delikatny EJ, Poptani H, Glickson JD. *In vivo* monitoring response to chemotherapy of human diffuse large B-cell lymphoma xenografts in SCID mice by ^1H and ^{31}P MRS. *Acad Radiol.* 2007; 14:1531–9. [PubMed: 18035282]

Hugg JW, Maudsley AA, Weiner MW, Matson GB. Comparison of k-space sampling schemes for multidimensional MR spectroscopic imaging. *Magn Reson Med.* 1996; 36:469–73. [PubMed: 8875420]

Hurd RE, Yen YF, Mayer D, Chen A, Wilson D, Kohler S, Bok R, Vigneron D, Kurhanewicz J, Tropp J, Spielman D, Pfefferbaum A. Metabolic imaging in the anesthetized rat brain using hyperpolarized $[1-^{13}\text{C}]$ pyruvate and $[1-^{13}\text{C}]$ ethyl pyruvate. *Magn Reson Med.* 2010; 63:1137–43. [PubMed: 20432284]

Iorio E, Mezzanzanica D, Alberti P, Spadaro F, Ramoni C, D'Ascenzo S, Millimaggi D, Pavan A, Dolo V, Canevari S, Podo F. Alterations of choline phospholipid metabolism in ovarian tumor progression. *Cancer Res.* 2005; 65:9369–76. [PubMed: 16230400]

Jackel MC, Kopf-Maier P, Baumgart F, Ziessow D, Tausch-Treml R. Value of ^{31}P NMR spectroscopy in predicting the response of a xenografted human hypopharynx carcinoma to irradiation. *J Cancer Res Clin Oncol.* 2000; 126:325–31. [PubMed: 10870642]

Jaspers K, Aerts HJ, Leiner T, Oostendorp M, van Riel NA, Post MJ, Backes WH. Reliability of pharmacokinetic parameters: small vs. medium-sized contrast agents. *Magn Reson Med.* 2009; 62:779–87. [PubMed: 19623622]

Jennings D, Hatton BN, Guo J, Galons JP, Trouard TP, Raghunand N, Marshall J, Gillies RJ. Early response of prostate carcinoma xenografts to docetaxel chemotherapy monitored with diffusion MRI. *Neoplasia.* 2002; 4:255–62. [PubMed: 11988845]

Jensen JH, Chandra R. MR imaging of microvasculature. *Magn Reson Med.* 2000; 44:224–30. [PubMed: 10918321]

Jensen JH, Helpert JA. MRI quantification of non-Gaussian water diffusion by kurtosis analysis. *NMR in biomedicine.* 2010; 23:698–710. [PubMed: 20632416]

Jensen JH, Helpert JA, Ramani A, Lu H, Kaczynski K. Diffusional kurtosis imaging: the quantification of non-gaussian water diffusion by means of magnetic resonance imaging. *Magn Reson Med.* 2005; 53:1432–40. [PubMed: 15906300]

Johannesson H, Axelsson O, Karlsson M. Transfer of para-hydrogen spin order into polarization by diabatic field cycling. *Cr Phys.* 2004a; 5:315–24.

- Johannesson, H.; Axelsson, O.; Karlsson, M.; Goldman, M. Methods to convert para-hydrogen spin order into hetero nuclei polarization for in vivo detection. Proc 21st Annual Meeting ESMRMB; 2004b.
- Jordan BF, Black K, Robey IF, Runquist M, Powis G, Gillies RJ. Metabolite changes in HT-29 xenograft tumors following HIF-1 α inhibition with PX-478 as studied by MR spectroscopy in vivo and ex vivo. NMR Biomed. 2005; 18:430–9. [PubMed: 16206237]
- Jordan BF, Crokart N, Baudelet C, Cron GO, Ansiaux R, Gallez B. Complex relationship between changes in oxygenation status and changes in R*2: the case of insulin and NS-398, two inhibitors of oxygen consumption. Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine/Society of Magnetic Resonance in Medicine. 2006; 56:637–43.
- Katz-Brull R, Degani H. Kinetics of choline transport and phosphorylation in human breast cancer cells; NMR application of the zero trans method. Anticancer Res. 1996; 16:1375–80. [PubMed: 8694504]
- Kelley DA, Wald LL, Star-Lack JM. Lactate detection at 3T: compensating J coupling effects with BASING. J Magn Reson Imaging. 1999; 9:732–7. [PubMed: 10331771]
- Kerbel RS. Tumor angiogenesis. N Engl J Med. 2008; 358:2039–49. [PubMed: 18463380]
- Keshari KR, Wilson DM, Chen AP, Bok R, Larson PE, Hu S, Van Crielinge M, Macdonald JM, Vigneron DB, Kurhanewicz J. Hyperpolarized [2-13C]-fructose: a hemiketal DNP substrate for in vivo metabolic imaging. J Am Chem Soc. 2009; 131:17591–6. [PubMed: 19860409]
- Kettunen MI, Hu DE, Witney TH, McLaughlin R, Gallagher FA, Bohndiek SE, Day SE, Brindle KM. Magnetization transfer measurements of exchange between hyperpolarized [1-13C]pyruvate and [1-13C]lactate in a murine lymphoma. Magn Reson Med. 2010; 63:872–80. [PubMed: 20373388]
- Kiessling F, Farhan N, Lichy MP, Vosseler S, Heilmann M, Krix M, Bohlen P, Miller DW, Mueller MM, Semmler W, Fusenig NE, Delorme S. Dynamic contrast-enhanced magnetic resonance imaging rapidly indicates vessel regression in human squamous cell carcinomas grown in nude mice caused by VEGF receptor 2 blockade with DC101. Neoplasia. 2004; 6:213–23. [PubMed: 15153333]
- Kiessling F, Le-Huu M, Kunert T, Thorn M, Vosseler S, Schmidt K, Hoffend J, Meinzer HP, Fusenig NE, Semmler W. Improved correlation of histological data with DCE MRI parameter maps by 3D reconstruction, reslicing and parameterization of the histological images. Eur Radiol. 2005; 15:1079–86. [PubMed: 15747142]
- Kim H, Morgan DE, Zeng H, Grizzle WE, Warram JM, Stockard CR, Wang D, Zinn KR. Breast tumor xenografts: diffusion-weighted MR imaging to assess early therapy with novel apoptosis-inducing anti-DR5 antibody. Radiology. 2008; 248:844–51. [PubMed: 18710978]
- Kim JH, Im GH, Yang J, Choi D, Lee WJ, Lee JH. Quantitative dynamic contrast-enhanced MRI for mouse models using automatic detection of the arterial input function. NMR Biomed. 2012a; 25:674–84. [PubMed: 21954069]
- Kim S, Decarlo L, Cho GY, Jensen JH, Sodickson DK, Moy L, Formenti S, Schneider RJ, Goldberg JD, Sigmund EE. Interstitial fluid pressure correlates with intravoxel incoherent motion imaging metrics in a mouse mammary carcinoma model. NMR Biomed. 2012b; 25:787–94. [PubMed: 22072561]
- Kohler SJ, Yen Y, Wolber J, Chen AP, Albers MJ, Bok R, Zhang V, Tropp J, Nelson S, Vigneron DB, Kurhanewicz J, Hurd RE. In vivo 13 carbon metabolic imaging at 3T with hyperpolarized 13C-1-pyruvate. Magn Reson Med. 2007; 58:65–9. [PubMed: 17659629]
- Koutcher JA, Alfieri AA, Devitt ML, Rhee JG, Kornblith AB, Mahmood U, Merchant TE, Cowburn D. Quantitative changes in tumor metabolism, partial pressure of oxygen, and radiobiological oxygenation status postirradiation. Cancer Res. 1992; 52:4620–7. [PubMed: 1511430]
- Koutcher JA, Alfieri AA, Matei C, Meyer KL, Street JC, Martin DS. Effect of 6-aminonicotinamide on the pentose phosphate pathway: ³¹P NMR and tumor growth delay studies. Magn Reson Med. 1996; 36:887–92. [PubMed: 8946354]
- Koutcher JA, Motwani M, Zakian KL, Li XK, Matei C, Dyke JP, Ballon D, Yoo HH, Schwartz GK. The *in vivo* effect of bryostat-1 on paclitaxel-induced tumor growth, mitotic entry, and blood flow. Clin Cancer Res. 2000; 6:1498–507. [PubMed: 10778982]

- Koutcher JA, Okunieff P, Neuringer L, Suit H, Brady T. Size dependent changes in tumor phosphate metabolism after radiation therapy as detected by ^{31}P NMR spectroscopy. *Int J Radiat Oncol Biol Phys.* 1987; 13:1851–5. [PubMed: 3679923]
- Kristensen CA, Askenasy N, Jain RK, Koretsky AP. Creatine and cyclocreatine treatment of human colon adenocarcinoma xenografts: ^{31}P and ^1H magnetic resonance spectroscopic studies. *British journal of cancer.* 1999; 79:278–85. [PubMed: 9888469]
- Larocque MP, Syme A, Allalunis-Turner J, Fallone BG. ADC response to radiation therapy correlates with induced changes in radiosensitivity. *Med Phys.* 2010a; 37:3855–61. [PubMed: 20831093]
- Larocque MP, Syme A, Yahya A, Wachowicz K, Allalunis-Turner J, Fallone BG. Temporal and dose dependence of T2 and ADC at 9.4 T in a mouse model following single fraction radiation therapy. *Med Phys.* 2009; 36:2948–54. [PubMed: 19673193]
- Larocque MP, Syme A, Yahya A, Wachowicz K, Allalunis-Turner J, Fallone BG. Monitoring T2 and ADC at 9.4 T following fractionated external beam radiation therapy in a mouse model. *Phys Med Biol.* 2010b; 55:1381–93. [PubMed: 20150684]
- Larson KB, Perman WH, Perlmutter JS, Gado MH, Ollinger JM, Zierler K. Tracer-kinetic analysis for measuring regional cerebral blood flow by dynamic nuclear magnetic resonance imaging. *J Theor Biol.* 1994; 170:1–14. [PubMed: 7967632]
- Larson PE, Bok R, Kerr AB, Lustig M, Hu S, Chen AP, Nelson SJ, Pauly JM, Kurhanewicz J, Vigneron DB. Investigation of tumor hyperpolarized $[1-^{13}\text{C}]$ -pyruvate dynamics using time-resolved multiband RF excitation echo-planar MRSI. *Magn Reson Med.* 2010; 63:582–91. [PubMed: 20187172]
- Lauterbur PC. Image Formation by Induced Local Interactions: Examples Employing Nuclear Magnetic Resonance. *Nature.* 1973; 242:190–1.
- Le Bihan D, Breton E, Lallemand D, Aubin ML, Vignaud J, Laval-Jeantet M. Separation of diffusion and perfusion in intravoxel incoherent motion MR imaging. *Radiology.* 1988; 168:497–505. [PubMed: 3393671]
- Le Bihan D, Breton E, Lallemand D, Grenier P, Cabanis E, Laval-Jeantet M. MR imaging of intravoxel incoherent motions: application to diffusion and perfusion in neurologic disorders. *Radiology.* 1986; 161:401–7. [PubMed: 3763909]
- Le HC, Lupu M, Kotedia K, Rosen N, Solit D, Koutcher JA. Proton MRS detects metabolic changes in hormone sensitive and resistant human prostate cancer models CWR22 and CWR22r. *Magn Reson Med.* 2009; 62:1112–9. [PubMed: 19780165]
- Leach MO. Introduction to in vivo MRS of cancer: new perspectives and open problems. *Anticancer Res.* 1996; 16:1503–14. [PubMed: 8694519]
- Leach MO, Brindle KM, Evelhoch JL, Griffiths JR, Horsman MR, Jackson A, Jayson GC, Judson IR, Knopp MV, Maxwell RJ, McIntyre D, Padhani AR, Price P, Rathbone R, Rustin GJ, Tofts PS, Tozer GM, Vennart W, Waterton JC, Williams SR, Workman P. The assessment of antiangiogenic and antivasular therapies in early-stage clinical trials using magnetic resonance imaging: issues and recommendations. *British journal of cancer.* 2005; 92:1599–610. [PubMed: 15870830]
- Lee SC, Huang MQ, Nelson DS, Pickup S, Wehrli S, Adegbola O, Poptani H, Delikatny EJ, Glickson JD. *In vivo* MRS markers of response to CHOP chemotherapy in the WSU-DLCL2 human diffuse large B-cell lymphoma xenograft. *NMR Biomed.* 2008; 21:723–33. [PubMed: 18384181]
- Lee SC, Poptani H, Pickup S, Jenkins WT, Kim S, Koch CJ, Delikatny EJ, Glickson JD. Early detection of radiation therapy response in non-Hodgkin's lymphoma xenografts by in vivo ^1H magnetic resonance spectroscopy and imaging. *NMR Biomed.* 2010; 23:624–32. [PubMed: 20661875]
- Lee SJ, Kim JH, Kim YM, Lee GK, Lee EJ, Park IS, Jung JM, Kang KH, Shin T. Perfusion MR imaging in gliomas: comparison with histologic tumor grade. *Korean J Radiol.* 2001; 2:1–7. [PubMed: 11752962]
- Lemaire L, Howe FA, Rodrigues LM, Griffiths JR. Assessment of induced rat mammary tumour response to chemotherapy using the apparent diffusion coefficient of tissue water as determined by diffusion-weighted ^1H -NMR spectroscopy in vivo. *MAGMA.* 1999; 8:20–6. [PubMed: 10383089]

- Li AX, Wojciechowski F, Suchy M, Jones CK, Hudson RH, Menon RS, Bartha R. A sensitive PARACEST contrast agent for temperature MRI: Eu3+-DOTAM-glycine (Gly)-phenylalanine (Phe). *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine/Society of Magnetic Resonance in Medicine*. 2008; 59:374–81.
- Li C, Penet MF, Wildes F, Takagi T, Chen Z, Winnard PT, Artemov D, Bhujwalla ZM. Nanoplex delivery of siRNA and prodrug enzyme for multimodality image-guided molecular pathway targeted cancer therapy. *ACS nano*. 2010; 4:6707–16. [PubMed: 20958072]
- Ling W, Regatte RR, Navon G, Jerschow A. Assessment of glycosaminoglycan concentration in vivo by chemical exchange-dependent saturation transfer (gagCEST). *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105:2266–70. [PubMed: 18268341]
- Liu G, Li Y, Sheth VR, Pagel MD. Imaging in vivo extracellular pH with a single paramagnetic chemical exchange saturation transfer magnetic resonance imaging contrast agent. *Molecular imaging*. 2012; 11:47–57. [PubMed: 22418027]
- Loveless ME, Halliday J, Liess C, Xu L, Dortch RD, Whisenant J, Waterton JC, Gore JC, Yankeelov TE. A quantitative comparison of the influence of individual versus population-derived vascular input functions on dynamic contrast enhanced-MRI in small animals. *Magn Reson Med*. 2012; 67:226–36. [PubMed: 21688316]
- Lustig M, Donoho DL, Santos JM, Pauly JM. Compressed sensing MRI. *Ieee Signal Proc Mag*. 2008; 25:72–82.
- Lyng H, Haraldseth O, Rofstad EK. Measurement of cell density and necrotic fraction in human melanoma xenografts by diffusion weighted magnetic resonance imaging. *Magn Reson Med*. 2000; 43:828–36. [PubMed: 10861877]
- Maeda M, Matsumine A, Kato H, Kusuzaki K, Maier SE, Uchida A, Takeda K. Soft-tissue tumors evaluated by line-scan diffusion-weighted imaging: influence of myxoid matrix on the apparent diffusion coefficient. *Journal of magnetic resonance imaging : JMRI*. 2007; 25:1199–204. [PubMed: 17520732]
- Mahmood U, Alfieri AA, Thaler H, Cowburn D, Koutcher JA. Radiation dose-dependent changes in tumor metabolism measured by ³¹P nuclear magnetic resonance spectroscopy. *Cancer Res*. 1994; 54:4885–91. [PubMed: 8069854]
- Mahmood U, Street JC, Matei C, Ballon D, Martin DS, Koutcher JA. *In vivo* detection by ³¹P NMR of pentose phosphate pathway block secondary to biochemical modulation. *NMR in biomedicine*. 1996; 9:114–20. [PubMed: 8892397]
- Mansson S, Johansson E, Magnusson P, Chai CM, Hansson G, Petersson JS, Stahlberg F, Golman K. ¹³C imaging-a new diagnostic platform. *Eur Radiol*. 2006; 16:57–67. [PubMed: 16402256]
- Marzola P, Degrossi A, Calderan L, Farace P, Crescimanno C, Nicolato E, Giusti A, Pesenti E, Terron A, Sbarbati A, Abrams T, Murray L, Osculati F. In vivo assessment of antiangiogenic activity of SU6668 in an experimental colon carcinoma model. *Clin Cancer Res*. 2004; 10:739–50. [PubMed: 14760097]
- Marzola P, Degrossi A, Calderan L, Farace P, Nicolato E, Crescimanno C, Sandri M, Giusti A, Pesenti E, Terron A, Sbarbati A, Osculati F. Early antiangiogenic activity of SU11248 evaluated in vivo by dynamic contrast-enhanced magnetic resonance imaging in an experimental model of colon carcinoma. *Clin Cancer Res*. 2005; 11:5827–32. [PubMed: 16115922]
- McGrath DM, Bradley DP, Tessier JL, Lacey T, Taylor CJ, Parker GJ. Comparison of model-based arterial input functions for dynamic contrast-enhanced MRI in tumor bearing rats. *Magn Reson Med*. 2009; 61:1173–84. [PubMed: 19253360]
- McMahon MT, Gilad AA, Zhou J, Sun PZ, Bulte JW, van Zijl PC. Quantifying exchange rates in chemical exchange saturation transfer agents using the saturation time and saturation power dependencies of the magnetization transfer effect on the magnetic resonance imaging signal (QUEST and QUESP): Ph calibration for poly-L-lysine and a starburst dendrimer. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine/Society of Magnetic Resonance in Medicine*. 2006; 55:836–47.
- Misselwitz B, Schmitt-Willich H, Ebert W, Frenzel T, Weinmann HJ. Pharmacokinetics of Gadomer-17, a new dendritic magnetic resonance contrast agent. *MAGMA*. 2001; 12:128–34. [PubMed: 11390268]

- Moffat BA, Chenevert TL, Lawrence TS, Meyer CR, Johnson TD, Dong Q, Tsien C, Mukherji S, Quint DJ, Gebarski SS, Robertson PL, Junck LR, Rehemtulla A, Ross BD. Functional diffusion map: a noninvasive MRI biomarker for early stratification of clinical brain tumor response. *Proc Natl Acad Sci U S A*. 2005; 102:5524–9. [PubMed: 15805192]
- Moore A, Marecos E, Bogdanov A Jr, Weissleder R. Tumoral distribution of long-circulating dextran-coated iron oxide nanoparticles in a rodent model. *Radiology*. 2000; 214:568–74. [PubMed: 10671613]
- Mori N, Delsite R, Natarajan K, Kulawiec M, Bhujwala ZM, Singh KK. Loss of p53 function in colon cancer cells results in increased phosphocholine and total choline. *Mol Imaging*. 2004; 3:319–23. [PubMed: 15802048]
- Morse DL, Raghunand N, Sadarangani P, Murthi S, Job C, Day S, Howison C, Gillies RJ. Response of choline metabolites to docetaxel therapy is quantified *in vivo* by localized (31)P MRS of human breast cancer xenografts and *in vitro* by high-resolution (31)P NMR spectroscopy of cell extracts. *Magn Reson Med*. 2007; 58:270–80. [PubMed: 17654590]
- Muruganandham M, Alfieri AA, Matei C, Chen Y, Sukenick G, Schemainda I, Hasmann M, Saltz LB, Koutcher JA. Metabolic signatures associated with a NAD synthesis inhibitor-induced tumor apoptosis identified by 1H-decoupled-31P magnetic resonance spectroscopy. *Clin Cancer Res*. 2005; 11:3503–13. [PubMed: 15867253]
- Muruganandham M, Koutcher JA, Pizzorno G, He Q. In vivo tumor lactate relaxation measurements by selective multiple-quantum-coherence (Sel-MQC) transfer. *Magn Reson Med*. 2004; 52:902–6. [PubMed: 15389963]
- Naressi A, Couturier C, Devos JM, Janssen M, Mangeat C, de Beer R, Graveron-Demilly D. Java-based graphical user interface for the MRUI quantitation package. *MAGMA*. 2001; 12:141–52. [PubMed: 11390270]
- Neeman M, Dafni H, Bukhari O, Braun RD, Dewhirst MW. In vivo BOLD contrast MRI mapping of subcutaneous vascular function and maturation: validation by intravital microscopy. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine/ Society of Magnetic Resonance in Medicine*. 2001; 45:887–98.
- Negendank W, Li CW, Padavic-Shaller K, Murphy-Boesch J, Brown TR. Phospholipid metabolites in 1H-decoupled 31PMRS in vivo in human cancer: implications for experimental models and clinical studies. *Anticancer Res*. 1996; 16:1539–44. [PubMed: 8694523]
- Ni Y, Wang H, Chen F, Li J, DeKeyser F, Feng Y, Yu J, Bosmans H, Marchal G. Tumor models and specific contrast agents for small animal imaging in oncology. *Methods*. 2009; 48:125–38. [PubMed: 19328231]
- Noebauer-Huhmann IM, Szomolanyi P, Juras V, Kraff O, Ladd ME, Trattnig S. Gadolinium-based magnetic resonance contrast agents at 7 Tesla: in vitro T1 relaxivities in human blood plasma. *Investigative radiology*. 2010; 45:554–8. [PubMed: 20697225]
- Noh DY, Ahn SJ, Lee RA, Park IA, Kim JH, Suh PG, Ryu SH, Lee KH, Han JS. Overexpression of phospholipase D1 in human breast cancer tissues. *Cancer letters*. 2000; 161:207–14. [PubMed: 11090971]
- Nordsmark M, Grau C, Horsman MR, Jorgensen HS, Overgaard J. Relationship between tumour oxygenation, bioenergetic status and radiobiological hypoxia in an experimental model. *Acta Oncol*. 1995; 34:329–34. [PubMed: 7779418]
- O'Connor JP, Naish JH, Parker GJ, Waterton JC, Watson Y, Jayson GC, Buonaccorsi GA, Cheung S, Buckley DL, McGrath DM, West CM, Davidson SE, Roberts C, Mills SJ, Mitchell CL, Hope L, Ton NC, Jackson A. Preliminary study of oxygen-enhanced longitudinal relaxation in MRI: a potential novel biomarker of oxygenation changes in solid tumors. *International journal of radiation oncology, biology, physics*. 2009; 75:1209–15.
- Ogan MD. Albumin labeled with Gd-DTPA: an intravascular contrast-enhancing agent for magnetic resonance blood pool imaging: preparation and characterization. *Investigative radiology*. 1988; 23:961. [PubMed: 3204001]
- Ogawa S, Lee TM, Nayak AS, Glynn P. Oxygenation-sensitive contrast in magnetic resonance image of rodent brain at high magnetic fields. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine/Society of Magnetic Resonance in Medicine*. 1990; 14:68–78.

- Okunieff P, McFarland E, Rummeny E, Willett C, Hitzig B, Neuringer L, Suit H. Effects of oxygen on the metabolism of murine tumors using in vivo phosphorus-31 NMR. *American journal of clinical oncology*. 1987; 10:475–82. [PubMed: 3687832]
- Okunieff PG, Koutcher JA, Gerweck L, McFarland E, Hitzig B, Urano M, Brady T, Neuringer L, Suit HD. Tumor size dependent changes in a murine fibrosarcoma: use of in vivo 31P NMR for non-invasive evaluation of tumor metabolic status. *Int J Radiat Oncol Biol Phys*. 1986; 12:793–9. [PubMed: 3710861]
- Ostergaard L. Principles of cerebral perfusion imaging by bolus tracking. *J Magn Reson Imaging*. 2005; 22:710–7. [PubMed: 16261573]
- Ottobri L, Martelli C, Trabattini DL, Clerici M, Lucignani G. In vivo imaging of immune cell trafficking in cancer. *Eur J Nucl Med Mol Imaging*. 2011; 38:949–68. [PubMed: 21170525]
- Pacheco-Torres J, Calle D, Lizarbe B, Negri V, Ubide C, Fayos R, Larrubia PL, Ballesteros P, Cerdan S. Environmentally sensitive paramagnetic and diamagnetic contrast agents for nuclear magnetic resonance imaging and spectroscopy. *Current topics in medicinal chemistry*. 2011; 11:115–30. [PubMed: 20809891]
- Pathak AP, Schmainda KM, Ward BD, Linderman JR, Reborek KJ, Greene AS. MR-derived cerebral blood volume maps: issues regarding histological validation and assessment of tumor angiogenesis. *Magn Reson Med*. 2001; 46:735–47. [PubMed: 11590650]
- Patlak CS, Blasberg RG, Fenstermacher JD. Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. *J Cereb Blood Flow Metab*. 1983; 3:1–7. [PubMed: 6822610]
- Peng XH, Qian X, Mao H, Wang AY, Chen ZG, Nie S, Shin DM. Targeted magnetic iron oxide nanoparticles for tumor imaging and therapy. *Int J Nanomedicine*. 2008; 3:311–21. [PubMed: 18990940]
- Persigehl T, Bieker R, Matuszewski L, Wall A, Kessler T, Kooijman H, Meier N, Ebert W, Berdel WE, Heindel W, Mesters RM, Bremer C. Antiangiogenic tumor treatment: early noninvasive monitoring with USPIO-enhanced MR imaging in mice. *Radiology*. 2007a; 244:449–56. [PubMed: 17562810]
- Persigehl T, Matuszewski L, Kessler T, Wall A, Meier N, Ebert W, Berdel WE, Heindel W, Mesters R, Bremer C. Prediction of antiangiogenic treatment efficacy by iron oxide enhanced parametric magnetic resonance imaging. *Invest Radiol*. 2007b; 42:791–6. [PubMed: 18007150]
- Pickup S, Zhou R, Glickson J. MRI estimation of the arterial input function in mice. *Acad Radiol*. 2003; 10:963–8. [PubMed: 13678084]
- Pikkemaat JA, Wegh RT, Lamerichs R, van de Molengraaf RA, Langereis S, Burdinski D, Raymond AY, Janssen HM, de Waal BF, Willard NP, Meijer EW, Grull H. Dendritic PARACEST contrast agents for magnetic resonance imaging. *Contrast media & molecular imaging*. 2007; 2:229–39. [PubMed: 17937448]
- Pintaske J, Martirosian P, Graf H, Erb G, Lodemann KP, Claussen CD, Schick F. Relaxivity of Gadopentetate Dimeglumine (Magnevist), Gadobutrol (Gadovist), and Gadobenate Dimeglumine (MultiHance) in human blood plasma at 0.2, 1.5, and 3 Tesla. *Investigative radiology*. 2006; 41:213–21. [PubMed: 16481903]
- Podo F. Tumour phospholipid metabolism. *NMR Biomed*. 1999; 12:413–39. [PubMed: 10654290]
- Podo F, Canevari S, Canese R, Pisanu ME, Ricci A, Iorio E. MR evaluation of response to targeted treatment in cancer cells. *NMR Biomed*. 2011; 24:648–72. [PubMed: 21387442]
- Prichard JW, Alger JR, Behar KL, Petroff OA, Shulman RG. Cerebral metabolic studies in vivo by 31P NMR. *Proc Natl Acad Sci U S A*. 1983; 80:2748–51. [PubMed: 6573678]
- Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magn Reson Med*. 1993; 30:672–9. [PubMed: 8139448]
- Raatschen HJ, Simon GH, Fu Y, Sennino B, Shames DM, Wendland MF, McDonald DM, Brasch RC. Vascular permeability during antiangiogenesis treatment: MR imaging assay results as biomarker for subsequent tumor growth in rats. *Radiology*. 2008; 247:391–9. [PubMed: 18372448]
- Ragan DK, Lai SY, Bankson JA. Fast, reproducible measurement of the vascular input function in mice using constrained reconstruction and cardiac sampling. *NMR Biomed*. 2010
- Raghunand N. Tissue pH measurement by magnetic resonance spectroscopy and imaging. *Methods in molecular medicine*. 2006; 124:347–64. [PubMed: 16506429]

- Raghunand N, Gillies RJ. pH and chemotherapy. *Novartis Found Symp.* 2001; 240:199–211. discussion 65–8. [PubMed: 11727930]
- Rakow-Penner R, Daniel B, Glover GH. Detecting blood oxygen level-dependent (BOLD) contrast in the breast. *Journal of magnetic resonance imaging : JMRI.* 2010; 32:120–9. [PubMed: 20578018]
- Ramirez de Molina A, Gutierrez R, Ramos MA, Silva JM, Silva J, Bonilla F, Sanchez JJ, Lacal JC. Increased choline kinase activity in human breast carcinomas: clinical evidence for a potential novel antitumor strategy. *Oncogene.* 2002; 21:4317–22. [PubMed: 12082619]
- Reichardt W, Juettner E, Uhl M, Elverfeldt DV, Kontny U. Diffusion-weighted imaging as predictor of therapy response in an animal model of Ewing sarcoma. *Investigative radiology.* 2009; 44:298–303. [PubMed: 19346963]
- Remmele S, Dahnke H, Flacke S, Soehle M, Wenningmann I, Kovacs A, Traber F, Muller A, Willinek WA, Konig R, Clusmann H, Gieseke J, Schild HH, Murtz P. Quantification of the magnetic resonance signal response to dynamic (C)O(2)-enhanced imaging in the brain at 3 T: R*(2) BOLD vs. balanced SSFP. *Journal of magnetic resonance imaging : JMRI.* 2010; 31:1300–10. [PubMed: 20512881]
- Rijpkema M, Kaanders JH, Joosten FB, van der Kogel AJ, Heerschap A. Effects of breathing a hyperoxic hypercapnic gas mixture on blood oxygenation and vascularity of head-and-neck tumors as measured by magnetic resonance imaging. *International journal of radiation oncology, biology, physics.* 2002; 53:1185–91.
- Robey IF, Baggett BK, Kirkpatrick ND, Roe DJ, Dosesu J, Sloane BF, Hashim AI, Morse DL, Raghunand N, Gatenby RA, Gillies RJ. Bicarbonate increases tumor pH and inhibits spontaneous metastases. *Cancer Res.* 2009; 69:2260–8. [PubMed: 19276390]
- Robinson SP, Barton SJ, McSheehy PM, Griffiths JR. Nuclear magnetic resonance spectroscopy of cancer. *Br J Radiol.* 1997; 70(Spec No):S60–9. [PubMed: 9534719]
- Robinson SP, Kalber TL, Howe FA, McIntyre DJ, Griffiths JR, Blakey DC, Whittaker L, Ryan AJ, Waterton JC. Acute tumor response to ZD6126 assessed by intrinsic susceptibility magnetic resonance imaging. *Neoplasia.* 2005; 7:466–74. [PubMed: 15967099]
- Rodrigues LM, Robinson SP, McSheehy PM, Stubbs M, Griffiths JR. Enhanced uptake of ifosfamide into GH3 prolactinomas with hypercapnic hyperoxic gases monitored *in vivo* by (31)P MRS. *Neoplasia.* 2002; 4:539–43. [PubMed: 12407448]
- Rohrer M, Bauer H, Mintonovitch J, Requardt M, Weinmann HJ. Comparison of magnetic properties of MRI contrast media solutions at different magnetic field strengths. *Investigative radiology.* 2005; 40:715–24. [PubMed: 16230904]
- Ronen SM, Leach MO. Imaging biochemistry: applications to breast cancer. *Breast Cancer Res.* 2001; 3:36–40. [PubMed: 11250743]
- Roth Y, Tichler T, Kostenich G, Ruiz-Cabello J, Maier SE, Cohen JS, Orenstein A, Mardor Y. High-b-value diffusion-weighted MR imaging for pretreatment prediction and early monitoring of tumor response to therapy in mice. *Radiology.* 2004; 232:685–92. [PubMed: 15215551]
- Ruiz-Cabello J, Cohen JS. Phospholipid metabolites as indicators of cancer cell function. *NMR Biomed.* 1992; 5:226–33. [PubMed: 1449961]
- Saitoh J, Sakurai H, Suzuki Y, Muramatsu H, Ishikawa H, Kitamoto Y, Akimoto T, Hasegawa M, Mitsuhashi N, Nakano T. Correlations between *in vivo* tumor weight, oxygen pressure, ³¹P NMR spectroscopy, hypoxic microenvironment marking by beta-D-iodinated azomycin galactopyranoside (beta-D-IAZGP), and radiation sensitivity. *Int J Radiat Oncol Biol Phys.* 2002; 54:903–9. [PubMed: 12377344]
- Seierstad T, Folkvord S, Roe K, Flatmark K, Skretting A, Olsen DR. Early changes in apparent diffusion coefficient predict the quantitative antitumoral activity of capecitabine, oxaliplatin, and irradiation in HT29 xenografts in athymic nude mice. *Neoplasia.* 2007; 9:392–400. [PubMed: 17534444]
- Seth P, Grant A, Tang J, Vinogradov E, Wang X, Lenkinski R, Sukhatme VP. On-target inhibition of tumor fermentative glycolysis as visualized by hyperpolarized pyruvate. *Neoplasia.* 2011; 13:60–71. [PubMed: 21245941]

- Sharma RK, Jain V. Radiotherapeutic response of Ehrlich ascites tumor cells perfused in agarose gel threads and implanted in mice. A ^{31}P MR spectroscopy study. *Strahlenther Onkol.* 2001; 177:212–9. [PubMed: 11370557]
- Shen J, Petersen KF, Behar KL, Brown P, Nixon TW, Mason GF, Petroff OA, Shulman GI, Shulman RG, Rothman DL. Determination of the rate of the glutamate/glutamine cycle in the human brain by *in vivo* ^{13}C NMR. *Proc Natl Acad Sci U S A.* 1999; 96:8235–40. [PubMed: 10393978]
- Shin JH, Lee HK, Kwun BD, Kim JS, Kang W, Choi CG, Suh DC. Using relative cerebral blood flow and volume to evaluate the histopathologic grade of cerebral gliomas: preliminary results. *AJR Am J Roentgenol.* 2002; 179:783–9. [PubMed: 12185064]
- Shungu DC, Glickson JD. Band-selective spin echoes for *in vivo* localized ^1H NMR spectroscopy. *Magn Reson Med.* 1994; 32:277–84. [PubMed: 7984059]
- Slotboom J, Mehlkopf AF, Bovee WMMJ. The Effects of Frequency-Selective RF Pulses on J-Coupled Spin-Systems. *Journal of Magnetic Resonance, Series A.* 1994; 108:38–50.
- Smith MA, Koutcher JA, Zakian KL. J-difference lactate editing at 3.0 Tesla in the presence of strong lipids. *J Magn Reson Imaging.* 2008; 28:1492–8. [PubMed: 19025937]
- Smith TA, Eccles S, Ormerod MG, Tombs AJ, Titley JC, Leach MO. The phosphocholine and glycerophosphocholine content of an oestrogen-sensitive rat mammary tumour correlates strongly with growth rate. *British journal of cancer.* 1991; 64:821–6. [PubMed: 1931601]
- Song HT, Jordan EK, Lewis BK, Gold E, Liu W, Frank JA. Quantitative T_2^* imaging of metastatic human breast cancer to brain in the nude rat at 3 T. *NMR in biomedicine.* 2011; 24:325–34. [PubMed: 20949637]
- Soto GE, Zhu Z, Evelhoch JL, Ackerman JJ. Tumor ^{31}P NMR pH measurements *in vivo*: a comparison of inorganic phosphate and intracellular 2-deoxyglucose-6-phosphate as pHnmr indicators in murine radiation-induced fibrosarcoma-1. *Magn Reson Med.* 1996; 36:698–704. [PubMed: 8916020]
- Spees WM, Evelhoch JL, Thompson PA, Sloop DJ, Ackerman JJ. Defining the pH_i-hyperthermia sensitivity relationship for the RIF-1 tumor *in vivo*: a ^{31}P MR spectroscopy study. *Radiat Res.* 2005; 164:86–99. [PubMed: 15966768]
- Stabi KL, Bendz LM. Ferumoxytol use as an intravenous contrast agent for magnetic resonance angiography. *Ann Pharmacother.* 2011; 45:1571–5. [PubMed: 22045905]
- Star-Lack J, Spielman D, Adalsteinsson E, Kurhanewicz J, Terris DJ, Vigneron DB. *In vivo* lactate editing with simultaneous detection of choline, creatine, NAA, and lipid singlets at 1.5 T using PRESS excitation with applications to the study of brain and head and neck tumors. *J Magn Reson.* 1998; 133:243–54. [PubMed: 9716465]
- Steen RG. Response of solid tumors to chemotherapy monitored by *in vivo* ^{31}P nuclear magnetic resonance spectroscopy: a review. *Cancer Res.* 1989; 49:4075–85. [PubMed: 2663140]
- Stejskal EO, Tanner JE. Spin Diffusion Measurements: Spin Echoes in the Presence of a Time-Dependent Field Gradient. *Journal of Chemical Physics.* 1965; 42:288–+.
- Stoyanova R, Brown TR. NMR spectral quantitation by principal component analysis. III. A generalized procedure for determination of lineshape variations. *J Magn Reson.* 2002; 154:163–75. [PubMed: 11846573]
- Street JC, Mahmood U, Matei C, Koutcher JA. *In vivo* and *in vitro* studies of cyclophosphamide chemotherapy in a mouse mammary carcinoma by ^{31}P NMR spectroscopy. *NMR in biomedicine.* 1995; 8:149–58. [PubMed: 8771089]
- Street JC, Szwergold BS, Matei C, Kappler F, Mahmood U, Brown TR, Koutcher JA. Study of the metabolism of choline and phosphatidylcholine in tumors *in vivo* using phosphonium-choline. *Magn Reson Med.* 1997; 38:769–75. [PubMed: 9358451]
- Su MY, Cheung YC, Fruehauf JP, Yu H, Nalcioglu O, Mechetner E, Kyshtoobayeva A, Chen SC, Hsueh S, McLaren CE, Wan YL. Correlation of dynamic contrast enhancement MRI parameters with microvessel density and VEGF for assessment of angiogenesis in breast cancer. *Journal of magnetic resonance imaging : JMRI.* 2003; 18:467–77. [PubMed: 14508784]
- Su MY, Muhler A, Lao X, Nalcioglu O. Tumor characterization with dynamic contrast-enhanced MRI using MR contrast agents of various molecular weights. *Magn Reson Med.* 1998; 39:259–69. [PubMed: 9469709]

- Sugahara T, Korogi Y, Kochi M, Ikushima I, Shigematu Y, Hirai T, Okuda T, Liang L, Ge Y, Komohara Y, Ushio Y, Takahashi M. Usefulness of diffusion-weighted MRI with echo-planar technique in the evaluation of cellularity in gliomas. *Journal of magnetic resonance imaging : JMRI*. 1999; 9:53–60. [PubMed: 10030650]
- Sukstanskii AL, Ackerman JJ, Yablonskiy DA. Effects of barrier-induced nuclear spin magnetization inhomogeneities on diffusion-attenuated MR signal. *Magn Reson Med*. 2003; 50:735–42. [PubMed: 14523959]
- Svensson J, Mansson S, Johansson E, Petersson JS, Olsson LE. Hyperpolarized ^{13}C MR angiography using trueFISP. *Magn Reson Med*. 2003; 50:256–62. [PubMed: 12876701]
- Taupitz M, Wagner S, Hamm B, Binder A, Pfefferer D. Interstitial MR lymphography with iron oxide particles: results in tumor-free and VX2 tumor-bearing rabbits. *AJR Am J Roentgenol*. 1993; 161:193–200. [PubMed: 8517301]
- Terpstra M, Gruetter R, High WB, Mescher M, DelaBarre L, Merkle H, Garwood M. Lactate turnover in rat glioma measured by in vivo nuclear magnetic resonance spectroscopy. *Cancer Res*. 1998; 58:5083–8. [PubMed: 9823316]
- Terreno E, Castelli DD, Milone L, Rollet S, Stancanelli J, Violante E, Aime S. First ex-vivo MRI co-localization of two LIPOCEST agents. *Contrast media & molecular imaging*. 2008; 3:38–43. [PubMed: 18335476]
- Thoeny HC, De Keyzer F, Chen F, Ni Y, Landuyt W, Verbeken EK, Bosmans H, Marchal G, Hermans R. Diffusion-weighted MR imaging in monitoring the effect of a vascular targeting agent on rhabdomyosarcoma in rats. *Radiology*. 2005a; 234:756–64. [PubMed: 15734932]
- Thoeny HC, De Keyzer F, Vandecaveye V, Chen F, Sun X, Bosmans H, Hermans R, Verbeken EK, Boesch C, Marchal G, Landuyt W, Ni Y. Effect of vascular targeting agent in rat tumor model: dynamic contrast-enhanced versus diffusion-weighted MR imaging. *Radiology*. 2005b; 237:492–9. [PubMed: 16192323]
- Thomlinson RH, Gray LH. The histological structure of some human lung cancers and the possible implications for radiotherapy. *British journal of cancer*. 1955; 9:539–49. [PubMed: 13304213]
- Thorek DL, Chen AK, Czupryna J, Tsourkas A. Superparamagnetic iron oxide nanoparticle probes for molecular imaging. *Ann Biomed Eng*. 2006; 34:23–38. [PubMed: 16496086]
- Ting YL, Sherr D, Degani H. Variations in energy and phospholipid metabolism in normal and cancer human mammary epithelial cells. *Anticancer Res*. 1996; 16:1381–8. [PubMed: 8694505]
- Tkac I, Starcuk Z, Choi IY, Gruetter R. In vivo ^1H NMR spectroscopy of rat brain at 1 ms echo time. *Magn Reson Med*. 1999; 41:649–56. [PubMed: 10332839]
- Tofts PS, Brix G, Buckley DL, Evelhoch JL, Henderson E, Knopp MV, Larsson HB, Lee TY, Mayr NA, Parker GJ, Port RE, Taylor J, Weisskoff RM. Estimating kinetic parameters from dynamic contrast-enhanced T(1)-weighted MRI of a diffusable tracer: standardized quantities and symbols. *Journal of magnetic resonance imaging : JMRI*. 1999; 10:223–32. [PubMed: 10508281]
- Tofts PS, Kermode AG. Measurement of the blood-brain barrier permeability and leakage space using dynamic MR imaging. 1. Fundamental concepts. *Magn Reson Med*. 1991; 17:357–67. [PubMed: 2062210]
- Tropres I, Grimault S, Vaeth A, Grillon E, Julien C, Payen JF, Lamalle L, Decors M. Vessel size imaging. *Magn Reson Med*. 2001; 45:397–408. [PubMed: 11241696]
- Tropres I, Lamalle L, Farion R, Segebarth C, Remy C. Vessel size imaging using low intravascular contrast agent concentrations. *MAGMA*. 2004a; 17:313–6. [PubMed: 15580376]
- Tropres I, Lamalle L, Peoc'h M, Farion R, Usson Y, Decors M, Remy C. In vivo assessment of tumoral angiogenesis. *Magn Reson Med*. 2004b; 51:533–41. [PubMed: 15004795]
- Tuncbilek N, Karakas HM, Altaner S. Dynamic MRI in indirect estimation of microvessel density, histologic grade, and prognosis in colorectal adenocarcinomas. *Abdominal imaging*. 2004; 29:166–72. [PubMed: 15290941]
- Turetschek K, Huber S, Floyd E, Helbich T, Roberts TP, Shames DM, Tarlo KS, Wendland MF, Brasch RC. MR imaging characterization of microvessels in experimental breast tumors by using a particulate contrast agent with histopathologic correlation. *Radiology*. 2001; 218:562–9. [PubMed: 11161179]

- Turetschek K, Preda A, Novikov V, Brasch RC, Weinmann HJ, Wunderbaldinger P, Roberts TP. Tumor microvascular changes in antiangiogenic treatment: assessment by magnetic resonance contrast media of different molecular weights. *Journal of magnetic resonance imaging : JMRI*. 2004; 20:138–44. [PubMed: 15221819]
- Ungersma SE, Pacheco G, Ho C, Yee SF, Ross J, van Bruggen N, Peale FV Jr, Ross S, Carano RA. Erratum to: Ungersma SE, Pacheco G, Ho C, Yee SF, Ross J, van Bruggen N, Peale FV Jr, Ross S, Carano RA. Vessel imaging with viable tumor analysis for quantification of tumor angiogenesis. *Magn Reson Med* 2010;63:1637–1647. *Magn Reson Med*. 2011; 65:889–99. [PubMed: 21442797]
- Van Den Thillart G, Van Waarde A. Nuclear magnetic resonance spectroscopy of living systems: applications in comparative physiology. *Physiological reviews*. 1996; 76:799–837. [PubMed: 8757789]
- van Dijke CF, Brasch RC, Roberts TP, Weidner N, Mathur A, Shames DM, Mann JS, Demsar F, Lang P, Schwickert HC. Mammary carcinoma model: correlation of macromolecular contrast-enhanced MR imaging characterizations of tumor microvasculature and histologic capillary density. *Radiology*. 1996; 198:813–8. [PubMed: 8628876]
- van Laarhoven HW, Gambarota G, Lok J, Lammens M, Kamm YL, Wagener T, Punt CJ, van der Kogel AJ, Heerschap A. Carbogen breathing differentially enhances blood plasma volume and 5-fluorouracil uptake in two murine colon tumor models with a distinct vascular structure. *Neoplasia*. 2006; 8:477–87. [PubMed: 16820094]
- van Zijl PC, Jones CK, Ren J, Malloy CR, Sherry AD. MRI detection of glycogen in vivo by using chemical exchange saturation transfer imaging (glycoCEST). *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104:4359–64. [PubMed: 17360529]
- van Zijl PC, Yadav NN. Chemical exchange saturation transfer (CEST): what is in a name and what isn't? *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine/Society of Magnetic Resonance in Medicine*. 2011; 65:927–48.
- Varallyay CG, Muldoon LL, Gahramanov S, Wu YJ, Goodman JA, Li X, Pike MM, Neuwelt EA. Dynamic MRI using iron oxide nanoparticles to assess early vascular effects of antiangiogenic versus corticosteroid treatment in a glioma model. *J Cereb Blood Flow Metab*. 2009; 29:853–60. [PubMed: 19142191]
- Varallyay P, Nesbit G, Muldoon LL, Nixon RR, Delashaw J, Cohen JI, Petrillo A, Rink D, Neuwelt EA. Comparison of two superparamagnetic viral-sized iron oxide particles ferumoxides and ferumoxtran-10 with a gadolinium chelate in imaging intracranial tumors. *AJNR Am J Neuroradiol*. 2002; 23:510–9. [PubMed: 11950637]
- Vassallo P, Matei C, Heston WD, McLachlan SJ, Koutcher JA, Castellino RA. AMI-227-enhanced MR lymphography: usefulness for differentiating reactive from tumor-bearing lymph nodes. *Radiology*. 1994; 193:501–6. [PubMed: 7972768]
- Vaupel P. Is there a critical tissue oxygen tension for bioenergetic status and cellular pH regulation in solid tumors? *Experientia*. 1996; 52:464–8. [PubMed: 8641384]
- Viale A, Reineri F, Santelia D, Cerutti E, Ellena S, Gobetto R, Aime S. Hyperpolarized agents for advanced MRI investigations. *Q J Nucl Med Mol Imaging*. 2009; 53:604–17. [PubMed: 20016452]
- Vogel-Claussen J, Gimi B, Artemov D, Bhujwala ZM. Diffusion-weighted and macromolecular contrast enhanced MRI of tumor response to antivasular therapy with ZD6126. *Cancer Biol Ther*. 2007; 6:1469–75. [PubMed: 17881899]
- von Morze C, Larson PE, Hu S, Yoshihara HA, Bok RA, Goga A, Ardenkjaer-Larsen JH, Vigneron DB. Investigating tumor perfusion and metabolism using multiple hyperpolarized (13)C compounds: HP001, pyruvate and urea. *Magn Reson Imaging*. 2012; 30:305–11. [PubMed: 22169407]
- Wade TP, Kozlowski P. Longitudinal studies of angiogenesis in hormone-dependent Shionogi tumors. *Neoplasia*. 2007; 9:563–8. [PubMed: 17710159]
- Walker-Samuel S, Leach MO, Collins DJ. Evaluation of response to treatment using DCE-MRI: the relationship between initial area under the gadolinium curve (IAUGC) and quantitative pharmacokinetic analysis. *Phys Med Biol*. 2006; 51:3593–602. [PubMed: 16825751]

- Wang H, Li J, Chen F, De Keyzer F, Yu J, Feng Y, Nuyts J, Marchal G, Ni Y. Morphological, functional and metabolic imaging biomarkers: assessment of vascular-disrupting effect on rodent liver tumours. *Eur Radiol.* 2010; 20:2013–26. [PubMed: 20182730]
- Wang YX. Superparamagnetic iron oxide based MRI contrast agents: Current status of clinical application. *Quant Imaging Med Surg.* 2011; 1:35–40. [PubMed: 23256052]
- Wang Z, Su MY, Najafi A, Nalcioğlu O. Effect of vasodilator hydralazine on tumor microvascular random flow and blood volume as measured by intravoxel incoherent motion (IVIM) weighted MRI in conjunction with Gd-DTPA-Albumin enhanced MRI. *Magn Reson Imaging.* 2001; 19:1063–72. [PubMed: 11711230]
- Warburg O. On the origin of cancer cells. *Science.* 1956; 123:309–14. [PubMed: 13298683]
- Ward CS, Venkatesh HS, Chaumeil MM, Brandes AH, Vancricking M, Dafni H, Sukumar S, Nelson SJ, Vigneron DB, Kurhanewicz J, James CD, Haas-Kogan DA, Ronen SM. Noninvasive detection of target modulation following phosphatidylinositol 3-kinase inhibition using hyperpolarized ^{13}C magnetic resonance spectroscopy. *Cancer Res.* 2010; 70:1296–305. [PubMed: 20145128]
- Ward KM, Aletras AH, Balaban RS. A new class of contrast agents for MRI based on proton chemical exchange dependent saturation transfer (CEST). *Journal of magnetic resonance.* 2000; 143:79–87. [PubMed: 10698648]
- Weidner N. Intratumor microvessel density as a prognostic factor in cancer. *The American journal of pathology.* 1995; 147:9–19. [PubMed: 7541613]
- Weinmann HJ, Brasch RC, Press WR, Wesbey GE. Characteristics of gadolinium-DTPA complex: a potential NMR contrast agent. *AJR Am J Roentgenol.* 1984; 142:619–24. [PubMed: 6607655]
- Weissleder R, Hahn PF, Stark DD, Rummeny E, Saini S, Wittenberg J, Ferrucci JT. MR imaging of splenic metastases: ferrite-enhanced detection in rats. *AJR Am J Roentgenol.* 1987; 149:723–6. [PubMed: 3498320]
- Wendland MF, Iyer SB, Fu KK, Lam KN, James TL. Correlations between *in vivo* ^{31}P MRS measurements, tumor size, cell survival, and hypoxic fraction in the murine EMT6 tumor. *Magn Reson Med.* 1992; 25:217–32. [PubMed: 1614307]
- Wilson DM, Hurd RE, Keshari K, Van Crielkinge M, Chen AP, Nelson SJ, Vigneron DB, Kurhanewicz J. Generation of hyperpolarized substrates by secondary labeling with $[1,1\text{-}^{13}\text{C}]$ acetic anhydride. *Proc Natl Acad Sci U S A.* 2009; 106:5503–7. [PubMed: 19276112]
- Wilson DM, Keshari KR, Larson PE, Chen AP, Hu S, Van Crielkinge M, Bok R, Nelson SJ, Macdonald JM, Vigneron DB, Kurhanewicz J. Multi-compound polarization by DNP allows simultaneous assessment of multiple enzymatic activities *in vivo*. *J Magn Reson.* 2010; 205:141–7. [PubMed: 20478721]
- Witney TH, Kettunen MI, Day SE, Hu DE, Neves AA, Gallagher FA, Fulton SM, Brindle KM. A comparison between radiolabeled fluorodeoxyglucose uptake and hyperpolarized (^{13}C) -labeled pyruvate utilization as methods for detecting tumor response to treatment. *Neoplasia.* 2009; 11:574–82. 1 p following 82. [PubMed: 19484146]
- Witney TH, Kettunen MI, Hu DE, Gallagher FA, Bohndiek SE, Napolitano R, Brindle KM. Detecting treatment response in a model of human breast adenocarcinoma using hyperpolarised $[1\text{-}(^{13}\text{C})\text{pyruvate}]$ and $[1,4\text{-}(^{13}\text{C})\text{fumarate}]$. *British journal of cancer.* 2010
- Wu EX, Tang H, Jensen JH. Applications of ultrasmall superparamagnetic iron oxide contrast agents in the MR study of animal models. *NMR in biomedicine.* 2004; 17:478–83. [PubMed: 15526349]
- Yablonskiy DA, Bretthorst GL, Ackerman JJ. Statistical model for diffusion attenuated MR signal. *Magn Reson Med.* 2003; 50:664–9. [PubMed: 14523949]
- Yablonskiy DA, Haacke EM. Theory of NMR signal behavior in magnetically inhomogeneous tissues: the static dephasing regime. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine/Society of Magnetic Resonance in Medicine.* 1994; 32:749–63.
- Yablonskiy DA, Neil JJ, Raichle ME, Ackerman JJ. Homonuclear J coupling effects in volume localized NMR spectroscopy: pitfalls and solutions. *Magn Reson Med.* 1998; 39:169–78. [PubMed: 9469698]
- Yamamoto D, Tanaka K, Nakai K, Baden T, Inoue K, Yamamoto C, Takemoto H, Kamato K, Hirata H, Morikawa S, Inubushi T, Hioki K. Synergistic effects induced by cycloprodigiosin

- hydrochloride and epirubicin on human breast cancer cells. *Breast Cancer Res Treat.* 2002; 72:1–10. [PubMed: 12000216]
- Yang C, Karczmar GS, Medved M, Stadler WM. Multiple reference tissue method for contrast agent arterial input function estimation. *Magn Reson Med.* 2007; 58:1266–75. [PubMed: 17969061]
- Yankeelov TE, Luci JJ, Lepage M, Li R, Debusk L, Lin PC, Price RR, Gore JC. Quantitative pharmacokinetic analysis of DCE-MRI data without an arterial input function: a reference region model. *Magn Reson Imaging.* 2005; 23:519–29. [PubMed: 15919597]
- Zakian KL, D'Angelica M, Matei C, Yoo HH, Ercolani M, Delman K, Fong Y, Koutcher JA. A quantitative assessment of liver metabolites during jaundice using three dimensional phosphorus chemical shift imaging. *Magn Reson Imaging.* 2000; 18:181–7. [PubMed: 10722978]
- Zhang F, Zhu L, Huang X, Niu G, Chen X. Differentiation of reactive and tumor metastatic lymph nodes with diffusion-weighted and SPIO-enhanced MRI. *Mol Imaging Biol.* 2013; 15:40–7. [PubMed: 22588595]
- Zhang JL, Sigmund EE, Chandarana H, Rusinek H, Chen Q, Vivier PH, Taouli B, Lee VS. Variability of renal apparent diffusion coefficients: limitations of the monoexponential model for diffusion quantification. *Radiology.* 2010; 254:783–92. [PubMed: 20089719]
- Zhang PJ, Run WWPL, Wang CB, Deng XX, Wang BB, Chen BBJJ, Liu HY, Dong ZN, Zhang XJ, Tian YP. Peripheral blood mRNA expression patterns to differentiate hepatocellular carcinoma from other hepatic diseases. *Frontiers in bioscience.* 2012; 4:620–30.
- Zhao D, Jiang L, Hahn EW, Mason RP. Comparison of 1H blood oxygen level-dependent (BOLD) and 19F MRI to investigate tumor oxygenation. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine/Society of Magnetic Resonance in Medicine.* 2009; 62:357–64.
- Zhao M, Pipe JG, Bonnett J, Evelhoch JL. Early detection of treatment response by diffusion-weighted 1H-NMR spectroscopy in a murine tumour in vivo. *British journal of cancer.* 1996; 73:61–4. [PubMed: 8554985]
- Zhou J, Blakeley JO, Hua J, Kim M, Laterra J, Pomper MG, van Zijl PC. Practical data acquisition method for human brain tumor amide proton transfer (APT) imaging. *Magn Reson Med.* 2008; 60:842–9. [PubMed: 18816868]
- Zhou J, Payen JF, Wilson DA, Traystman RJ, van Zijl PC. Using the amide proton signals of intracellular proteins and peptides to detect pH effects in MRI. *Nature medicine.* 2003; 9:1085–90.
- Zhou J, Tryggstad E, Wen Z, Lal B, Zhou T, Grossman R, Wang S, Yan K, Fu DX, Ford E, Tyler B, Blakeley J, Laterra J, van Zijl PC. Differentiation between glioma and radiation necrosis using molecular magnetic resonance imaging of endogenous proteins and peptides. *Nature medicine.* 2011; 17:130–4.
- Zhou J, Wilson DA, Sun PZ, Klaus JA, Van Zijl PC. Quantitative description of proton exchange processes between water and endogenous and exogenous agents for WEX, CEST, and APT experiments. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine/Society of Magnetic Resonance in Medicine.* 2004; 51:945–52.
- Zhou R, Bansal N, Leeper DB, Glickson JD. Intracellular acidification of human melanoma xenografts by the respiratory inhibitor m-iodobenzylguanidine plus hyperglycemia: a ³¹P magnetic resonance spectroscopy study. *Cancer Res.* 2000; 60:3532–6. [PubMed: 10910065]

Fig. 1a:

Fig. 1b:

Fig. 1.
Fig. 1a: ^1H MR spectrum from a mouse brain obtained on a 7.0 Tesla scanner using a PRESS spectroscopic imaging pulse sequence with TR = 2500 ms, TE = 12 ms, nominal voxel size = 4.8 μL , VAPOR water suppression and outer volume suppression. ALA, alanine; Cho, choline; Cr, creatine; GABA, γ -aminobutyric acid; Gln, glutamine; Ino, myo-inositol; Lac, lactate; MM, macromolecules; NAA, N-acetyl aspartate; NAAG, N-acetylaspartyl glutamate; PCr, phosphocreatine; Tau, taurine. Figure modified with permission from Simoes et al. *Neuromethods* (2012).

Fig. 1b: High-resolution *in vivo* rat brain spectrum obtained on a 14.1 T MR spectrometer. Approximately 20 metabolites were detected using an ultrashort echo time (2.8 ms) and TR = 4 s. Voxel size was $3 \times 4 \times 4 \text{ mm}^3$ and 480 data frames were averaged. Figure generously

supplied by V. Mlynarik, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland.

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Fig. 2.

Proton spectrum from a mouse with fatty liver. A) liver tissue volume, B) ^1H spectrum from indicated tissue volume showing water and lipid CH_3 peaks, C) vertically zoomed spectrum showing multiple lipid peaks. Data were obtained on a 7.0 Tesla Bruker scanner using PRESS with $\text{TE/TR} = 13.4 \text{ ms}/5000 \text{ ms}$, respiratory triggering, spectral width/points = 4000 Hz/2048 pts, 64 acquisitions, and voxel size = $4.67 \times 2.9 \times 3 = 40.6 \text{ mm}^3$. TUFA, total unsaturated fatty acids; PUFA, polyunsaturated fatty acids; LIP, lipid; 1 hydrogen at α -methylene to carboxyl at 2.22 ppm ($\text{COO}-\text{CH}_2-\text{CH}_2$), 2 Allylic hydrogen ($-\text{CH}_2-\text{CH}=\text{CH}-$) at 2.02 ppm.

Fig. 3.

Example of a metabolite map generated from proton CSI data in a tumor xenograft. Left, proton CSI spectral grid overlaid on corresponding T_1 -weighted image of a prostate cancer xenograft in the mouse. CSI images were acquired with FOV $32 \times 32 \text{ mm}^2$, 16×16 phase encoding steps, slice thickness 4 mm, TR = 1s, TE = 75 ms, spectral width 2000 Hz, 8 data frames averaged. Right, corresponding tCho metabolite map generated by integration of the tCho peak area in each spectral voxel. Adapted from (Le *et al.*, 2009).

Fig. 4.

A schematic of the dynamic nuclear polarization process. At normal thermal equilibrium there is almost an equal distribution of nuclear spins in the low and high energy spin states in the presence of an external B_0 field, with a correspondingly miniscule net spin polarization. At the low temperature of 4 K, doped free electron spins achieve a near complete polarization. By irradiating at the frequency of the electron resonance frequency, one pumps the nuclear spin distribution to a hyperpolarized state, a few percent to close to 100% polarization, through spin polarization transfer from the pre-polarized electron spin reservoir

Fig. 5.

Representative *in vivo* ¹H-decoupled ³¹P MR spectra of an MCa tumor implanted on the foot pad of a CH3/He mouse (A) fed a normal choline-containing diet and (B) a phosphonium choline (Chop)-supplemented diet (phosphonium region of the spectrum at ~ 28 ppm – 22 ppm not shown here). Using the phosphonium analog of choline, the metabolism of phospholipid precursors could be followed *in vivo* through the detection of the phosphonium analogs of these phospholipid precursors (e.g. PC). Signal assignments are as follows: PE, phosphoethanolamine; PC, phosphocholine; PChoP, phosphoryl moiety of the phosphonium analog of PC; Pi, inorganic phosphate; GPE, glycerophosphoethanolamine; GPC, glycerophosphocholine; PCr, phosphocreatine; α-/β-NDP, α-/β-nucleoside diphosphate; α-/β-/γ-NTP, α-/β-/γ-nucleoside triphosphate; NAD(H), overlapping signals of nicotinamide adenine dinucleotide (NAD⁺, NADH) and Nicotinamide adenine dinucleotide phosphate (NADP⁺, NADPH); DPDE, diphosphodiester. Adapted with permission from (Street *et al.*, 1997).

Fig. 6. Effect of combination chemotherapy on tumor metabolism. Representative *in vivo* ^{31}P MR spectra (no ^1H decoupling) of human diffuse large B-cell lymphoma xenografts in SCID mice (**A**) before and (**B**) after three cycles of combination chemotherapy with cyclophosphamide, hydroxy doxorubicin, Oncovin, prednisone, and bryostatin 1 (CHOPB). The treatment of tumors with CHOPB reduced significantly tumoral PME/ β -NTP ratio when compared to untreated tumors. Signal assignments as in Fig. 5. Adapted with permission from (Huang *et al.*, 2007).

Fig. 7. pH measurement by ^{31}P MRS – (A) In vivo ^{31}P MR spectrum (no ^1H decoupling) of a tumor xenograft in a SCID mouse. Signal assignments: 3-APP, exogenous pH marker 3-aminopropylphosphonate; Pi, inorganic phosphate (other signals assigned as in Fig. 3). (B) Distributions of tumoral pH (pH_{Pi}, mainly intracellular) and extracellular tumoral pH (pH_{3APP}) in a tumor xenograft, obtained by conversion of ^{31}P chemical shifts of inorganic phosphate and 3-APP, respectively and plotted as corrected signal intensity versus pH. The pH distributions (obtained after intensity corrections) are the result of the tumoral pH heterogeneity, as well as signal broadening due for one to T_2 relaxation and, in case of 3-APP, also due to its multiplet structure from ^1H coupling. Adapted with permission from (Raghunand, 2006).

Fig. 8.

Log-plot of concentrations that yield a 5% CEST effect for different chemical moieties. The plots for proton and molecular exchange agents are dependent on molecular size. The compartmental exchange curve is a function of particle radius, affecting both exchange rate and number of protons. Reproduced with permission from Van Zijl and Yadav, (van Zijl and Yadav, 2011)

Fig. 9. The effect of pH on detectability of the CEST effect on two Yb(III)- DOTAM complexes. Reproduced with permission from (Pikkemaat *et al.*, 2007).

Fig. 10. Detection of Yb based PARACEST agent with a wider pH range. Figure 9B is the initial pH map and figure 9C is the pH map with statistically significant amide and amine CEST effects. Reproduced with permission (Liu *et al.*, 2012)

Fig. 11.

CEST imaging poly-L-lysine, LRP phantoms and control cell extracts. Fig 11a shows phantom layout, Fig 11b displays the reference image at $Dw = -3.76$ ppm, Fig 11c is the difference image for $Dw = \pm 3.76$ ppm, and Fig 11d is a t-test map comparing $Dw = \pm 3.76$ ppm. (e) Signal intensity difference for different samples, including four LRP clones.

Fig. 12.

In vivo CEST imaging on a rat brain with the LRP expressing tumor cells and control tumor cells, transfected with an empty vector, implanted in opposite spheres of a mouse brain. The expression of LRP in the tumor is detected specifically with CEST imaging. Reproduced with permission (Terreno *et al.*, 2008)

Fig. 13.

TOFTS-MODEL. Schematic of the Tofts-Kety model. Tissue (dashed box) consists of two compartments, plasma compartment with fractional volume v_p , and extravascular compartment with fractional volume v_e . The contrast is delivered with the arterial input function (AIF) at the concentration $C_A(t)$. The contrast first enters the plasma compartment and leaks into the EES at the rate determined by K^{trans} and back at the rate of k_{ep} .

Fig. 14.

Population average vascular input functions (VIF) measured in mice using LMW contrast agent Gd-DTPA (a) and MMW contrast agent P846 (b) and an example of two individual VIFs (red and blue curves) versus population average VIF (black curve) measured with Gd-DTPA (c). Adapted with permission from (Loveless *et al.*, 2012).

Fig. 15. Maps of K^{trans} , v_e , histological section and a 3-color image of a subcutaneous V-27 melanoma xenograft grown in nu/nu mice. The 3-color map indicates voxels with unphysiological parameter values ($v_e < 0.05$ and $v_e > 0.35$, red), low K^{trans} ($K^{trans} < 0.05 \text{ min}^{-1}$, green) and remaining voxels (blue). Adapted with permission from (Egeland *et al.*, 2012).

Fig. 16.

Normalized changes of ADC measured in HT29 colorectal xenografts in BALB/c nu/nu mice after 4 days (A) and 11 days (B) of treatment versus tumor doubling growth delay after chemotherapy alone (Cap – capecitabine, Oxa – oxaliplatin) or chemotherapy combined with irradiation (IR, 2 Gy for 5 days, 5 days/week for 2 weeks starting on day 2). Adapted with permission from (Seierstad *et al.*, 2007).