Contrast Media Research 2019 "Ettore Majorana" Foundation and International Centre for Scientific Culture, Erice, Sicily, Italy, November 10–15, 2019 Conveners: Carlo C. Quattrocchi, MD, PhD and Silvio Aime, PhD

MONDAY, NOVEMBER 11
SESSION 1: X-RAY AGENTS, SAFETY, AND NEW APPLICATIONS

001:

Management of Hypersensitivity Reactions to Contrast Media: Multidisciplinary Netherlands Guideline.

A.J. van der Molen, I.A Dekkers, R.W.F. Geenen, H.M. Dekker, and For the Guideline Committee Safe Use of Contrast Media Part 2 of the Radiological Society of the Netherlands. *A.J.van_der_Molen@lumc.nl.*

Purpose: The aim was to present the recommendations of the new guideline in the Netherlands on management and follow-up of hypersensitivity reactions (HSRs) to contrast media (CM).

Methods and Materials: A multidisciplinary guideline committee systematically searched multiple literature databases using evidence-based medicine principles via answerable clinical questions in 5 topics: management of acute/late HSR, laboratory tests in HSR, skin testing in HSR, and use of premedication after HSR. Scientific evidence from the literature was rated according to the Appraisal of Guidelines for Research & Evaluation II instrument. Resulting conclusions from the scientific evidence was merged with expert opinion in the guideline committee and local factors into recommendations in each of these fields to improve the safe use of contrast agents.

Results: The management of acute and late HSR to CM still relies on a combination of intravenous fluids and oxygen in association with drugs given intramuscularly or intravenously (for mild reactions topical steroids, H1-antihistamines, and ß2-sympathicomimetics and for severe reactions primarily epinephrine). Within 1 to 2 hours after a moderately severe to severe HSR to CM, it is advisable to do serum tryptase testing. Because of a limited capacity for allergologic skin testing in the Netherlands, only severe or breakthrough HSRs should be analyzed by a specialist in drug allergy using repeated serum tryptase measures and skin patch and/or intradermal tests with a panel of contrast agents, either iodine- or gadolinium-based. Such analysis will provide the radiologist or cardiologist with suggestions for optimal CM choice and HSR management during future imaging. The use of steroid and antihistamine premedication can be limited to patients that have experienced severe HSR in the past or for situations in which the culprit agent is not known. Detailed management algorithms and flowcharts will be presented.

Conclusions: Updated multidisciplinary guidelines for management of HSR to CM show a larger role for use of an alternative contrast agent as well as serum tryptase measurement and allergologic skin testing. There is a much reduced role for steroid and antihistamine premedication.

002:

Preclinical Models of Acute Kidney Injury With an Improved Iodinated Contrast Agent

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Purpose: Iodinated contrast agents may place patients with certain risk factors at an increased risk for acute kidney injury during cardiac imaging procedures. Previous studies suggest that the addition of sulfobutylether β-cyclodextrin (Captisol) to a clinically administered dose of iohexol has an improved kidney safety profile. We examined the effects of Captisol-enabled (CE) iohexol in mouse, rat, and a newly established rabbit model of kidney injury.

Methods and Materials: Rats and mice were deprived of water for 20 hours and injected IP with indomethacin (10 mg/kg) and *N*-nitro-L-arginine methyl ester (10 mg/kg). Animals were subsequently injected intravenous (IV) with CE-iohexol or iohexol (both at 1.5 g I/kg), and kidneys were collected for histopathological analysis. Rabbits were injected IV with aristolochic acid (0.5 mg/kg). Seven days later, rabbits received IV iohexol or CE-iohexol (7.5 g I/kg). Rabbits were killed after 48 hours. Kidneys and blood were collected. Histopathology was conducted on hematoxylin and eosin and periodic acid-Schiff–stained slides. Slides were scored by a trained pathologist in a blinded fashion.

Results: Statistically significant increases in renal tubular dilatation, vacuolization, and loss of brush border in rodent kidneys were observed following administration of iohexol. Pathological changes were significantly reduced following administration of CE-iohexol in comparison to iohexol. Similar results were observed in a pilot study with iohexol in rabbits. Tubular dilatation, loss of brush border, and vacuolization were greater in iohexol treated rabbits relative to vehicle controls. A larger, confirmatory study was performed in rabbits with iohexol alone or in combination with low, mid, or high concentrations of Captisol. Change in serum creatinine from baseline was significantly elevated by iohexol relative to vehicle controls at 6, 24, and 48 hours ($P < 0.05$). Change in serum creatinine from baseline after CE-iohexol administration was not significantly greater than vehicle for any of the 3 tested doses. Kidney weights were significantly ($P < 0.01$) elevated relative to vehicle controls after iohexol administration, but no significant effects were observed following CE-iohexol administration.

Conclusions: Iohexol has detrimental effects on kidney pathology, serum creatinine, and kidney weight in rodent and rabbit models of acute kidney injury. CE-iohexol has reduced impact on kidney pathology, serum creatinine, and kidney weight. CE-iohexol may be a safer contrast agent for patients at risk of acute kidney injury.

003:

Crossover Study Comparing Bioavailability of Captisol-Enabled Iohexol Injection to Reference Iohexol Injection in Healthy Subjects

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Purpose: Iodinated contrast agents may place patients with certain risk factors at an increased risk for acute kidney injury during cardiac imaging procedures. Studies in renally compromised mice and rats demonstrated that the addition of sulfobutylether β-cyclodextrin (Captisol) to a clinically administered dose of iohexol significantly reduced renal pathology scores and increased survival in rats from 50% to 88%. A phase 1, single-center, randomized, double-blind, 2-period crossover study was conducted to determine relative bioavailability of Captisol-enabled (CE) iohexol and a reference iohexol injection (Omnipaque) after intravenous (IV) administration in healthy adults.

Methods and Materials: A total of 24 subjects were enrolled in the study as 2 groups of 12 subjects in 2 treatment periods. Subjects received each of the following treatments as a single IV dose (80 mL infused over 20 seconds): CE-iohexol, 755 mg/mL iohexol (350 mg I/mL)/50 mg Captisol/mL; Omnipaque, 755 mg/mL iohexol (350 mg I/mL). Serial blood samples were collected for iohexol plasma concentration determination, and safety was assessed during the 48 hours after each dose. Subjects were discharged on day 3.

Results: Twenty-two subjects completed the study; 2 subjects were withdrawn for technical reasons. Bioequivalence was demonstrated by calculation of geometric mean ratios (GMRs) between CE-iohexol and Omnipaque for key pharmacokinetic parameters. The geometric mean values for area under the concentration-time curve for time 0-infinity ($AUC_{0\text{-inf}}$) for CE-iohexol and Omnipaque were 11,216 and 11,213 h·μg/mL, respectively, and a GMR of 1.00 (94.12% confidence interval, 0.98–1.02). The geometric mean values for maximum concentration (C_{max}) for CE-iohexol and Omnipaque were 6243 and 6231 μg/mL, respectively, and a GMR of 1.00 (94.12% confidence interval, 0.95–1.06). Other PK parameters, including time to maximum observed concentration (T_{max}), half-life ($t_{1/2}$), and elimination rate constant (K_{el}), were similar between treatments. All treatment-emergent adverse events during the study were mild to moderate in severity and in line with the known safety profile of Omnipaque. No subject had a serious adverse event or discontinued from the study due to an adverse event.

Conclusions: The observed PK profile supports clinical development of CE-iohexol as a next-generation contrast agent with a reduced risk of renal toxicity (NCT03869983).

004:

Renally Clearable Silver Sulfide Nanoparticles for Dual-Energy Mammography and Other Modalities

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Purpose: Dual-energy mammography (DEM) and other techniques such as MRI, CT, and near infrared fluorescence (NIRF) imaging have been proposed as alternatives for women with dense breasts as conventional mammography is not effective in these women. However, currently available contrast agents suffer from safety/performance issues. Recently, we found that silver-based nanoparticles have strong contrast for DEM. We hypothesized that silver sulfide nanoparticles (SSNPs) would provide a low-cost DEM contrast agent, with good biocompatibility. Herein, we first develop an all-in-one nanoparticle (AION) for flexible breast cancer detection with DEM, CT, MRI, and NIRF imaging. We then present ultrasmall SSNP that can be cleared renally to avoid long-term body retention and increase their translational potential.

Methods and Materials: AIONs were formed by encapsulating hydrophobic 5-nm SSNP, 10-nm iron oxide nanoparticles, and DiR NIRF dye in PEGylated phospholipids. An array of hydrophilic, ultrasmall SSNP was synthesized via a viscosity-mediated, thermal decomposition method by modulating the reaction time. The nanoparticles were characterized using TEM, fluorimetry, SEM/EDX, XRD, and DLS. Contrast production was evaluated using clinical DEM, CT, and MRI scanners, and an IVIS Spectrum system. Silver ion leaching was assessed via ICP-OES, and biocompatibility was examined using Renca, J774A.1, and HepG2 cells. In vivo imaging and biodistribution studies were performed in female nude mice.

Results: AIONs were 90 nm in size (B), whereas hydrophilic SSNP were between 2 to 10 nm (C). Silver ion leaching was minimal compared with pure silver nanoparticles for both nanoparticle types. AIONs and SSNPs were found to be biocompatible and have generated strong contrast for DEM, CT, MRI, and NIRF, and DEM and CT, respectively, via phantom imaging. AIONs enhanced the tumor contrast as evidenced by in vivo imaging of a murine model of breast cancer with all imaging modalities (D). Biodistribution results revealed that higher amounts of AION remained in the RES organs, whereas retention was significantly lower with sub–5-nm SSNP (E). Kidney filtration and urinary excretion of SSNP were observed in CT and NIRF imaging (F). Eighty-five percent of injected dose was eliminated within 24 hours of injection, which is among the best clearance of all nanoparticles reported to date (ie, 20%–75% of injected dose).

Conclusions: AIONs have potential as a multimodal agent for a range of breast cancer imaging methods, whereas renally clearable, sub–5-nm SSNP have potential as safe DEM, CT, and NIRF contrast agents with promise for future translation.

005:

Allergic Hypersensitivity to Iodine- or Gadolinium-Based Contrast Media: Analysis of Cross-Reactivity Reactions Occurrence in the CIRTACI Study

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Purpose: The aim of this study was to analyze cross-reactivity reactions of the prospective CIRTACI multicenter study, which included the characterization of immediate hypersensitivity reactions to iodinated- (ICM) and gadolinium-based contrast media (GBCM).

Methods and Materials: Among 245 skin-tested patients (ICM = 209; GBCM = 36), allergic immediate hypersensitivity to ICM was identified in 41 (19.6%) and to GBCM in 10 (27.8%). Skin cross-reactivity was diagnosed with positive intradermal test (IDT) for the diluted 1/10 solution and for the pure one.

Results: Skin cross-reactivity with diluted solutions of the nonadministered 9 ICM or 4 GBCM, respectively, was positive in 16 patients (31.4%): 11 (26.8%) to ICM (6 to 1 ICM, 1 to 2, 2 to 3, and 2 to 4) and in 5 (50.0%) to GBCM (4 to 1 GBCM, and 1 to 4).

With pure solutions, more cross-reactivity was found: IDTs were positive in 32 patients (62.7%); 25 (61%) to ICM (9 to 1 ICM, 4 to 2, 5 to 3, and 7 to 4 to 7 ICM) and 7 (70.0%) to GBCM (2 to 1 GBCM, 2 to 2, 2 to 3, and 1 to 4). The frequency of cross-reactivity was not different between ICM and GBCM ($P = 0.30$). There was no specific relationship between monomer and dimer cross-reactivity. The reacting groups described in reference 2 were not identified.

Conclusions: Cross-reactivity to ICM and GBCM is more frequent using a pure solution compared with the diluted one, indicating a need to change the diagnostic criteria of the skin testing guidelines. The molecular mechanisms underlying cross-reactivity need to be further investigated.

MONDAY, NOVEMBER 11
SESSION 2: MRI AGENTS: SAFETY, AND BIODISTRIBUTION

006:

Safe Use of Gadolinium-Based Contrast Agents: Multidisciplinary Netherlands Guideline

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Purpose: The aim of this study was to present the recommendations of the new guideline in the Netherlands on the safe use of gadolinium-based contrast agents (GBCAs), prevention of postcontrast acute kidney injury and nephrogenic systemic fibrosis (NSF), and minimizing gadolinium deposition.

Methods and Materials: A multidisciplinary guideline committee systematically searched the literature using evidence-based medicine principles via answerable clinical questions. Scientific evidence from the literature was rated according to the AGREE II instrument (Appraisal of Guidelines for Research & Evaluation II). Resulting conclusions from the scientific evidence was merged with expert opinion in the guideline committee and local factors into recommendations in each of these fields to improve the safe use of contrast agents.

Results: Macrocyclic GBCAs are considered to have a higher thermodynamic and kinetic stability than linear GBCAs. Since the implementation of the European Medicines Agency ruling, only macrocyclic GBCAs are available for general use in MRI, whereas linear GBCAs are limited to specific hepatobiliary or arthrographic indications. In general, it is recommended to use the lowest GBCA dose needed for diagnosis, but more study is needed in this field. Optimal GBCA dosing based on patient weight should be taken into account in local dosing protocols for diagnostic MRI examinations. When used in standard (0.1 mmol/kg) doses, the allowed GBCAs are less nephrotoxic than iodine-based contrast media and do not require any prophylactic measures to prevent postcontrast acute kidney injury in high-risk patients (eGFR <30 mL/min/1.73m^2). GBCAs should not be used for interventional procedures to replace iodine-based CM.

For the prevention of NSF and minimization of possible deposition of gadolinium, a strict indication for contrast-enhanced MRI is needed. For the allowed GBCA, the risk of NSF is very low, even in patients with eGFR less than 30 mL/min/1.73m^2 and dialysis patients. At the present, no further recommendations can be given to minimize gadolinium deposition.

Conclusions: Updated multidisciplinary guidelines for safe use GBCA stress a strict indication for contrast-enhanced MRI. With currently allowed GBCA given at appropriate doses, the risk for postcontrast acute kidney injury or NSF is very low. GBCAs should not be used for interventional procedures.

007:

Structure and Function of Dentate Nuclei and Cerebellum of Patients With MRI Evidence of Gadolinium Deposition: What to Expect From In Vivo Imaging

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Purpose: The aim of this study was to provide a structural and functional analysis of possible tissue changes of dentate nuclei and cerebellum in patients with magnetic resonance imaging (MRI) evidence of gadolinium deposition in the brain.

Methods and Materials: The analysis included gadolinium-exposed patients with MR evidence of deposition in the dentate nuclei of the cerebellum and gadolinium-naive control subjects.

Images were acquired using scanner with a field strength of 1.5-T (Siemens Avanto and Siemens Aera, Erlangen, Germany).

The investigation included unenhanced T1-weighted images, apparent diffusion coefficient maps obtained using diffusion-weighted imaging, and resting-state blood oxygenation level-dependent functional MRI. The threshold of statistical significance was defined as $P < 0.05$.

Results: We did not find neither structural nor functional significant difference (P = nonsignificant) directly related to gadolinium deposition between gadolinium-exposed with hyperintense dentate nuclei on T1-weighted images and controls.

Conclusions: Our results suggest no significant changes of tissue integrity in patients with MRI evidence of gadolinium deposition in the brain.

008:

Gadolinium Mapping in the Pig Brain by LA-ICP-MS After Multiple Administrations of Linear or Macrocyclic Gadolinium-Based Contrast Agents

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Purpose: This study aims to map and quantify Gd in the larger and gyrencephalic brain of pigs by high-resolution laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) after repeated exposure to gadolinium-based contrast agents. The study compares the Gd distribution in pigs to lissencephalic rat brains, which is currently the most widely used animal species for the evaluation of Gd retention.

Methods and Materials: Goettingen minipigs received 6 weekly intravenous injections of gadodiamide or gadobutrol at a dose of 0.33 mmol Gd/kg body weight (equivalent to a triple standard dose in humans after body-surface adaption) and were killed 15 weeks after the last injection. Brain hemispheres were sectioned in frontal and sagittal planes and the spatial distribution of Gd, and the endogenous element iron (Fe) was quantified and analyzed using LA-ICP-MS with a resolution of 50 μm or less.

Results: The larger brain size of pigs improved the level of anatomical detail obtained by LA-ICP-MS compared with rodents. In example, we were able to visualize larger blood vessels within the tissue by analyzing the iron distribution. In agreement with studies in rats, Gd accumulated in the deep cerebellar nuclei (DCN) after exposure to gadodiamide but not gadobutrol. Interestingly, the Gd distribution within the DCN of gadodiamide exposed pigs was not homogenously distributed. The DCN contained small Gd hot spots with concentrations greater than 50 nmol/g and a more homogenous Gd background concentration of less than 5 nmol/g. The small Gd hotspots were also found in the adjacent white matter, but not the lower background Gd. Apart from the DCN, additional not yet described brain regions such as the colliculus inferior were identified that specifically accumulated Gd after exposure to gadodiamide but not after gadobutrol. The overall Gd pattern observed in gadodiamide animals only partially overlapped with the distribution of Fe indicating Gd presence outside the vasculature.

Conclusions: The distribution and concentration of Gd in the brain was analyzed in larger animal species with closer resemblance to the human brain than rodents. Both the gadolinium-based contrast agent class difference and the anatomical distribution were comparable to rats. After exposure to gadodiamide, we observed that within the DCN region gadolinium is present in different concentrations most likely representing different Gd species. The Gd hot spots presumably account for Gd precipitates.

009:

GBCAs With Reduced Kinetic Stability Induce Mitochondrial Toxicity and Cell Death in Human Neurons at Clinical Levels

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Purpose: This preclinical study was devised to investigate potential cellular toxicity in human neurons induced by GBCAs used for contrast-enhanced magnetic resonance imaging (MRI). Neurons modeling a subset of those in the basal ganglia were tested, as the basal ganglion is 1 of the 2 brain regions that display the greatest T1-dependent signal hyperintensity changes.

Methods and Materials: Eight GBCAs were tested. Dopaminergic neurons modeling a subset of those in the basal ganglia were differentiated from an established human neuroblastoma cell line and exposed to increasing concentrations of each agent for 7 days. The tested dosages ranged from clinically relevant concentrations measured in some autopsy patients who had received repeated injections of contrast for MRI, to higher concentrations to reveal dose-dependent toxicity trends. Cell death, mitochondrial membrane potential, mitochondrial oxidative capacity, and mitochondrial function measured by oxygen consumption were quantified in negative controls and cells treated with each GBCA or the osmolality control mannitol.

Results: Mannitol caused no change from negative controls in any of the tests, at any concentration tested. For all GBCAs, cell death increased with exposure dose, with toxicity at clinically relevant doses for some linear agents and one macrocyclic agent. Reduction of mitochondrial membrane potential and oxidative respiratory function also increased with dose.

Conclusions: In human neurons modeling a subset of those in the basal ganglia, our results demonstrate a toxic effect of gadolinium-containing MRI contrast agents on mitochondrial respiratory function and cell viability. Toxicity increases as agent concentration increases and varies with between agents.

010:

Pathways of Gadomer-17 Elimination From Cerebrospinal Fluid After Intraventricular Infusion or Injection Into the Cisterna Magna—Ultra-High-Field Magnetic Resonance Imaging Investigation

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Purpose: The aim of this study was to elucidate pathways of cerebrospinal fluid (CSF) circulation and outflow, with focus on potential routes and mechanisms of gadolinium-based contrast agent (GBCA) uptake.

Methods and Materials: Two experiments were conducted in mice under general anesthesia. First, we injected GBCA into the cisterna magna and performed dynamic magnetic resonance imaging of the head during a 50-minute time frame (n = 5). Second, we infused GBCA after intraventricular cannulation and performed dynamic whole-body magnetic resonance imaging with a greater than 100-minute time frame (n = 5).

We used Gadomer-17 (GM17, 25 mM; NanoPET Pharma GmbH, Germany) as contrast agent applied with a NanoJet syringe (Chemyx, USA) injecting 5 μL at 1 μL/min (first group) or 6 μL at 0.1 μL/min (second group). Images were acquired with high-resolution T1-weighted 3D-FLASH sequences on a 9.4-T animal scanner (Bruker, Germany). Reconstruction and analysis were performed with Horos v2.0.1 (Horos Project).

Results: In animals injected into the cisterna magna, GM17 outflow occurs via nerve roots and the cribriform plates, subsequently following peripheral lymph vessels to submaxillary and deep cervical lymph nodes. In animals infused into the first ventricle, additional GM17 outflow into the subarachnoidal space of the spine, the central canal, peripheral lymph vessels, and sacral and iliac lymph nodes is observed. In these whole-body imaging experiments profound excretion into the urinary bladder is detectable from 30 minutes after complete intraventricular infusion of contrast medium. Distribution of GM17 to the cerebrum could not be detected. During the short time frame experiments, no GM17 transfer from the cisterna magna to the ventricles was detected as well.

Discussion: Results suggest that GM17 is eliminated from CSF in accordance with animal experiments demonstrating strong elimination of GBCA administrated intravenously, when mimicking sleep conditions by narcosis. However, this effect has also been described as a result of drastic reduction of CSF flow through the brain during general anesthesia. Mechanisms underlying CSF clearance are to date not fully understood. In particular, this holds true considering the connection between CSF circulation and the glymphatic pathway. In our investigation, transport of GM17 from the CSF into neocortical perivascular space and through the brain parenchyma could not be demonstrated. While this study in part supports preclinical and clinical studies on entry of GBCA into brain parenchyma, further investigations are needed to completely clarify underlying physiological principles.

MONDAY, NOVEMBER 11
SESSION 3: NEW MRI AGENTS

011:

Macrocyclic Paramagnetic Agents for MRI: Determinants of Relaxivity and Strategies for Their Improvement

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Purpose: Commercially available macrocyclic contrast agents (CAs) are well appreciated for their inertness and chemical stability, but they are characterized by relaxivity (r_1) values generally lower than linear agents and thus by a reduced diagnostic potential. This study aims to dissect the contributions to the longitudinal r_1 of 2 commercial CAs, gadoterate meglumine and gadoteridol, and then on the basis of acquired inputs, to synthesize/characterize a novel macrocyclic agent (Gd-Phen-DO3A) having superior r_1.

Methods and Materials: A deep relaxometric characterization was carried out, consisting in (1) the acquisition of nuclear magnetic resonance dispersion profiles at 37°C of 1 mM Gd solution in saline, in saline added with 35 g/L of human serum albumin (HSA), in human plasma (HP), and in a ionized simulated body fluid (i-SBF, which mimics the same ionic content of HP); (2) the measurement of r_1 in saline, in a viscous simulated body fluid (which mimics the same viscosity of HP), and in HP at 37°C and 0.47 T; (3) the study of longitudinal relaxation rate ($R_1 = 1/T_1$) as a function of HSA concentration at 37°C of 0.1 mM Gd solution in saline; and (4) the study of R_1 as a function of pH at 37°C of 1 mM Gd solution in saline and in i-SBF. Results have been interpreted to evince the main determinants to the observed r_1 values.

Results: In viscous simulated body fluid or in the presence of HSA, r_1 is enhanced for all complexes, reflecting the viscosity increase and a weak interaction with proteins.

The CAs further differentiate in plasma, with an r_1 increase (vs saline) of approximately 1, 1.5, and 2.5 $mM^{-1}s^{-1}$ for gadoterate meglumine, gadoteridol, and Gd-Phen-DO3A, respectively. R_1 versus pH curves in i-SBF indicate that proton exchange sizably contributes to the r_1 of gadoteridol and Gd-Phen-DO3A.

Conclusions: The major contributions to r_1 in the physiological environment have been highlighted, namely, increased viscosity, complex-protein interaction, and proton exchange. The control of these terms allows the design of novel macrocyclic structures with enhanced r_1 as a result of an improved interaction with plasma's macromolecules and the shift of the proton exchange to physiological pH. The results of the present study give rise to a greater interest on the exploiting the proton exchange as additional route to boost r_1 of CAs.

012:

Divalent Europium-Based Contrast Agents for Magnetic Resonance Imaging

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Purpose: This study explores the properties of europium-based contrast agents as alternatives to gadolinium-based contrast agents and the ability of europium-based agents to image hypoxia. There is a need to study the in vitro and in vivo properties of europium-based agents in assessing the viability of these molecules to serve as contrast agents.

Methods and Materials: Ligands and metal complexes were prepared using standard chemical synthetic techniques. Air-sensitive manipulations were performed in a wet glovebox under an atmosphere of nitrogen gas. Samples were sealed using paraffin wax before removal from the glovebox. Concentrations of europium were measured using inductively coupled plasma mass spectrometry or energy-dispersive x-ray fluorescence spectroscopy. Cyclic voltammetry was performed using a glassy carbon working electrode, platinum wire auxiliary electrode, and Ag/AgCl reference electrode. All animal studies were done in accordance with protocols preapproved by the Institutional Animal Care and Use Committee of Baylor College of Medicine. T_1-weighted and ^{19}F-MRI scans were performed with a Bruker BioSpec 9.4 T horizontal bore MRI scanner.

Results: We have studied the physicochemical properties of several europium-containing complexes and performed initial imaging experiments in vivo. The data indicate that divalent europium rivals gadolinium with respect to relaxivity and that divalent europium can be used to image hypoxic regions in vivo. By taking multiple modalities into account, ratiometric probes have been studied with the intention of removing the need to know concentration in vivo.

Conclusions: The results demonstrate that europium-based contrast agents are a promising alternative to gadolinium-based agents for specific purposes such as in the imaging of hypoxic regions of tumors. Future research is being focused on overcoming chemical issues related to delivery methods in vivo.

013:

Impact of Molecule Size and Hydration Number on the Enhancement Properties of Experimental Gd-Based Contrast Agents at 9.4 T

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Purpose: The aim of this study was to evaluate in vitro and in vivo the enhancement properties of different Gd-based contrast agents with different molecular weights (MWs) and hydration numbers (q).

We compared the experimental compounds P846 (MW, 3.5 kDa; q = 2) and gadopiclenol (MW, 0.97 kDa; q = 2) with the clinically approved low-molecular, extracellular agents gadopentetate (MW, 0.94 kDa; q = 1) and gadoterate (MW, 0.56 kDa; q = 2) at 9.4 T.

Methods and Materials: All experiments were performed with a 9.4-T animal scanner (Bruker, Germany). We performed relaxometry measurements for all contrast agents in human plasma at 37°C using an IR-RARE sequence. In addition, all contrast agents were evaluated in vivo in a rat model of liver metastases calculating signal-to-noise ratios, contrast-to-noise ratios, and lesion enhancement for liver parenchyma and tumors based on region of interest measurements.

Results: Longitudinal relaxivities (r_1) of the experimental compounds were higher as compared with the low-molecular agents. However, r_1 of gadoterate and gadopentetate dropped only moderately at 9.4 T as compared with lower field strengths (gadopentetate: r_1 [at 9.4 T], 3.4 $mM^{-1}s^{-1}/r_1$ [at 1.5 T], 4.1 $mM^{-1}s^{-1}$/gadoterate: r_1 [at 9.4 T], 3.1 $mM^{-1}s^{-1}/r_1$ [at 1.5 T], 3.6 $mM^{-1}s^{-1}$). In distinction, r_1 of P846 remarkably decreases at 9.4 T compared with 1.5 T (P846: r_1 [at 9.4 T], 6.4 $mM^{-1}s^{-1}/r_1$ [at 1.5 T], 32 $mM^{-1}s^{-1}$). In this study, gadopiclenol provided the highest r_1 at 9.4 T with a less apparent reduction of r_1 as compared with lower field strength (gadopiclenol: r_1 [at 9.4 T], 8.7 $mM^{-1}s^{-1}/r_1$ [at 1.5 T], 12.7 $mM^{-1}s^{-1}$).

For the intraindividual comparison in vivo, P846 and gadopiclenol provided significantly higher signal-to-noise ratio, contrast-to-noise ratio, and lesion enhancement as compared with the low-molecular control agents ($P < 0.001$ for all parameters and examination time points).

Conclusions: Based on the higher hydration number, r_1 of P846 and gadopiclenol are markedly higher and provide superior enhancement in vivo as compared with the reference contrast agents gadopentetate and gadoterate. However, the macromolecular agent P846 shows a marked decrease of r_1 from 1.5 T to 9.4 T. This effect is less apparent for the low-molecular agents gadopiclenol, gadopentetate, and gadoterate. Thus, creating molecules with moderately increased size and hydration number, as implemented in gadopiclenol, seems to be a promising way to develop highly effective Gd-based contrast agents for a broad spectrum of field strengths.

014:

Precision MRI Enabled by Protein MRI Contrast Agents

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Purpose: Precision medicine to chronic diseases, especially for cancer and fibrosis, requires noninvasive precision diagnostics. Noninvasive precision imaging capable of early detection, staging, and molecular subtyping/stratification of patients is a major unmet need. Rapid development and approval of precision medicine also requires precision imaging to evaluate drug efficacy at animal and patient levels.

Methods and Materials: In this study, I will first introduce our pioneering strategy to develop a novel class of protein MRI contrast agents (ProCA) to enable precision imaging by MRI. We have successfully designed an array of ProCAs with significant improvement of both r_1 and r_2 relaxivities at both 1.4 and 7.0 T and the capability to quantify key biomarkers expressed on various types of cancers, metastases, stroma, and fibrosis, with significant improvement in sensitivity and specificity. Importantly, these biomarker targeted contrast agents exhibit strong metal stability and selectivity against transmetallation.

Results: The biomarker targeted contrast agents enable the first robust detection of early-stage liver and lung fibrosis from several different mouse models via dual contrast modes. By probing tumor microenvironment, we are able to detect premalignant dysplasia hepatocellular carcinoma nodules, micrometastasis, and tumor permeability via novel dynamic molecular imaging. We have achieved direct visualization and 3-dimensional mapping of differential molecular radiomics in various types of fibrosis and cancers.

Conclusions: Rapid development of ProCAs for IND-enabled regulatory studies is expected to have broad applications in early detection, monitoring progression, image-guided intervention, patient stratification, and accelerated facilitation of therapeutic drug development.

015:

Next-Generation Cancer Magnetic Resonance Imaging With Long-Acting Tumor-Targeted Alkylphosphocholine Gd-Chelates

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Purpose: The aims of this study was were synthesize, characterize, and validate a broad-spectrum tumor-targeted macrocyclic alkylphosphocholine (APC) chelate magnetic resonance imaging (MRI) contrast agent, Gd-NM600, and to identify and track the chemical speciation and spatial localization of this new chelate in order to understand its Gd deposition properties.

Methods and Materials: Gd-DO3A-APC or Gd-NM600 is an extension of our APC analog technology, which has previously demonstrated broad-spectrum cancer-specific uptake in both primary and metastatic tumors in clinical trials. We added a chelating moiety capable of binding gadolinium and many other metals for cancer-targeted MRI. The T1-weighted relaxivities were characterized in water and plasma at 1.5 T and 3.0 T. Tumor uptake and subcellular localization studies of Gd-NM600 were performed using transmission electron microscopy. We imaged (dose 0.12 g/kg) over 10 different rodent models of human cancer and compared the T1-weighted imaging results with that of a commercial macrocyclic chelate, Gd-DOTA. Finally, MALDI mass spectrometry imaging (MSI) was used to characterize the biodistribution and the chemical speciation of Gd-NM600.

Results: Gd-NM600 exhibits high T1 relaxivities (approximately 16.4 s-1/mM at 1.5 T) and excellent tumor uptake (3.95 %ID/g at 48 hours). In rodent models of cancer, significant uptake, prolonged tumor retention, and minimal uptake saturability of Gd-NM600 were observed. Broad-spectrum tumor-specific uptake was demonstrated

in over 10 different human cancer models. Cancer cell uptake of Gd-NM600 via endosomal internalization and processing was revealed with transmission electron microscopy. MALDI-MSI successfully interrogated the spatial localization and chemical speciation of Gd-containing compounds.
Conclusions: Gd-NM600, a macrocyclic, tumor-targeted Gd chelate, achieves prolonged broad-spectrum tumor uptake and retention. Gd-NM600 targets via a distinct cancer-targeting mechanism of APC chelates that is not receptor-mediated, high capacity, and appears to be selective for a broad array of cancers. In addition, we demonstrate a specific and sensitive method to identify and spatially map the presence and the chemical form of our compound in tissue samples through MALDI-MSI analysis, a new promising tool for studying the clinically problematic Gd deposition phenomenon in normal tissue. In addition to affording a significant high-resolution alternative to PET for oncologic imaging, a tumor-specific, long-acting MRI contrast agent would enable much better tumor location and motion management and treatment planning for new MR-guided external beam radiotherapy systems.

MONDAY, NOVEMBER 11
SESSION 4: ULTRASOUND AGENTS

016:

The Conception of Ultrasound Contrast Agents as Social Process

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Purpose: The aim of this study was to appreciate the conception of echo-contrast agents as a social process.
Materials and Methods: Publications, patents, and letters from historical protagonists were examined.
Results and Conclusions: Technological innovation is a social process involving interference of distinct knowledge cultures of individual and institutional protagonists. A particular process model passed plausibility tests in the case of x-ray contrast agents (CAs). Now the same was found to hold for the genesis phase of echographic CAs. Analogical reasoning lead from accidental observation of echographic clouds at vascular saline injection sites (Joyner, 1963) to the use of saline-associated microbubbles as echocardiographic CA (Holmes, 1967). Solutions of indocyanine green and dextrose, or diluted blood, simply shaken or treated with ultrasound, became also used. In 1984, a committee of the American Society of Echocardiography summarized the safety of the technology. For the technology to get out of stagnation, input from knowledge cultures other than the academic ones was obligatory, in particular, entrepreneurship, engineering, and patenting. Exploration of methods for measuring local blood pressure by ultrasonic resonance and cavitation of microbubbles emerged from analogous studies of cavitation-mediated erosion of ship propellers. In natural waters, microbubbles were long known to be stabilized by natural surfactants. From there, it was a small step to the nascent technological vision of stabilized microbubbles as CA. This step was made by N. Rasor (1977) at Rasor Associates Inc. Prototypical products were patented. In 1981, the intellectual property was sold to Schering AG, which developed galactose microcrystals. In 1991, it offered the first commercial CA, initially without (Echovist), and in 1995 with palmitic acid as stabilizer (Levovist). Schering AG's entrance into the field coincided with additional actors joining the vision of CA. In 1983, the academic clinician Feinstein invented the creation of microbubbles by ultrasonication, and in 1985, he patented sonicated 5% serum albumin. Molecular Biosystems Inc in 1987 patented its own version thereof and its manufacturing. Named Albunex, in 1994, it was launched by Mallinckrodt as the first CA in the United States. By 1995, a dozen of companies had embraced the vision. Benefitting from ever improving equipment and the first CA, echographers developed new indications and overcame their aversions against CA. The author has no direct or indirect financial interest in the products under investigation or subject matter discussed in the manuscript.

017:

Targeting Microbubbles to the Tumor Vasculature via Microbubble-Lectin-Endothelium Biomechanics

P. Zhang, A. Khan, G.B. Diakova, and A.L. Klibanov. *sklib1@gmail.com; sasha@virginia.edu.*
Purpose: Contrast agents for molecular ultrasound imaging aim at molecular biomarkers overexpressed on tumor vascular endothelium: VEGFR2, alpha$_V$beta$_3$, VCAM-1, and so on. We propose an alternative contrast ultrasound tumor imaging technique, based on distinct vascular biomechanics. Tumor neovasculature is abnormal and meandering, unlike the organized linear vasculature in normal tissues. Blood velocity in tumor vasculature is lower than in the norm. Tomato lectin (TL, *Lycopersicon esculentum*, a 71-KDa protein) is a known vascular endothelium stain. We report TL-decorated microbubbles (MBs) that selectively adhere in the tumor areas with slow blood flow: they may have some affinity to vascular endothelium in all vessels, but they would be sheared off endothelium in the conditions of high flow in the normal vasculature, so adhesion occurs preferentially in low shear flow areas.
Methods and Materials: Decafluorobutane MBs were made with DSPC/PEG stearate shell. Biotinylated MBs, prepared by sonication, contained biotin-PEG-DSPE (1:20 mass ratio to DSPC); biotinylated TL was attached to MBs via streptavidin. Alternatively, MBs suitable for practical translation, streptavidin-free, were prepared by Vialmix amalgamation: preparation medium was aqueous saline with propylene glycol, DSPC, PEG stearate, and DSPE-PEG-TL, covalently coupled. In vivo study was performed on MC38 murine colon adenocarcinoma (a generous gift by J. Schlom, NCI) subcutaneous hind limb tumor in C57BL/6 mice under isoflurane. Ultrasound imaging was performed with Siemens Sequoia 512 (15L8 probe, 7 MHz, CPS mode, MI 0.2). Contralateral leg muscle served as a negative control.
Results: Tomato lectin coupling to MB shell was confirmed by fluorescence microscopy and spectroscopy; ~2.10^4 molecules per MB were attached, via streptavidin-biotin or via a PEG-tethered DSPE lipid anchor. Tumor interior blood velocity, assessed by tracking of circulating MBs, was significantly lower than in the periphery, or in the control leg muscle. TL-MB adhered on MVEC cells in a parallel plate flow chamber at 0.25 and 0.5 dyn/cm^2 but not at 4 dyn/cm^2. TL-MBs selectively bound to tumor vasculature and delineated murine tumor 10 minutes after intravenous bolus of contrast; signal in the control leg muscle was minimal ($P < 0.01$).
Conclusions: Tomato lectin–decorated MBs adhere to tumor vasculature, with its slow blood flow, and not in normal vessels. This ultrasound contrast, based on tumor vasculature biomechanics, may become a general tool for solid tumor imaging, if tumor-specific molecular biomarkers are not easily targetable.

018:

Quantitative In Vitro Evaluation of Ultrasound Contrast-Specific Mode Sensitivity

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Purpose: The aim of this study was to explore the sensitivity of ultrasound contrast-specific modes from 4 high-end clinical scanners over a range of parameters such as imaging depth, flow rate, vessel diameter, and contrast agent.
Methods and Materials: A flow phantom was constructed within a Perspex tank (10 × 11 × 20 cm) with 6 C-flex tubes suspended horizontally across the tank and exiting through engineered holes in the Perspex ends. Three tubes were of internal diameter (ID) 1.6 mm and the remaining 3 of ID 3 mm. Tubes of each diameter were centered at depths of 1, 5, and 8 cm from the surface of the tank. Once the tubes were in position, the tank was then filled with an agar-based tissue-mimicking material. A reservoir of degassed water or diluted contrast agents (SonoVue Bracco) or Definity (Lantheus Medical Imaging) was continuously stirred and pumped sequentially through each of the tubes at 2 flow settings—0.25 mL/s and 1.5 mL/s using a Micropump suction pump. All tubes within the phantom were scanned at all depths using 4 transducers: phase inversion with curvilinear probe (GE LogiqE9, C2-9), cadence contrast pulse sequencing with curvilinear probe (Siemens, 6C1), phase inversion with linear array probe (Philips, L9-3), and second harmonic imaging with linear array probe (Esaote, LA523). Images were acquired when the tubes were full of water and when contrast enhancement had reached maximum backscatter intensity, which was assessed visually by the operator. The images were downloaded in DICOM format and analyzed using Analyze 11.0 (Mayo Clinic Rochester, MN) by one observer who was not blinded to the study. Nine regions of interest (ROIs) were placed on contrast-enhanced image, and mean intensity of the final 18 ROIs were exported to an excel spread sheet. Mean intensity in the 3 ROIs within the tubing acquired using contrast-specific software were compared.
Results: For the Siemens, Philips, and Logiq9 scanners, there was no significant difference in intensity when different flow rates or contrast agent were used within the same tube. For all scanners, maximum enhancement was observed from the most superficial pipes but the Philips scanner and Logiq9 scanner yielding significantly higher enhancement compared with the Siemens scanner at all depths. Interestingly, the Esaote scanner demonstrated the highest enhancement under all conditions with Definity in the most superficial tube, but Definity could not be visualized in deeper tubes.
Conclusions: Different contrast-specific modes present different sensitivity for microbubble contrast harmonics according to depth.

019:

Controlled Microbubble Inflation for Ultrasound Tumor Imaging and Therapy

C.J. Brambila, J. Lux, R.F. Mattrey, D. Boyd, M.A. Borden, and C. de Gracia Lux. *Robert.Mattrey@UTSouthwestern.edu.*

Purpose: Acoustic droplet vaporization was introduced over 20 years ago to embolize tumors with large microbubbles (MBs) noninvasively by converting 2-μm perfluorocarbon (PFC) droplets with ultrasound (US) in the feeding artery, and more recently cavitating 300-nm nanodroplets (NDs) to improve detection. Acoustic droplet vaporization has yet to reach clinical testing because of critical efficacy and safety issues. We introduce a novel theranostic platform that uses PFC NDs to inflate PFC-filled bubbles without the need for US activation and with possibly less adverse effects.

Methods and Materials: Nanodroplets, MBs, and nanobubbles were formulated using described procedures and characterized using tunable resistive pulse sensing, DLS, and Multisizer 4 Coulter counter systems. To quantify MB inflation, we used a multiwavelength BD Accuri C6 flow cytometer. We validated MB inflation using an Axio A1 upright fluorescence microscope equipped with a high-speed ORCA-spark Digital 2.3 megapixel CMOS camera and the HCImage Live software. US imaging at 8 MHz used a clinical Acuson Sequoia 512 system equipped with a 15L8 transducer. In vivo studies used intravital microscopy of nude mice.

Results: Ultrasound imaging confirmed that when liquid PFC NDs come in close proximity to PFC gas-filled nanobubbles, the latter inflate by 2 orders of magnitude to become visible on US without the need for US activation. Microscopy showed MB expansion under stationary and flow conditions, and showed that inflated MBs can occlude a 200-μm tube. Flow cytometry showed that not only did PFC transfer from NDs to MBs, but also the shell lipids and lipophilic payloads transferred. When NDs were targeted to MBs, the same rate and degree of inflation occurred at 1/10th the ND dose. In the hope of controlling MB inflation, we show that formulation parameters affect the rate of inflation, but only polymeric MB shells limited the ultimate size of inflated MBs, enabling safe diagnostic applications. Using nude mice, we confirmed that MB inflation occurs in vivo.

Conclusions: These results explain in part the observed clinical adverse effects of perfluoropentane emulsions (Echogen) and point toward a potentially safe theranostic tool for clinical translation. For the purpose of tumor ablation, the only available MBs when NDs are administered should be adherent in tumors, and for the purpose of diagnostic use, inflated MBs must not exceed 3 to 5 μm to eliminate potential adverse effects. It is promising to note that NDs targeted to MBs can accomplish the theranostic goals at 1/10th the ND dose to further limit adverse effects. The study was supported by CPRIT RR150010.

TUESDAY, NOVEMBER 12
SESSION 5: NEW AGENTS, APPLICATIONS

020:

Ferumoxytol Improves Evaluation of Vascular Involvement in MRI of Sarcomas in Children/Adolescents

F. Siedek, A.M. Muehe, A.J. Theruvath, R. Avedian, A. Pribnow, S.L. Spunt, T. Liang, C. Farrell, and H.E. Daldrup-Link. *heiked@stanford.edu.*

Purpose: Small molecular gadolinium (Gd)-chelates for magnetic resonance imaging (MRI) have been associated with adverse effects, such as nephrogenic systemic fibrosis, in patients with renal failure and Gd deposition in the brain after repetitive administration. Thus, alternatives to Gd-chelates with comparable or even better disease assessment are urgently needed. The purpose of our study was to compare the value of ferumoxytol (FMX)- and Gd-chelate–enhanced MRI for evaluation of bone and soft tissue sarcomas in pediatric and adolescent patients. We hypothesized that tumor size and assessment of tumor vascular involvement are equivalent or superior on FMX-enhanced MRI scans compared with Gd-enhanced MRI scans.

Methods and Materials: In this institutional review board–approved retrospective study, we analyzed MRI data of 22 patients (16 males; mean age, 15.3 ± 5.0 years) with newly diagnosed bone or soft tissue sarcoma, who underwent both an FMX-MRI and Gd-MRI within an interval of 4 weeks or less. Magnetic resonance imaging sequences included T1-liver acquisition with volume acceleration (LAVA), T1-spinecho (SE), and fat-saturated T2-fast SE (FSE) sequences. In the FMX-MRI protocol, all 3 sequences were ferumoxytol-enhanced, whereas in the Gd-MRI protocol, T1-SE and T2-FSE were unenhanced. Three-dimensional tumor volumes, signal-to-noise ratios (SNRs) of the primary tumor, and adjacent vessels and corresponding contrast-to-noise ratios (CNRs) were compared between FMX-MRI and Gd-MRI scans using multivariate analysis with a generalized linear model. In addition, morphologic parameters regarding neurovascular involvement and tumor thrombi were rated by 4 readers using a Likert scale. Corresponding results were pooled across readers and compared between FMX- and Gd-MRI scans using multivariate analysis with a mixed-effect model.

Results: Ferumoxytol MRI and Gd-MRI did not show significantly different tumor volumes on T1-LAVA ($P = 0.721$), T1-SE ($P = 0.290$), and T2-FSE ($P = 0.609$) sequences. Compared with Gd-MRI, FMX-MRI showed equal tumor SNR on T1-SE ($P = 0.104$) and T2-FSE ($P = 0.305$) and significantly lower tumor SNR on T1-LAVA ($P < 0.001$). Ferumoxytol MRI showed significantly higher tumor-to-vessel ($P = 0.003$) CNR on T1-LAVA (see image). Neurovascular bundle involvement and tumor thrombi were assessed with significantly higher confidence on FMX-MRI (both $P < 0.001$) compared with Gd-MRI.

Conclusions: Ferumoxytol MRI allows equal evaluation of tumor size of bone and soft tissue sarcomas and better assessment of neurovascular involvement and tumor thrombi compared with Gd-MRI. Thus, FMX-MRI is a valid alternative for tumor staging in pediatric patients with bone and soft tissue sarcoma.

021:

Parametric Study of Flow Processes for the Synthesis of Iron Oxide Nanoparticles in Polyol Media

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Purpose: Many synthetic methods were developed to produce magnetic iron oxide nanoparticles (ie, maghemite and magnetite) with good control over size, shape, and composition. The thermal decomposition appears as the best procedure to obtain crystalline and monodisperse nanoparticles. However, even if the technique arouse a great interest from the scientific community, it is important to note that most of the studies imply production at the lab-scale (hundred milligram-scale). The transposition to industrial production requires the development of scale-up challenges ensuring reproducibility and cost-effectiveness, as well as the use of innocuous reagents. Since then, we propose a scale-up approach based on the continuous flow process technology and synthesized particles in polyol media to obtained hydrophilic nanoparticles. The influence of the different synthesis parameters over the particle's physico-chemical properties is reported.

Methods and Materials: Iron oxide nanoparticles were synthesized by thermal decomposition method in a continuous flow process in polyol media. The particles obtained were purified by diethylether extraction, precipitation in acetone/diethylether, and acid washing. The particles were redispersed in water and centrifugated before characterization by dynamic light scattering, transmission electron microscopy, vibrating sample magnetometer, and relaxometric measurements.

Results and Conclusions: During the syntheses, a 1-m capillary reactor with an internal diameter of 1 mm and 2.4 mm were used. The flow rate, the ratio of iron precursor/stabilizing agent, and the solvent nature were varied. At the end of the process, water-stable particles were obtained. Depending on the experimental conditions, various particles properties (ie, magnetization, relaxivities, and size distribution) were observed. According to the application, the synthesis conditions can be adapted to obtain particles having the desired size and properties.

022:

Renal Secreted Sub–5-nm Iron Oxide Nanoparticles: Improved Targeting, Delivery, and T1-Weighted MRI

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Purpose: The unique chemical and physical properties of magnetic iron oxide nanoparticles (IONPs) offer many advantages for noninvasive, biomarker-targeted MRI applications. However, slow clearance, long liver retention time, and dominant dark T2-weighed contrast are not desirable. We have developed sub–5-nm unltrafine iron oxide nanoparticles (uIONPs) that offer T1-weighted contrast and favorable pharmacokinetics with renal clearance while maintaining surface functionalization for biomarker targeting. The size-dependent effect on MRI contrast, tumor delivery, and targeting were investigated using multimodal imaging in tumor-bearing animal models.

Methods and Materials: Oligosaccharide-coated uIONPs with a core size of 3 nm were first synthesized after our previously published method. Hydroxyl groups of oligosaccharide coatings were partially ammoniated with ammonia hydroxide solution. Fluorescent dye TRITC or FITC can be conjugation on ammoniated uIONPs. Similarly, transferrin-targeting transferrin receptor was conjugated on uIONP. IONPs with 10 and 20 were prepared as controls for studying the size effects. uIONPs and IONPs before and after functionalization were characterized using TEM, DLS, and fluorescence microscopy. r_1 and r_2 relaxivities of uIONPs and IONPs were measured at 3 T. Transferrin receptor targeting specificity was confirmed using cancer cell lines and blocking experiments. T1-weighted contrast was tested in mice bearing intracranial

U87 brain tumor and subcutaneous 4T1 breast tumor using the T1-weighted spin echo sequence and 3-T MRI scanner. Renal clearance and intratumoral delivery were investigated with MRI, NIR imaging, and multiphoton microscopy.
Results: uIONPs were highly stable in aqueous solution and in blood. With Gd-DTPA comparable r_1 value (5 mM $mM^{-1}s^{-1}$ and r_2/r_1 at 3.2), uIONP enhanced brain tumors and breast tumors in T1-weighted spin echo imaging 20 minutes after the intravenous injection. Multiphoton imaging and confocal fluorescence imaging tracking of equal amounts of active targeting and nontargeting uIONPs (or IONPs) labeled with different fluorescent dyes coinjected into the same mice bearing 4T1 mammary tumors at 1, 3, and 24 hours after coinjection revealed that active targeting uIONPs exhibited 6 times higher level of tumor retention with deeper penetration comparing to nontargeting uIONPs at 24 hours after coinjection. However, accumulation of active targeting IONPs with a 30-nm core is only 1.5 times higher than nontargeting IONPs.
Conclusions: With renewed interests in using IONP for clinical imaging, uIONP provides a promising platform for T1-weighted MRI with expanded capabilities and good biosafety profile.

023:

ZD2-(^{68}Ga-NOTA) Specific to EDB Fibronectin for PET Imaging of Pancreatic Cancer

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Purpose: Currently, there is an unmet clinical need for novel imaging technologies for accurate detection of pancreatic cancer at treatable stages. PET is a quantitative and sensitive imaging modality and routinely used for cancer molecular imaging. Accurate early detection of pancreatic cancer with a size of 10 mm or less could dramatically improve patient survival after timely treatment. The goal of this work is to develop a novel PET probe specific to extradomain B fibronectin in tumor microenvironment for accurate early detection and diagnosis of aggressive tumors, including pancreatic cancer.
Methods and Materials: A targeted ligand ZD2-NOTA was synthesized by conjugation of a macrocyclic ligand NOTA to a linear ZD2 peptide (Thr-Val-Arg-Thr-Ser-Ala-Asp) via a 6-aminohexanoic acid spacer. ZD2-(^{68}Ga-NOTA) was synthesized by relabeling of ZD2-NOTA with $^{68}GaCl_3$ in a high purity under GMP conditions. Mouse pancreatic cancer models were developed by subcutaneous inoculation of BxPC3 and Capan-1 human pancreatic cancer cells. The expression of EDB-FN was determined in the cancer cells and tumor tissues by Western blotting and immunohistochemistry. PET/CT images were acquired with the mice bearing BxPC3 and Capan-1 xenografts on a microPET/CT scanner after 1 and 2 hours after intravenous injection of the tracer.
Results: The targeted ligand ZD2-NOTA was synthesized in a high purity of 98% and characterized by HPLC and MALDI-TOF. ZD2-(^{68}Ga-NOTA) was also prepared in high yield and purity. High expression of EDB-FN was shown in the cancer cells and tumor tissues. ZD2-(^{68}Ga-NOTA) resulted in significantly higher uptake in both the BxPC3 and Capan-1 tumor xenografts than normal organs and tissues, including the brain, heart, liver, and muscle, at 1 hour postinjection in mice. The tumor-to-muscle uptake ratio was at least 5-fold for the tracer in both tumors. PET with ZD2-(^{68}Ga-NOTA) was able to clearly delineate the small pancreatic tumors with minimal background noise in normal tissues, including the liver. Substantial tumor uptake is still visible at 2 hours postinjection.
Conclusions: The peptide-targeted PET probe ZD2-(^{68}Ga-NOTA) specific to EDB-FN is an effective sensitive detection of pancreatic cancer and has potential to be translated for early detection and clinical management of pancreatic cancer.

024:

Mechanistic Studies on a Novel Hybrid Cell-Penetrating Peptide

L. Gong, S. Kothandaraman, D. Dusane, P. Stoodley, and M.F. Tweedle. *Michael.tweedle@osumc.edu.*
Purpose: The first promising HNSCC (head and neck squamous cell carcinoma)-targeted peptide HN1 was discovered by phage display but was hampered by a slow internalization process. 4lphf-HN17 (4Iph)(f)LPNSNHIKQGL (f, fmoc; 4Iph, 4-iodophenyl) was developed to overcome this flaw. Dye-labeled 4lphf-HN17 internalizes into HNSCC cells 26-fold faster than dye-labeled HN1 in 1 hour. We studied its uptake in live Cal-27 cells in the presence of endocytosis inhibitors, uptake and internalization kinetics, cell viability, and membrane integrity.
Methods and Materials: Synthesis and other studies are reported in Kothandaraman (CMR 2019). IR800 and Cy5 conjugates were studied for plate reader or confocal microscopy, respectively. Human HSNCC Cal-27 (ATCC) was maintained by standard cell culture methods. Confocal fluorescence microscopy used an Olympus FluoView FV1000. Four common endocytosis pathways facilitate cell-penetrating processes: clathrin-dependent, caveolin-dependent, clathrin/caveolin-independent, and macropinocytosis. A nonendocytosis pathway can involve direct membrane transduction. Specific pathway inhibitors were used to block the relevant processes: 10 nM sodium azide (blocks endocytosis by depleting the cellular ATP pool), 100 nmol/mL nocodazole (inhibits clatherin-coated pits formation), 5 μM methyl-beta-cyclodextrin (inhibits the lipid raft-mediated caveolae pathway), 50 μM chlorpromazine (inhibits clathrin-independent pathway), and 5 mM amiloride (inhibits macropinocytosis). Propidium iodide was used to detect membrane permeability. Resazurin was used to detect cell viability. Quantitative assessment of 4lphf-HN17-FITC cell association was performed on a flow cytometer.
Results and Conclusions: Cell uptake kinetics showed accumulation of 4lphf-HN17-Cy5 in cytosol detectable in 5 minutes. IC50 for cell viability at 24 hours was 20 μM (at 1 hour, 50 uM failed to kill cells). At 4°C, all uptake is blocked; otherwise we were unable to block internalization. Propidium iodide did not penetrate the cell membrane unless 5 uM peptide was added, whereupon in stained the nuclei, suggesting either edosomal escape or direct internalization facilitated by 4lphf-HN17. The data suggest that 4lphf-HN17-Cy5 cell internalization occurs through either a direct transduction process or an unusual endocytotic pathway, or both. There is a significant concentration window between internalization and toxicity.

025:

Prepolarized MRI at Earth Field to Seek New Contrasts Linked to Molecular Events for Very Early Detection of Pathologies: PRIMOGAIA Project

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Purpose: PrimoGaia (FETOPEN project, Horizon 2020–Work Programme 2018–2020; Future and Emerging Technologies) opens up a new pathway in magnetic resonance imaging (MRI) by initiating the concept of in vivo "enzymatic imaging" for a better understanding of human physiology, early detection and prognosis of diseases, and monitoring of therapeutic treatment. The main objective is to overcome current boundaries by making it possible to map and quantify the activity of an enzyme in a pathological tissue.
Methods and Materials: It will be accomplished by building an MRI instrumentation operating at earth field in order to allow the use of 70 to 150 MHz frequency for saturating the electron paramagnetic resonance (EPR) transition of nitroxyl radicals. Enzyme activity is assessed by the use of suitable probes designed to generate the radical upon action of the enzyme of interest. Upon saturation of the radical EPR resonance, polarization is transferred to the protons of the water molecules (Overhauser MRI).
Results: The enhancement factor will be high (more than 2 orders of magnitude). The polarized water signal thus reports on the local concentration of the radicals that reflect the enzymatic activity. Three lines of activity will be addressed to generate the radicals of interest, namely, (1) using a radical-containing molecular precursor that, upon the action of specific enzyme, yields a radical whose absorption frequency is sufficiently different to be selectively irradiated (U Marseille); (2) the use of paramagnetic impurities on nanodiamond surfaces to increase the OMRI effect (U Mons); and (3) the use of radical precursors as "prodrugs" generating a signal only after their activation (U Torino). The overall methodology will be much less expensive than the current clinical scanners and will allow distribution in developing countries. PrimoGaia brings together an interdisciplinary consortium of research teams from 4 academics (CNRS Marseille, U Mons, U Torino [reagents], CNRS Bordeaux [EPR unit, sequences]), Fraunhofer (physics), and 2 companies ("Stelar" [magnetic unit] and "Pure Device," an innovative SME [MRI instrumentation]). The project will be coordinated by the CNRS (Bordeaux).

TUESDAY, NOVEMBER 12
SESSION 6: THERANOSTIC AGENTS 1

026:

Prostate-Specific Membrane Antigen Targeted Gold Nanoparticles as X-ray CT Contrast Agents in Targeted Radiotherapy: Does Size Matter?

D. Luo, X. Wang, S. Zeng, G. Ramamurthy, C. Burda, and J.P. Basilion. *james.basilion@case.edu.*
Purpose: X-rays were first discovered in 1896 by Dr Roentgen and were first tried as a cancer therapy shortly after in 1899 and remain a main component of the radiotherapeutic approaches to treat prostate cancer. Although radiosensitizers have

been developed to increase the efficacy of radiotherapy, no radiosensitizer has been developed to selectively target prostate cancer. The purpose of this work is to develop a prostate cancer selective radiosensitizer to improve radiotherapy of prostate cancer.
Methods and Materials: Gold is a strong absorber of x-ray radiation and can be used to amplify the therapeutic efficacy of x-ray therapy. Here we have targeted gold-nanoparticles (AuNPs) and gold nanoclusters (AuNCs) and utilized them to sensitize tissues to x-ray therapy. For the first time, we systematically investigate both AuNP size and a new targeting ligand on cell uptake, tumor targeting, and radiotherapy efficacy. AuNPs and AuNCs were conjugated with a ligand highly selective for prostate-specific membrane antigen (PSMA) and their uptake and ability to increase radiosensitivity measured both in vitro and in vivo. AuNPs ranging in size from 2 to 19 nm core diameter and AuNCs (1.7 nm diameter) were investigated.
Results: With increasing AuNP size, the total amount of gold internalized was slightly improved. However, enhancement of radiotherapy was significantly more pronounced by internalization of smaller PSMA targeted-AuNPs. The smaller AuNPs that were targeted with PSMA-1 also showed a high degree of selectivity for tumors that overexpressed the PSMA receptor. Interestingly, the AuNPs showed significant uptake and clearance through the RES and liver while the much smaller AuNCs showed primarily renal clearance with much less RES excretion, but still remained effective as radiosensitizers.
Conclusions: These data suggest that small targeted AuNPs may be more effective radiosensitizers. Further reduction of the size of the nanoparticles, that is, gold nanoclusters, significantly altered their excretion profile. The more rapid excretion of the AuNCs may serve to reduce off target radiosensitivity and also, due to reduced gold deposition in nontargeted tissues, a reduction in elemental gold toxicity.

027:

Alginate Microcapsules for Enhanced Magnetic Hyperthermia Treatment

Y. Fu, P. Korangath, C. Weiss, R. Ivkov, and D.L. Kraitchman. *dkraitc1@jhmi.edu.*
Purpose: Nonablative heat therapy, also known as hyperthermia (HT), for locally advanced or recurrent cancers is an established treatment modality that can enhance the potency of radiation therapy and chemotherapy without inducing significant adverse effects. An increase in the local temperature between 41°C and 46°C enhances tissue blood perfusion and inhibits DNA damage repair, improving treatment response rates and increasing overall survival. Magnetic hyperthermia therapy (MHT) involves the delivery of magnetic iron-oxide nanoparticles (MIONs) to tumors and the application of alternating magnetic fields to create local HT. However, to achieve an effective MION dose to a tumor, large quantities of MIONs must be infused intravenously. Alternatively, direct intratumoral delivery requires slow infusions to overcome the intrinsic tumor hydrostatic pressure. With either method, persistence of the MIONs intratumorally may be short-lived. The purpose of the current study was to create a microbead that could be used to deliver a high payload of MION intratumorally in a minimally invasive manner.
Methods and Materials: Magnetic hyperthermia therapy microcapsules (MHTCs) containing alginate and BNF-Starch MIONs (Micromed Partikeltechnology) were generated using an electrostatic droplet method. Briefly, alginate was mixed with 1.92 (low concentration) to 28.92 (high concentration) mg/mL iron in 1.2% (vol/vol) alginate that was subsequently extruded through a 25-gauge blunt-end needle into a $CaCl_2$ solution followed by crosslinking with poly-L-lysine. Magnetic hyperthermia therapy microcapsule morphology was determined from optical imaging (Nikon Eclipse Ti-U). In vitro imaging of a 6-well plate phantom containing various concentrations of MHTCs was performed on a 1.5-T MR scanner (Espree, Siemens Healthcare). In vivo, MHT was performed after percutaneous delivery of MHTCs to the thigh of anesthetized mice. Temperature adjacent to the MHTCs was measured using a fiber optic probe. Core rectal temperature was also measured during MHT.
Results: Magnetic hyperthermia therapy microcapsule average diameter was 161 ± 3.7 μm. In vitro imaging demonstrated a large susceptibility artifact on T2*-weighted imaging such that single, low-concentration MHT caps were visible. At a 0.57 mg Fe/100 μL loading of MHTCs, no heating was observed in vivo. Definitive heating of muscle occurred with high-concentration MHTCs at 600 V and was sustained at 300 V. At necropsy, the nanoparticles were intact and confined to the injection site.
Conclusions: Magnetic hyperthermia therapy using MION-loaded alginate capsules shows promise for oncological and potentially nononcological applications.

028:

Diagnostic and Theranostic Applications of an Ultrasound Contrast Agent Nanoplatform

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Purpose: Recently, several investigations have proposed perfluorocarbon (PFC)-based nanodroplets as potential new technology for ultrasound (US) imaging. Currently, octafluoropropane, decafluorobutane, and dodecafluoropentane, or a combination of those PFCs, are used for the preparation of nanodroplets. However, decafluorobutane and dodecafluoropentane nanodroplets require a higher mechanical index for US imaging, and octafluoropropane nanodroplets are known to have insufficient stability that limit their biological application. We have developed a new methodology to produce stable, low-boiling point nanoscale phase-change agents that can serve as a platform technology for imaging and therapy. The purpose of these studies was to characterize the biophysical and chemical properties of these novel nanodroplets and test them in a variety of applications in in vitro models.
Methods and Materials: The stability of the formulation was tested at different temperatures (4°C, 20°C, and 37°C) and time points using a nanosize analyzer. The morphology and the size of the nanodroplets were visualized by transmission electron microscopy. Raman spectroscopy was used to quantify the gas content of the different formulations at different time points. Stable formulations of nanodroplets were achieved by our proprietary innovative methodology.
Results: Particle size distribution showed that the size of the droplets remained similar at the different conditions tested. Transmission electron microscopy images showed spherical and stable nanodroplets at room temperature. Our new methodology generated nanodroplets that remained more stable after incubation at 37°C. Raman results confirmed that the PFC gas content remained the same in the novel formulation as compared with previous formulation. Finally, in order to validate the therapeutic use of the nanodroplets, we have functionalized these nanodroplets for the detection of tau aggregates and fibrin clots.
Conclusions: We present preliminary results regarding their effects on these protein aggregates upon US activation. In conclusion, we have developed a strong multifunctional nanoplatform using a novel methodology. These results show promising applicability for enhanced theranostic of the phase change ultrasound contrast agents.

029:

Neurotheranostic for MRI of Dopamine Transporters and Treatment of Dopaminergic Neurodegeneration

G.D. Vitaliano, J. Kim, D. Mintzopoulos, C. Adam, G. Zeballos, and F. Vitaliano. *gvitaliano@mclean.harvard.edu.*
Purpose: Neurotheranostics (NTs) show great promise for diagnostic imaging and treatment of neurodegenerative disorders. Neurotheranostics consist of combined central nervous system diagnostic and therapeutic agents, and can be used to enhance magnetic resonance imaging (MRI) sensitivity and molecular targeting by delivering superparamagnetic iron oxide (SPIO) contrast agents along with antibodies. However, NTs cannot cross an intact blood-brain barrier (BBB) limiting their use in neurology/psychiatry. Our goal was to develop a novel NT for targeted, noninvasive delivery of DAT-antibody (DAT-Ab), SPIO, and brain-derived neurotrophic factor (BDNF) to dopamine brain regions.
Methods and Materials: Neurotheranostics were synthesized by conjugating DAT-Ab and SPIO for MRI (plus BDNF for treatment) to clathrin carrier using polyethylene glycols (PEGs) at 1:1:1:1 molar ratio, and NT's size and structure were characterized. For 7 days, iTat mice received intraperitoneally doxycycline (100 mg/kg per day) to induce neurotoxic HIV-Tat protein expression. Concurrently, Tat+ mice received intranasally saline or NTs (BDNF, 300 μg/kg per day). For MRI, wild-type C57BL/6J mice received one dose of intranasal saline or NTs (SPIO, 68 pmol, 5 0μL). Four hours after the last NT/saline dose, mouse brains were prepared for immunohistochemistry or ex vivo MRI. Voxel-wise R2* relaxation rates were obtained using a series of gradient-echo images (TR = 1.5 seconds; TE = 3.2, 4, 5, 6, 7, 8, 9, 10 milliseconds; 128 × 128 in-plane matrix; 0.2 mm resolution; 64 slices at 0.5 mm thickness; 7 averages). R2* values in the striatum (STR), substantia nigra (SN), and visual cortex (vCTX), a control region with low DAT expression, were calculated.
Results: The iron-stained brain slices contained NTs in brain regions rich in DAT (eg, STR). Striatal tyrosine hydroxylase densities were higher ($P = 0.0467$) in NT versus saline-treated Tat+ mice. MRI studies of NT with DAT-Ab-SPIO revealed that R2* values were significantly higher in the STR ($P = 0.0010$) and SN ($P = 0.0007$) compared with vCTX in animals that received NTs, but not in saline-treated animals. Neurotheranostics significantly increased R2* in the STR ($P < 0.0001$) and SN ($P = 0.0002$) compared with saline without significantly altering R2* in the vCTX.
Conclusions: Neurotheranostics successfully bypassed the BBB and delivered adequate concentrations of SPIO and BDNF to neurons expressing DAT in the mouse brain. Neurotheranostics enabled DAT detection using MRI and rescued striatal tyrosine hydroxylase-positive fibers from HIV/Tat neurotoxicity. Thus, clathrin-based NTs could assist in early detection and treatment of neurodegeneration and in monitoring disease progression and recovery processes.

030:

Ten New Clinical-Translational Applications of Nanoparticle-Enhanced Magnetic Resonance Imaging

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Purpose: Due to concerns of gadolinium (Gd)-deposition in the brain, superparamagnetic particles of iron oxide (SPIOs) are experiencing renewed interest as alternate contrast agents for magnetic resonance imaging. This presentation will discuss 10 relatively new imaging applications of SPIO, which take advantage of their unique properties and biodistribution.

Methods and Materials: Superparamagnetic particles of iron oxide have been developed over the past 3 decades, and multiple compounds, such as ferumoxtran, ferumoxtran-10, ferucarbotran, and ferumoxytol, have been tested in preclinical and clinical settings. Although first-generation SPIOs for clinical imaging were composed of relatively large nanoparticles (hydrodynamic diameter, >50 nm), which mainly distributed to the liver, second-generation SPIOs were smaller (hydrodynamic diameter, <50 nm) and enabled better vascular and tissue enhancement. This presentation will focus on our experience with ferumoxytol nanoparticles, which are Food and Drug Administration–approved for the treatment of anemia and can be used "off label" to produce strong T1- and T2-tissue enhancement on magnetic resonance images.

Results: After intravenous infusion, ferumoxytol nanoparticles do not extravasate due to their large size and, therefore, provide long-lasting blood-pool enhancement. Subsequently, the nanoparticles extravasate through discontinuous microvessels in reticuloendothelial system organs, such as the liver, spleen, and bone marrow, where they are phagocytosed by macrophages. The nanoparticles also extravasate slowly through discontinuous microvessels of malignant tumors, where they are phagocytosed by tumor-associated macrophages. This biodistribution enables relatively new clinical-translational applications, which lend themselves to further investigations:

1. Differentiation of benign and malignant tumors
2. Detection of tumor thrombi in peritumoral vessels
3. Diagnosing tumor joint infiltration
4. Imaging tumor necrosis
5. Imaging tumor response to TAM-modulating cancer immunotherapies
6. Tracking therapeutic T cells
7. Tracking stem cells
8. Imaging migraine
9. Investigating disease-specific protein coronas
10. Utilizing theranostic effects in patients

Conclusions: Iron oxide nanoparticles such as ferumoxytol can be used as alternative magnetic resonance contrast agents to Gd-chelates and provide new opportunities to achieve more sensitive and specific medical diagnoses. Future investigations have to compare magnetic resonance imaging properties of ferumoxytol with other new (or renewed) compounds, such as ferumoxtran-10 and Molday iron oxide nanoparticles.

WEDNESDAY, NOVEMBER 13
SESSION 7: BIORESPONSIVE MRI AGENTS

031:

Molecular Magnetic Resonance Imaging Using Redox Active Fe Complexes

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Purpose: The goal of this study is to demonstrate that rationally designed complexes of redox active iron (Fe) can switch from the low-relaxivity Fe^{2+} oxidation state to high-relaxivity Fe^{3+} oxidation state in the presence of reactive oxygen species (ROS) and can be used to visualize oxidizing microenvironments in vivo.

Methods and Materials: The T_1-relaxivity (r_1) of Fe^{2+} and Fe^{3+} complexes were measured by an inversion recovery experiment at 1.4 T, 4.7 T, and 11.7 T. Redox potentials were quantified using cyclic voltammery. The kinetics of H_2O_2-mediated oxidation of Fe^{2+} to Fe^{3+} was monitored by UV-vis spectroscopy. We used a model of pharmacologically induced acute pancreatitis (carulein/lipopolysaccharide i.p.). Mice were imaged at 4.7 T with a 2-dimensional T_1-weighted gradient echo sequence before and out to 30 minutes after intravenous injection of a 0.2 mmol/kg dose of the unoptimized prototype chelate Fe^{2+}-PyC3A (n = 7). Saline treated mice (n = 4) were also imaged following an equal dose of Fe^{2+}-PyC3A. For negative control, a group of caerulein/LPS-treated mice were treated with the nonresponsive agent Mn^{2+}-PyC3A. For the 3 groups, the post-pre injection change in pancreas-to-muscle contrast-to-noise ratios observed 6 to 12 minutes after contrast agent were recorded and compared by multiple comparisons one-way analysis of variance. Pancreatic tissue was harvested and analyzed for inflammation by H&E staining, for myeloperoxidase (MPO, a biomarker for neutrophil generated ROS) by immunohistochemical staining, and assayed for MPO via guaiacol spectrophotometric assay.

Results: Oxidation of Fe^{2+} to Fe^{3+} generates an order of magnitude relaxivity change between 1.4 T and 11.7 T. Rapid oxidation from Fe^{2+} to Fe^{3+} is achieved with hydrogen peroxide, and the reaction velocity correlates with the rate of enzymatic hydrogen peroxide production. Fe^{2+}-PyC3A provided signal enhancement in the oxidizing microenvironment of the inflamed pancreas, but not in normal pancreas or in other tissues. Inflamed pancreatic tissue was not contrast enhanced by negative control. After treatment with Fe^{2+}-PyC3A, peak contrast-to-noise ratio recorded in pancreatic tissue correlates tightly with pancreatic MPO activity levels ($r = 0.95$, $P < 0.0001$).

Conclusions: Reactive oxygen species–mediated oxidation of rationally designed Fe complexes provides an order of magnitude relaxivity increase and enables strong, selective signal enhancement of tissues with elevated levels of ROS in vivo. Redox-activated Fe complexes offer a new paradigm for biochemically responsive magnetic resonance imaging contrast agents.

032:

Mn^{2+}-Based Smart Magnetic Resonance Imaging Contrast Agent Candidates

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Purpose: Complexes of Mn^{2+} containing a water molecule in the inner coordination sphere of the metal ion have attracted considerable attention recently because they considered as safer alternatives to Gd^{3+}-based magnetic resonance imaging (MRI) contrast agents (CAs). Despite the intensive research conducted in the past 8 to 10 years, only a few ligands were found to possess proper balance between the seemingly contradictory requirements necessary for safe in vivo applications. The 1,4-disubstituted 1,4,7,10-tetraazacyclododecane derivatives possessing acetate (1,4DO2A) or amide ($1,4DO2AM^{Me}$) moieties are far the best candidates in this respect, yet certain applications (ie, targeting purposes or preparation of smart/responsive imaging agents) require better ligand platforms. Our approach on this avenue takes the advantage offered by the PCTA chelator forming thermodynamically stable Mn^{2+} complex of high inertness. By "truncating" the PCTA ligand, we have designed, synthesized, and studied several PC2A derivatives including a bifunctional ligand and monoaquated (q = 1) Mn^{2+} complexes applicable for angiographic and pH MRI.

Methods and Materials: Ligands were prepared using standard chemical synthetic techniques, and metal complexes were isolated and characterized by high-pressure liquid chromatography, mass spectrometry, and ^{1}H-relaxometry. The thermodynamic stability of the Mn^{2+} complexes was determined by the combination of pH-potentiometry and ^{1}H-relaxometry, whereas solvent exchange kinetics was studied via variable temperature ^{17}O-NMR method. Dissociation kinetics of the complexes was studied by studying metal exchange reactions with essential (Cu^{2+} and Zn^{2+}) metal ions, and their serum stability was also evaluated by using commercially available human blood serum. T1-weighted images of phantoms were acquired at 25°C, by using preclinical (Mediso NanoScan PET/MRI 1 T) and clinical (Siemens Magnetom Essenza 1.5 T and Philips Achivea 3 T) MRI scanners.

Results: The current study shows that the PC2A ligand forms a stable (log *K*[Mn(PC2A)] = 17.09, pMn = 8.64) complex with the Mn^{2+} ion, yet its dissociation remains relatively fast. Attachment of electron withdrawing groups (ie, para-nitrobenyl, 4-phenylbenzyl, etc) to the nitrogen atom being *trans*- to the pyridine nitrogen allowed us to improve the inertness of the corresponding Mn^{2+} complexes, and these modifications have also improved the interaction of the complexes with HSA. The [Mn(PC2A-BP)] complex possess good thermodynamic (log *K*[Mn(PC2A-BP)] = 14.86, pMn = 8.35), excellent dissociation kinetic (half-life about 72.5–95.0 hours at 37°C to minimize the rate of decomplexation in vivo), and relaxation parameters (r_{1p} = 3.8 vs r_{1p}^{bound} = 23.5 $mM^{-1}s^{-1}$). However, attachment of ethylamine moiety to the given nitrogen atom resulted in a stable (log *K*[Mn(PC2A-EA)] = 19.01, pMn = 9.27) pH-responsive Mn^{2+} chelate capable of responding to the changes in pH (r_{1p} = 3.6 vs 2.1 $mM^{-1}s^{-1}$) above pH > 5.8 where the deprotonation and coordination of the amine group occurs.

Conclusions: We have demonstrated that the PC2A platform can be used to build smart/responsible MRI CAs. A potential bifunctional ligand ($pNO_2BzPC2A$), angiographic (PC2A-BP), and pH-responsive (PC2A-Sa and PC2A-EA) MRI CA candidates possessing high stability, inertness, as well as excellent relaxation properties (all of these are being crucial for a chelate being considered for the in vivo applications) were designed, synthesized, and characterized.

033:

Environmentally Sensitive Protein-Based MRI Contrast Agents

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Purpose: Gd(III) complexes are widely used as contrast agents (CAs) in medical imaging. Increasing concerns over their safety could be addressed (1) by optimizing their relaxivity (ie, contrast that CAs generate per unit of concentration), which allows lowering their concentrations, and (2) by making them even more inert (ie, preventing metal release). A promising approach has been to engineer tight gadolinium-binding sites into proteins, resulting in both high relaxivity and high stability protein-based contrast agents. Our goal is to look for protein-based contrast agents that would not only be stable and but also could be allosterically modulated, leading to MRI sensors. These would be unique properties that are foundational for a new class of safe and smart contrast agents.

Methods and Materials: We synthesized our recently identified and characterized stable protein "contrast-*o*-phore" (a functional unit responsible for contrast), a self-assembled hexa-α-helical bundle that defines a water-filled cage with one captured Gd(III) ion in a state of low coordination with respect to nonwater ligands. We tested the behavior of this assembly in the presence of a series of ligands, measuring relaxivity and obtaining crystal structures.

Results: Despite its intrinsic stability (t1/2 >2 years), and very slow spontaneous release of metal, the structure is dynamic, with the size of the cage allosterically regulated by ligands in a manner that impacts the environment of the Gd(III) ions and its contrast up to 40% (from r1 ~140 ± 5 $mM^{-1}s^{-1}$ and r2 ~260 ± 0 $mM^{-1}s^{-1}$ per Gd (III) at 60 MHz or 1.4 T, 37°C). We show that some ligands can reach to Gd(III) in the cage and rearrange its coordination sphere.

Conclusions: Stably self-assembled hexa-α-helical bundles show great promise for engineering of smart contrast agents.

034:

A Platform of Bioresponsive MR Agents for Tracking Gene Therapy In Vivo.

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Purpose: The purpose of this abstract is to report a new series of magnetic resonance (MR) contrast agents for tracking gene therapy in vivo and to ultimately treat monogenic diseases. With mean survival rate of 5 years (and most cases are fatal), lysomal storage diseases are among the most dismal of prognosis in all of medicine. Lysomal storage diseases represent a large number of monogenetic diseases, and although rare, the prevalence is to hemophilia. As monogenetic diseases with clearly defined genotype-phenotype relations, lysosomal storage diseases are excellent candidates for gene therapy. The transformative results documented in an adeno-associated virus (AAV) gene therapy clinical trial in infants affected by spinal muscular atrophy demonstrated unequivocally the potential of in vivo gene transfer to treat monogenic neurological disorders.

To date, there is a lack of noninvasive ways to determine biodistribution or activity levels of these AAV therapies in patients. This is a significant hindrance, leaving investigators guessing which organs or structures are effectively treated, and due to the lag time associated with clinical disease progression, this limitation ultimately impacts the evolution of treatment modalities. In order to overcome these limitations, we have developed a new class of bioresponsive MR imaging agents to track enzymatic activity in any organ, peripheral nervous system, or central nervous system over time. MR imaging is an ideal technique for the study of neurological disorders.

Methods and Materials: Some time ago, we developed and successfully tested a MR agent activated by the enzyme β-glucuronidase (β-gluc). Our new synthesis builds upon our experience and insights we learned while designing, synthesizing, and evaluating new MR probes. Extending this work to incorporate new pendant sugars has made possible the evaluation of new enzymes and disease states. The agents (and intermediates) have been fully characterized by mass spec, relaxivity measurements, cell-uptake, enzyme kinetics, and in vivo imaging.

Results: To verify the contrast agent activity with mammalian β-gal, we tested the agent in GM1 mice ($\beta\text{-gal}^{-/-}$) treated with an AAV9 vector encoding β-gal. GM1 mice were injected with an AAV9-GLB1 intracranially (1E10vg, in the thalamus, unilateral) or intravenously (3E11 vg) as previously described. In animals that received intrathecal injection of contrast agent, strong enhancement of the CSF and brain parenchyma was observed. After intrathecal injection of contrast, no enhancement was observed in wild-type animals; however, after thalamic injection of gene therapy for β-gal, strong enhancement of the brain is observed, in areas with better contrast distribution after intrathecal delivery (lateral ventricles and ventral aspect of the brain).

Conclusions: We have developed a series of new MR contrast agents that can monitor the biodistrubution and activity levels of AAV gene therapy in mammals.

035:

Gadolinium-Chelated Poly(L-Glutamic Acid) for MR Imaging of Tumor-Infiltrating Macrophages in Response to Chemotherapy and Immunotherapy

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Purpose: Peripheral blood monocytes are recruited to solid tumors as a result of therapy-induced cell death, where they become tumor-associated macrophages (TAMs). The purpose of this study was to noninvasively assess changes in tumor accumulation of TAMs as a new way to measure early tumor response to anticancer therapies using a biodegradable, biocompatible macromolecular magnetic resonance contrast agent.

Methods and Materials: Gadolinium-chelated poly(L-glutamic acid) (PG-Gd) was synthesized and characterized for its molecular weight, Gd payload, relaxivity, and biodegradability. Biodistribution and T1-weighted magnetic resonance imaging (T1-MRI) were conducted in rodents and in rhesus monkeys. Dual optical/T1-MRI scans were acquired in breast tumor-bearing mice after treatment with taxanes. In addition, T1-MRI was performed in mice bearing syngeneic, orthotopically inoculated pancreatic ductal adenocarcinoma after combined local tumor ablation by irreversible electroporation, and anti-PD1 checkpoint blockade immunotherapy. Co-localization of PG-Gd with TAMs was investigated by immunohistochemical staining and fluorescently labeled PG-Gd.

Results: PG-Gd with molecular weight of ~50 KDa displayed significantly higher relaxivity than its small-molecular weight counterpart gadolinium diethylenetriaminepentacetate (gadopentetic acid). PG-Gd was degradable in the presence of lysosomal enzymes (eg, cathepsin B) and had prolonged blood circulation times both in mice and in monkeys. In monkeys, PG-Gd was completely clearly from the body within 7 days. Compared with untreated tumors, T1-MRI revealed increased uptake of PG-Gd in taxane-treated tumors and tumors treated with immunotherapy. Moreover, PG-Gd in tumors co-localized with TAMs. Our results suggest that infiltration of macrophages due to chemotherapy- or immunotherapy-induced cell death was partially responsible for increased PG-Gd uptake in treated tumors.

Conclusions: MRI techniques in conjunction with systemic administration of PG-Gd that are taken up by macrophages are a promising tool for assessing early treatment response to chemotherapy and immunotherapy.

036:

Functional MRI of Cerebral Ischemia With a Calcium Responsive Probe

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Purpose: Over two thirds of human ischemic stroke is caused by occlusion of the middle cerebral artery and its branches. The success of recovery from ischemic injury is heavily dependent on its timely detection. To this end, the ability to promptly monitor cerebral ischemia is of crucial importance, since the extent of caused brain injury depends primarily on the ischemia duration. For instance, the extracellular Ca^{2+} decreases dramatically during ischemia. Therefore, an imaging method that tracks in vivo variations of $[Ca^{2+}]_e$, thus enabling monitoring of the intensity and duration of cerebral ischemia, would be of paramount importance. Here we report development of an fMRI method to monitor the progress of ischemia by means of Ca-responsive MRI probe.

Methods and Materials: Two probes, responsive Gd_2L^1 or nonresponsive Gd_2L^2, as a control were prepared and compared in vitro. Subsequently, they were intracranially infused in Wistar rats using osmotic pumps. Cerebral ischemia was caused using remote transient middle cerebral artery occlusion (tMCAo). fMRI consisted of a series of T1-weighted MRI acquisitions during the preischemia, ischemia, and postischemia periods, whereas controls included infusion of probes, without tMCAo. Data analysis was based on K-means clustering and the signal detrending.

Results: The relaxometric titrations and MRI experiments on tube phantoms showed suitability of Gd_2L^1 to report on changes in [Ca2+], whereas Gd_2L^2 remained Ca-insensitive. When both probes were administered in vivo and MRI experiments were performed, clustering of the obtained T1-weighted MRI signals displayed the co-centric pattern. Detrended clustered signals showed up to 5% signal change for tMCAo experiments with Gd_2L^1, whereas those with Gd_2L^2 and control experiments showed no signal alterations. The MRI signal changes

completely followed the temporal pattern of tMCAo induction and the tissue reperfusion after the occluder retraction.

Conclusions: We developed an fMRI approach for monitoring of the cerebral ischemia using bioresponsive MRI probe. This method is successfully reporting on the physiological changes of tissue affected with ischemia; moreover, it detects the ischemic onset promptly, as well as changes during reperfusion, the feature that is crucial for the choice of therapy and subsequent recovery. This molecular fMRI technique could become important tool to study the extent of brain injury caused by the cerebral ischemia. In addition, it could allow the visualization and mapping of neural signaling directly by sensing its direct indicator—calcium, thus supplementing the use of conventional fMRI based on BOLD signal.

WEDNESDAY, NOVEMBER 13
SESSION 8: HETERONUCLEAR AND HYPERPOLARIZED AGENTS

037:

Functionalized Perfluorocarbon Nanoemulsions for Sensitive Fluorine-19 MRI Cell Detection

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Purpose: Advances in cell immunotherapy against cancer have stimulated the need for imaging tools to determine cell biodistribution and survival posttransfer. ^{19}F-MRI is an emerging method enabling background-free, quantitative hot-spot imaging of cell therapies. We describe next-generation perfluorocarbon nanoemulsion (NE) ^{19}F probes to detect cells in vivo with an order of magnitude sensitivity improvement. We use a 2-pronged approach for boosting limits of detection via (1) the incorporation of paramagnetic metal chelates into the NE fluorous phase and (2) formulation of NE to display cell-penetrating peptides to enhance cell uptake.

Methods and Materials: We synthesized fluorinated, metal-binding β-diketones conjugated to linear perfluoropolyether (PFPE) yielding "FETRIS" construct and blended this with unconjugated PFPE oil at various ratios. As a co-surfactant, we synthesized a modified peptide from the transactivator of transcription (TAT) component of the human immunodeficiency virus type 1. TAT residues 49 to 58 are positively charged and carry a signal facilitating endocytosis, and a fluorous anchor was covalently attached. The fluorous TAT and poloxamer surfactant was used to form NE with the blended PFPE oil. Metalation of NE occurred via the addition of Fe (III) into the aqueous buffer. Intracellular cell uptake of the probe ex vivo was studied in primary human chimeric antigen receptor (CAR) T cells. In vivo ^{19}F-MRI mouse studies using inoculated labeled CAR T cells were performed in a xenograft model of glioma at 11.7 T.

Results: Additional of Fe(III) into NE decreases the ^{19}F T1 >10-fold via the intermolecular paramagnetic relaxation enhancement mechanism, with modest T2 broadening. Shortening T1 increases the ^{19}F image sensitivity per time with repetitive signal averaging. By incorporating TAT, a labeling efficiency ~1012 fluorine atoms per CAR T cell was achieved, which is a >8-fold increase compared with NE without TAT. In vitro assays show that T cells are unaltered after NE labeling. The ^{19}F-MRI signal detected from TAT-labeled CAR T cells in mouse was >10 times higher than cells labeled with control NE (without Fe and TAT).

Conclusions: Lymphocytes are challenging to label and detect with MRI due to their weak phagocytic properties and small size. Using multipronged improvements to NE formulation via incorporation of Fe-chelate and TAT peptide, one can significantly enhance cell labeling and imaging sensitivity. These same agents should be useful for tagging other weakly phagocytic cells such as stem and progenitor cells.

038:

Nanoparticle-Based ^{19}F-MRI Contrast Agent With Tunable Chemical Switches

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Purpose: One of the great challenges in the postgenomic era is to clarify the biological significance of intracellular molecules directly in living cells. If we can visualize a molecule in action, it is possible to acquire biological information, which is unavailable if we deal with cell homogenates. One possible approach is to design and synthesize chemical probes that can convert biological information to chemical output. In this presentation, molecular design strategies for magnetic resonance imaging (MRI) probes are introduced.

Methods and Materials: ^{19}F-MRI is suitable for monitoring particular signals concerning biological phenomena because ^{19}F-MRI shows little endogenous background signals. We have developed the ^{19}F-MRI probes to detect protease activity and gene expression on the basis of paramagnetic resonance enhancement effect. However, ^{19}F-MRI probes have faced 2 challenges. First, ^{19}F-MRI has the low sensitivity. Second, the suppression of molecular mobility induced by the increase in molecular size shortens the transverse relaxation time (T_2), which is a crucial factor affecting the MRI contrast, resulting in attenuation of the MRI signals. To solve these challenges, we developed a novel ^{19}F-MRI contrast agent, fluorine-accumulated silica nanoparticle for MRI contrast enhancement, which is composed of a perfluorocarbon (PFC) core and a robust silica shell. This nanoprobe has advantages such as high sensitivity, stability, modification of the surface, and biocompatibility. The activatable derivative of nanoprobe will also be introduced. In vivo multicolor imaging is important for monitoring multiple biomolecular or cellular processes. We report several types of PFC-encapsulated silica nanoparticles that show ^{19}F-NMR peaks with different chemical shifts. Three of the nanoprobes were selected, which show the spectrally distinct ^{19}F-NMR peaks with sufficient sensitivities for in vivo multicolor ^{19}F-MRI. We demonstrate that these nanoprobes exhibit the ^{19}F-MRI signals with triple colors in a living mouse. Our in vivo multicolor system could be utilized for evaluating the effect of surface functional groups on the hepatic uptake in a mouse. This novel multicolor imaging technology will be a practical tool for elucidating in vivo biomolecular networks by ^{19}F-MRI.

Results: We developed a multifunctional PFC-based silica nanoparticle, which is made up of a liquid PFCE core and a stable silica shell. Various biomedical applications such as detection of enzyme activities, cancer imaging, and drug delivery were achieved by using surface-functionalized FLAMEs. We newly developed 4 types of PFC-encapsulated silica nanoparticles with different chemical shifts in addition to previously reported PFCE@SiO_2. We selected 3 nanoprobes (PFCE@SiO_2, TPFBME@SiO_2, and PFTBA@SiO_2), and these nanoprobes enabled the triple-color ^{19}F-MRI in vivo. The PFC-encapsulated silica nanoparticles developed in this study offer 2 key advantages as compared with the conventional PFC-encapsulated nanoemulsions: first, the surface modifiability through silane-coupling reaction and subsequent functionalization in organic solvent; and second, the biodistribution of the nanoprobes via intravenous administration may be easily controlled by attaching targeting ligands to the surface of the nanoparticle.

Conclusions: Our multicolor nanoprobes could be applied to investigate the delivery of nanoparticles with various functional groups not only to liver but also to other organs.

039:

Parahydrogen Hyperpolarized [1-^{13}C] Pyruvate for the Investigation of Altered Metabolic Pathways

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Purpose: Hyperpolarization methods allow direct in vivo observation of cellular metabolism by means of magnetic resonance. Dynamic nuclear polarization is the hyperpolarization technique that has been most widely applied as the in vivo administration of hyperpolarized [1-^{13}C] pyruvate can provide relevant information for the detection of pathologic states. A limitation to the widespread use of this approach relies on the fact that it requires access to complex and expensive instrumentation. Parahydrogen-induced polarization is an alternative route to hyperpolarization that has the advantage of being cheaper and easier to handle with respect to dynamic nuclear polarization.

It has been recently shown that ^{13}C-HP pyruvate can be obtained by means of this easy to handle and cost-effective hyperpolarization technique.

These studies aim to increase the hyperpolarization level and to obtain a solution of the HP metabolite completely free from toxic impurities. We also show that relevant metabolic information can be obtained from the studies carried out using this HP metabolite in cells cultures and in vivo.

Methods and Materials: The parahydrogen-induced polarization–SAH hyperpolarization method implies the hydrogenation, using para-enriched hydrogen, of an ester derivative of pyruvate in an organic, hydrophobic solvent. Chloroform, currently used as hydrogenation solvents, has been substituted to avoid traces of this solvent in the aqueous solution of the final HP product. The magnetic field cycling profile (step 2) has been modified to increase the hyperpolarization level.

Metabolic studies on cells have been carried out perfusing cells (~10M) suspended in their grow medium with the aqueous solution of hyperpolarized [1-^{13}C] pyruvate.

Results: Among the solvents used for the hydrogenation of the pyruvate derivative, toluene resulted to be the most suitable, thanks to its low solubility in water, its low toxicity, and to the high polarization level observed. Together with the studies carried out the polarization transfer step (step 2), we have been able to achieve 12% ^{13}C hyperpolarization on the hydrogenated ester.

Conclusions: The metabolic studies carried out on different lines of prostate cancer cells (LNCap, DU145, and PC3) show that the exchange rate of the ^{13}C-HP label between pyruvate and lactate is higher in DU145 and PC3 than in LNCap cells. This is in accordance with the fact that metabolic phenotype of the first 2 cells lines is glycolytic, whereas in the last cell line is oxidative.

040:

Hyperpolarized, ^{13}C-Labeled 2-Ketoglutarate Derivatives as Metabolic MR Probes for the Citric Acid Cycle

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Purpose: The citric acid cycle is a series of enzyme-catalyzed metabolic processes occurring in the mitochondria of all aerobic cells. The cycle oxidizes 2-carbon units (acetyl CoA) to produce carbon dioxide and adenosine triphosphate (ATP). Aberrant TCA cycle has been implicated in a number of diseases including cancer and diabetes. Thus, a noninvasive imaging technique that could be used to monitor TCA cycle activity in real time in vivo would be valuable in the clinical practice. In vivo ^{13}C magnetic resonance spectroscopy/imaging with ^{13}C-labeled metabolic tracers offers a convenient solution to this problem because the in vivo fate of the tracer and its downstream metabolites can be observed based on their ^{13}C-NMR spectra. The low sensitivity of ^{13}C-NMR can be overcome by a hyperpolarization technique such as dynamic nuclear polarization. In this project, we studied the feasibility of using hyperpolarized (HP) ^{13}C-labeled 2-ketoglutarate derivatives to monitor TCA cycle activity by ^{13}C magnetic resonance spectroscopy/imaging.

Methods and Materials: 2-Ketoglutaric acid esters labeled at C1 and C2 were synthesized starting from ^{13}C-labeled diethyl oxalate and diethyl succinate. The labeled esters were HP using the clinical GE SPINlab polarizer. The HP tracers were injected (tail vein) into normal rats, and the metabolism of the HP probes was followed by in vivo ^{13}C-MR spectroscopy in the liver in a 3-T clinical MRI scanner.

Results: The ^{13}C-tracer was designed to have the ^{13}C label at C1 and C2 of ketoglutarate in long T_1 positions to preserve the ^{13}C polarization. [1,2-$^{13}C_2$]-2-ketoglutaric acid diethyl ester was successfully polarized using the clinical dynamic nuclear polarization polarizer. The T_1 relaxation times of the ^{13}C labels were found to be around 40 seconds at 3 T. A few seconds after injection, HP-^{13}C signal of bicarbonate appeared around 162 ppm in the liver.

Conclusions: Ketoglutarate is decarboxylated by ketoglutarate dehydrogenase to produce CO_2 and succinyl CoA. The appearance of HP-^{13}C-bicarbonate indicates that the HP-ketoglutarate ester was taken up; the ester groups were cleaved by intracellular esterases and decarboxylated in the TCA cycle to produce bicarbonate. Thus, the HP-bicarbonate signal can be used to monitor TCA cycle activity in vivo by ^{13}C MR.

WEDNESDAY, NOVEMBER 13
SESSION 9: OPTICAL AGENTS

041:

Thermosensitive Biodegradable Copper Sulfide Nanoparticles for In Vivo Multispectral Optoacoustic Tomography

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Purpose: Copper sulfide nanoparticles (CuS NPs) have emerged as one of the most promising theranostic nanoplatforms for treating cancer, due to their relatively small size, facile chemistry, and excellent photothermal properties. Herein, we have designed a thermosenstive biodegradable CuS NPs, which can be more clinical relevant with lower adverse effects. Our goal was to investigate their in vitro and in vivo biodegradation profile, and apply it to real-time multispectral optoacoustic tomography (MSOT) in living animals.

Methods and Materials: Polyethylene glycol (PEG)–coated CuS NPs were synthesized via a 1-step reaction with $CuCl_2$ and Na_2S as the precursors and mPEG-SH as the surfactant. As-prepared CuS-PEG was incubated in water at different temperatures and pH to examine its in vitro biodegradation by transmission electron microscopy, UV-VIS-NIFR spectrometry, and inductively coupled plasma optical emission spectrometry (ICP-OES). CuS-PEG was injected into healthy mice, and livers of CuS-PEG NP-injected mice were investigated over time with ICP-OES for in vivo biodegradation. MSOT was performed in SKOV-3 tumor-bearing nude mice to validate the imaging capacity of the biodegradable CuS NPs.

Results: CuS-PEG NPs were successfully synthesized with superior solubility and size range from 10 to 12 nm in diameter. After incubating with water under different conditions, significant degradation was observed at 37°C, whereas minimal degradation was observed at 4°C, suggesting their thermosenstive biodegradability. Cu concentration in liver based on ICP-OES also demonstrated significant biodegradation (50.0 ± 0.4 μg NP per g tissue at 3 hours p.i. vs 8.2 ± 0.4 μg NP per g tissue at 7 days p.i.) of CuS-PEG in vivo. Dynamic MSOT showed manifestly enhanced signal in tumor, suggesting an excellent nanoplatform for imaging tumor. Of note, degraded Cu ions will not render MSOT signal, leading to more accurate imaging results in comparison to the imaging techniques that rely on radionuclides.

Conclusions: The degradation of CuS was controllable and temperature dependent, making it a clinically translatable in vivo theranostic platform with minimal adverse effects. MSOT with biodegradable CuS NPs could effectively enhance the tumor signal.

042:

Imaging of Sn-2 Lipase-Labile Phospholipid Prodrug in Cells Using Fluorescence Lifetime and Superresolution Microscopy

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Purpose: Targeted lipid-encapsulated nanoparticles incorporating drugs dissolved in the lipid surfactant are effective in vivo. However, pharmacokinetic studies revealed rapid diffusional loss during circulation with a small portion of the payload responsible for the therapeutic benefit. Circulatory drug loss was overcome by use of Sn-2 phosphatidylcholine prodrugs (PC-PDs). By design, the inactive PC-PD is liberated by intracellular lipases upon targeted delivery. The objective of this research was to elucidate pathways of prodrug cellular uptake, intracellular trafficking, and active drug liberation.

Methods and Materials: Three complementary optical-based imaging methods were used to delineate cellular handling of doxorubicin-prodrug (Dox-PD) and AlexaFluor-488-PD (AF488-PD): classic fluorescence microscopy, fluorescence lifetime imaging microscopy, and single-molecule superresolution microscopy.

Results: Phosphatidylcholine prodrugs entered the cell with no apparent spatial restrictions as opposed to previously reported phosphatidylethanolamine drug surrogates. The PC-PDs intercalated or fused into the outer cell membrane and transferred to the inner membrane leaflet by both ATP-dependent and ATP-independent mechanisms. From the inner membrane leaflet, the PC-PDs distributed broadly within the cytosolic membranes over the next 12 hours. A significant PC-PD pathway was from the inner cell membrane to the perinuclear Golgi-endoplasmic reticulum region. Enzymatically liberated drug was appreciated widely in the cytosol, but preferentially in the Golgi region. Free doxorubicin rapidly entered cells and localized to the nucleus, whereas DOX-PD rapidly entered the cells, but a significant portion remained within the cell membranes and a smaller portion entered the nucleus. Fluorescence lifetime imaging microscopy of cell nuclei incubated with doxorubicin or targeted DOX-PD nanoparticles showed similar fluorescence lifetimes indicative of the drug bound to dsDNA. The in vitro biopotency of the 2 drug delivery approaches measured as inhibition to cell proliferation favored the DOX-PD at 48 hours, suggesting that the formation of a prodrug intracellular drug reservoir within the cytosol membranes may increase drug effectiveness.

Conclusions: Contact-facilitated drug delivery mechanism of inactivated Sn-2 lipase-labile PC-PDs takes place by readily traversing the cell membrane with broad intracellular membrane distribution, particularly, near Golgi/endoplasmic reticulum, where enzymatic release of free drug affords cytosolic or nuclear targeting.

043:

Tracking of Dendritic Cells With Gold Nanoparticles

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Purpose: The aim of this study is to assess the methodology of tracking tumor exosome-loaded dendritic cell with gold nanoparticles (GNs).

Methods and Materials: Dendritic cell line (DC2.4) and breast cancer cell line (E0771, 4T1, MDA-MB-231) were used. DC2.4 cells stably expressing GFP-luciferase and E0771, 4T1, and MDA-MB-231 cells stably expressing exosomal CD63-RFP fusion protein were generated by lentiviral transduction. The exosomes carrying CD63-RFP fusion protein were purified from breast cancer cells using the Exo-spin Exosome Purification Kit. The size distribution and concentration of exosomes were determined using a NanoSight NS500. The tumor exosome uptake by DC2.4 cells was visualized using confocal laser scanning microscopy. Rod-shaped GNs (10 × 40 nm) with surface plasmon resonance at 808 nm were used for DC2.4 cell labeling. After GN-labeled and tumor exosome-stimulated DC2.4 cells into

the footpad of mice, tracking of DCs that migrated into axillary LNs of mice was evaluated using the Vevo2100 LAZR imaging system.
Results: Tumor exosomes (<50 μg/mL) promoted DC2.4 cell proliferation and migration capacities. Co-stimulatory molecules (*CD40*, *CD80*, and *CD86*) on DC2.4 cells, which activate T cells, were upregulated by tumor exosomes. A 12-hour incubation of GNs at 120 pM did not cause cytotoxicity and a significant alteration in the expression levels of CD40, CD86 on DC2.4 cells. The amplitude of PA signals in GN-labeled DC2.4 cell phantom was proportional to cell number. GN-labeled and tumor exosome-stimulated DC2.4 cell migration into lymph node was monitored in real time through in vivo photoacoustic imaging. Histological analysis of lymph nodes correlated well with photoacoustic imaging findings in vivo.
Conclusions: Direct labeling with GNs can be readily accessible for noninvasive tracking dendritic cell migration into lymph nodes using photoacoustic imaging.

044:

Targeted NIR Fluorophore for Intraoperative Detection of Choline Kinase α Expression in Lung Cancer

S.A. Osharovich, A.V. Popov, D.E. Holt, S. Singhal, and E.J. Delikatny. *delikatn@pennmedicine.upenn.edu.*
Purpose: Choline kinase (ChoKα) produces phosphocholine (PC) in the Kennedy pathway of lipid synthesis. ChoKα is associated with aggressive phenotype, high histological tumor grade, and poor clinical outcome in human cancers, and is overexpressed in 60% of human lung tumors. We synthesized a near-infrared fluorescent ChoKα inhibitor, JAS239, that can assess ChoKα levels in tumors using optical imaging. JAS239 competitively binds ChoKα, attenuates PC production, and accumulates in tumors in proportion to expression. Here, we measure ChoKα levels in murine lung cancer cells and canine lung tumors and evaluate JAS239 for detection of lung metastases in mice. Our objective is to validate JAS239 for murine lung cancer imaging for translation to intraoperative resection of lung tumors in canine patients.
Methods and Materials: JAS239 was synthesized as described. ChoKα expression was measured in murine lung cancer lines by Western blot. ChoKα inhibition was measured by 14PC production. Luciferase-transfected KLN-205 murine lung cancer cells were grown as flank tumors in DBA2 mice. KLN-205 tumors and metastases were detected using bioluminescence and near-infrared imaging of intravenous JAS239. Pharmacokinetics, biodistribution, and toxicology were determined. ChoKα was measured in spontaneous canine adenocarcinomas by Western blot and immunohistochemistry (IHC).
Results: KLN 205 cells showed the highest ChoKα levels of murine lung cancer cells. Treatment of KLN 205 cells with JAS239 significantly reduced 14C-PC production. Intravenous JAS239 in mice with KLN 205- Luc+ tumors led to peak tumor fluorescence at 4 hours with a tumor-to-muscle ratio of 2:1. JAS239 illuminated lung metastases and did not accumulate in normal lung. Bioluminescence showed co-localization with JAS239 fluorescence in lung metastases. JAS239 was nontoxic at 50-fold higher doses. In canine lung tumors, IHC showed significantly higher ChoKα compared with normal lung.
Conclusions: We identified KLN 205, a highly metastatic lung cancer line that overexpresses ChoKα. Co-localization of fluorescence and bioluminescence indicates that JAS239 could identify tumor margins and micrometastases, critical for translation to canine patients. Spontaneous canine lung tumors have elevated ChoKα and show clear tumor delineation by IHC. Canines are an excellent model of human disease, as they express similar levels of ChoKα, are genetically diverse, and are exposed to the same environment. ChoKα expression profiling will aid in intraoperative margin delineation of lung tumors in a real-time clinical setting.

045:

Synthesis and Characterization of Nonionic Near-Infrared Dye Molecules

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Purpose: Discussed herein are the syntheses and characterization of 6 dicyanine dyes that have been used in humans or that are designed with future clinical use in mind by virtue of their near infrared fluorescence emission (<800 nm ex; ~800 nm em) matching clinical imagers. Several novel nonionically solubilized dyes are included along with the more highly charged anionic and zwitterionic dyes. We test the dyes as free acids and after conjugating to the lysine of a novel hybrid-peptidic cell-penetrating peptide.
Methods and Materials: ZW800s were a gift from Curadel. The other known dyes were purchased as free acids and NHS analogs. The new dyes were synthesized by modified classical methods with HPLC purity (≥95% by near-infrared detection) and mass spectral identity. The hybrid-peptide, 4Iphf-HN17 (4Iph)(f)LPNSNHIKQGL (f, fmoc; 4Iph, 4-iodophenyl), was conjugated with the dyes at the lysine with either NHS or EDC coupling. A Cary Eclipse fluorescence spectrophotometer was used to obtain the absorption and emission data in PBS, methanol, and in FBS (contains albumin). Human HNSCC cell lines were purchased (ATCC). Nude mice were purchased at OSU. In vivo optical imaging was studied on mice xenografts with fluorescence detection by a Fluoptics Fluobeam imager.
Results: Emission intensity was maximal with the highly charged dyes but matched by NI800-SA. The NI800 dyes (structures will be disclosed) bear nonionic solubilizers designed to conjugation to a lysine without changing the overall charge of the peptide or protein. Although the strategy worked, the spectral results for all dyes varied with conjugation and media. Conjugation tended to reduce the optical signal intensity, while negative charge quantity and albumin's presence tended to increase the emission intensities. Tumor to background signal was also observed in tumor animals serially from 1 to 24 hours, to observe the effects of the dye charge on tumor to background and clearance in Cal-27 HNSCC xenograft mice between 1 and 24 hours.
Conclusions: The dye substituents are theoretically there for the sole purpose of solubilizing the hydrophobic dicyanines, but clearly also play a role in the other emission characteristics, probably through both prevention of dye-dye interactions and some direct electronic influence. Preliminary results from the in vivo mouse xenograft model suggest that the sulfonic acid conjugates 4IPhf-HN17-IR800 and 4IPhf-HN17-IRSO456 had an identical efficacy. The latter is a symmetrical dye that offers a large cost advantage. 4Iphf-HN17-ZW800 shows good imaging characteristics with the most rapid background clearance.

046:

Functionalized Silica Nanoplatform as Bimodal Contrast Agent for ^{1}H-MRI and Optical Imaging

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Purpose: The inherent low sensitivity of magnetic resonance imaging can be overcome by the use of paramagnetic contrast agents (CAs), typically gadolinium complexes. It is known that the effect of a Gd chelate on the longitudinal relaxation rate of water molecules depends on molecular weight of the system. In order to improve the relaxation process, we decided to perform a noncovalent confinement of small gadolinium Gd(III)-based CAs in a semipermeable nanosystem. Thanks to their exceptional properties (ie, biocompatibility, chemical stability, low toxicity), silica nanoparticles (SiO_2 NPs) have been chosen as a matrix.
Methods and Materials: SiO_2 NPs were obtained by reverse microemulsion procedure in the presence of a hydrosoluble paramagnetic CAs (Gd-HPDO3A). Bimodality was reached by introducing ZW800-1, a near-infrared emitting molecule, during the microemulsion. Then, the particle surface was modified by silanol-PEG chains to ensure aqueous stability. Functional groups were introduced by mean of a photochemical treatment in the presence of a diazirine system. The as-obtained platforms were characterized by dynamic light scattering (nuclear magnetic resonance spectroscopy, relaxometry measurements, UV-Vis and IR spectroscopies, transmission electron microscopy).
Results: A stable fluorescent paramagnetic nanoplatform was successfully prepared and completely characterized. Narrow size distribution SiO_2 NPs were obtained (D_H, 80 nm). Relaxometric measurements of the as-synthetized nanoplatform have proven its efficiency to decrease $T_{1,2}$ of endogenous water protons molecules. The fluorescent properties were kept after encapsulation of the ZW800-1.
Conclusions: In a near future, a biological vector (peptide targeting inflammation) will be grafted on the surface of our bimodal platform and its efficiency will be evaluated in vitro and in vivo.

WEDNESDAY, NOVEMBER 13
SESSION 10: THERANOSTICAGENTS 2

047:

Near-Infrared Photoimmunotherapy, a Highly Selective Theranostics, Can Cure Cancers Even With Distant Metastasis

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Purpose: Near-infrared photoimmunotherapy (NIR-PIT) is a new molecularly targeted cancer phototheranostics based on conjugating a near-infrared silica-phthalocyanine dye, IR700, to a monoclonal antibody targeting cell-surface molecules.
Methods and Materials: A phase 1 clinical trial of NIR-PIT with the cetuximab-IR700 (RM1929) targeting EGFR in patients with inoperable head and neck cancer started in April 2015, and is now in worldwide phase 3 trial with fast-track recognition in countries in North America, Asia, and Europe (https://clinicaltrials.gov/ct2/show/NCT03769506). When exposed to NIR light, the conjugate induces a highly selective necrotic/immunogenic cell death (ICD) only in target-positive, monoclonal antibody-IR700–bound cancer cells.
Results: Cell death occurs as early as 1 minute after exposure to NIR light and disappearing IR700 fluorescence. Meanwhile, immediately adjacent target-negative cells are unharmed. NIR-PIT–induced immunogenic cell death initiates anticancer host-immunity by recognizing released cancer-specific antigens with dendritic cells, resulted in repriming and proliferating multiclonal CD8+ T-cells. Furthermore, NIR-PIT can also target negative regulatory immune cells such as T_{reg} only in the tumor bed that promptly activates local tumor cell-specific $CD8^+$-T and NK cells and let these cells kill cancer cells in local tumor as well as in distant tumors of the same cell origin. When combined cancer-targeted NIR-PIT and immuno-activated NIR-PIT or other conventional immune activation therapies, large proportions of cancers can completely cure even together with distant metastasis without recurrence.
Conclusions: NIR-PITs selectively kill and eliminate molecularly targeted cancer or immunosuppressor cells that can be monitored by fluorescence imaging. When combined, cancer cell-targeting and immunosuppressor cell-targeting NIR-PITs that effectively induces innate and acquired immunity specifically against cancer cells growing in patients and vaccination against the tumor cells resulted in completely curing systemic cancers without recurrence.

048:

New Tumor-Selective APC Metal Chelates for PET Imaging and Targeted Radionuclide Immunotherapy

R. Hernandez, J. Grudzinski, R. Patel, K. Walker, C. Massey, E. Aluicio-Sarduy, J. Jeffery, A. Pinchuk, C. Capitini, B. Bednarz, P. Carlson, P. Sondel, Z. Morris, and J. Weichert. *jweichert@uwhealth.org.*

Purpose: We have demonstrated the utility and structural flexibility of APCs (alkylphosphocholines) as a molecular platform for the selective delivery of a variety of imaging and therapy radioisotopes as well as stable metals (Gd/Mn) to a broad spectrum of tumors for multimodality oncology imaging and therapy. Our theranostic premise is to use isosteric imaging/therapy pairs ($^{86/90}$Y, $^{64/67}$Cu, $^{44/47}$Sc, for example) to quantify in vivo tumor uptake and dosimetry to guide subsequent radio/immunomodulatory therapy. Our focus is to assess the ability of $^{86/90}$Y-NM600 to systemically stimulate the immune system to recognize and kill cancer cells in vivo and establish immune memory to that particular tumor phenotype.
Methods and Materials: DO3a-NM600 was radio-labeled with ^{86}Y for PET imaging of syngeneic mice with T-cell lymphoma, B78 melanoma, or Panc-02 pancreatic tumors. PET images were analyzed, and 4-dimensional Monte Carlo–based organ dosimetry for ^{90}Y-NM600 was calculated. Dose response (25–500 μCi) studies were performed in each model before combining with either IL-2 immunocytokine or anti–CTLA-4 immune checkpoint inhibitor therapy. Animals were monitored for tumor response and survival, and mechanistic studies were conducted to assess immune markers and response.
Results: Tumor response was significant in all tumor models at higher monotherapy dose levels (>200 μCi). T-cell lymphoma results were striking and afforded compete responses in 80% of the mice. Moreover, when rechallenged 90 days later, tumor cells failed to grow suggesting induction of immune memory. Furthermore, when splenocytes from the responder cohort were transplanted into naive mice followed by cancer cell inoculation, tumor cells again failed to grow suggesting that immune memory was transferable to other mice. Combination of NM600 with both immunotherapies also produced a high number of complete responders compared with controls. Notable was the finding that low tumor doses (2.5–5 Gy) of NM-600 in combination with immunotherapy were sufficient to significantly enhance responses compared with immunotherapy alone.
Conclusions: The differential uptake of ^{90}Y-NM600 in primary and malignant cancer cells provides an excellent therapeutic window that allows high curative rates in both localized and disseminated syngeneic mouse cancer models of this disease, with no relevant toxicities. We believe that these findings demonstrate for the first time the interplay of low-dose targeted radionuclide therapy (NM-600) with the innate immune system in obtaining an elevated degree of complete response as well as induction and potential transfer of immune memory.

049:

MR Imaging of Theranostic Gd-Based AGuIX Nanoparticles in Patients With 4 Histological Types of Brain Metastasis

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Purpose: The use of radiosensitizers is an effective approach to increase the curative efficacy of radiotherapy and to limit some indesirable adverse effects. We present here the main MRI findings obtained in the phase 1 Nano-Rad trial (NCT02820454) with intravenous administration of Gd-based theranostic nanoparticles (NPs) in patients with multiple brain metastases from 4 types of primary tumors (NSCLC, colon, melanoma, breast).
Methods and Materials: Patients (n = 15) with multiple brain metastases were recruited. The AGuIX NP (NHTherAguix, France) is composed of a polysiloxane core with an average 10 DOTA cyclic ligands chelating Gd ion. Its diameter is 4 ± 2 nm, its mass is about 10 kDa, and its relaxivity r_1 at 3 T is equal to 8.9 $mM^{-1}{\cdot}s^{1}$ per Gd ion. At D1, the patients were injected intravenous with solution of AGuIX nanoparticles at doses of 15, 30, 50, 75, or 100 mg/kg body weight. Two hours later, the patients followed a MRI session performed at 3 T, including 3D T_1 mapping. The patients then underwent a whole-brain radiation therapy (10 sessions of 30 Gy). MRI sessions were later on performed at D8, D28, and D100.
Results: Two hours post-NP injection, MRI signal enhancements (SEs) were observed for all types of brain metastases, all patients, and all doses administered. The SE was measured to be similar for AGuIX and Dotarem (Guerbet, France) administration. Tumor enhancements are exemplified in Figure 1 for each type of primary tumor. The SEs were found to increase with the administered dose of AGuIX NP. The mean AGuIX concentration in metastases of the patients administered with the highest dose were measured to range between 20 and 60 mg/L, with the same order of magnitude as obtained previously in animal models. Persistence of MRI enhancement was noticed in metastases at D8, 1 week after administration of NP, illustrating the accumulation and delayed clearance of NP from the tumors, as reported previously in animal models. A close to linearity positive correlation between the SE and the NP concentration was observed in the range of values measured.
Conclusions: The preliminary results of this clinical trial demonstrate that intravenous injection of AGuIX NP is effective for enhancing different types of brain metastases in patients. Beside, the preliminary results of the Nano-Rad phase 1 study demonstrated good tolerance of intravenous injection of AGuIX NP. These results and observations make it possible to confidently start a phase 2 clinical trial on the same indication (NANORAD2, NCT03818386).

050:

Necrosis-Targeted Radio-Ablation on Top of VDA-Induced Necrosis in Microcancers May Elicit Early Eradication of Solid Malignancies: A Future Preventive Anticancer Strategy?

S. Wang, G. Bormans, J. Swinnen, and Y. Ni. *yicheng.ni@kuleuven.be.*

Purpose: Despite recent advances, cancer still remains a major life threat with increasing societal burdens. Earlier, we have developed a small molecule dual targeting pan-anticancer theragnostic strategy called OncoCiDia, which is now under clinical trials. OncoCiDia combines tumor-necrotizing effect of a vascular disrupting agent (VDA) such as combretastatin A-4 phosphate (CA4P) on day 1 and targeted radiotherapy by using a necrosis-avid compound such as hypericin labeled with iodine-131 (^{131}I-Hyp) on day 2. Beta particles emitted from ^{131}I-Hyp kill residual cancer cells and gamma rays facilitate cancer imaging, hence a theragnostic approach. OncoCiDia is primarily considered as a palliative care for patients with late-stage cancers. However, bulky necrosis was surprisingly found after using CA4P among both primary and secondary microcancers in rats, which opposes prior knowledge but implies curative potentials of OncoCiDia for early-stage cancers.
Methods and Materials: Hepatocellular carcinoma and visceral tumors were modeled in rats (n = 30) by DENA gavage and rhabdomyosarcoma (R1) implantation respectively until 2 to 5 mm in diameter was reached as monitored by MRI. Rats were intravenously injected with CA4P at 20 mg/kg and euthanized next day with their tumor-carrying organs dissected for microangiography and histomorphology examinations. Curability of microcancers was estimated by mathematical modeling before in vivo radioactive tests.
Results: Nearly complete necrosis was found in 5 of 7 micro-hepatocellular carcinomas and all 5 visceral micro-R1 tumors. Structural vascularity was not evident by microangiography and hematoxylin-eosin histology, but densely positive CD34 immuno-staining appeared among those lesions. Such findings suggest that tumorous

neoendothelia were attributable to early cancer growth and vulnerable to VDA attack, resulting in bulky necrosis in microcancers. Based on mathematic modeling results, the residual viable tumoral cells could be well covered and destroyed within the 2-mm penetration distance of the high-energy beta particles, by applying one complete episode of OncoCiDia.

Conclusions: Necrosis induced by VDA in microcancers of less than 5 mm in diameter could be targeted by ^{131}I-Hyp, which may lethally eradiate the entire lesion with all remaining cancer cells. A PhD program is ongoing to prove or disprove this assumption. In combination with newly developed sensitive liquid biopsy techniques, OncoCiDia may help eradicate cancers at their infancy, that is, a preventive anticancer strategy for eliminating microsolid malignancies.

051:

Gadolinium-Free Alternative to General Purpose Gadolinium-Based Contrast Agents (GBCAs)

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Purpose: The safety of GBCAs has been called into question, first in the context of nephrogenic systemic fibrosis, and more recently with the realization that gadolinium in some form is retained in the body for long periods. We set out to develop an alternative MRI contrast agent that could provide the same diagnostic information as GBCAs but without using Gd as the source of signal generation.

Methods and Materials: We synthesized a hexadentate chelator, PyC3A, and its complex with Mn^{2+}. The thermodynamic stability and kinetic inertness of Mn-PyC3A was measured by pH titration and competition with Zn^{2+}, respectively. Relaxivity was determined in PBS and blood plasma at 37°C. Contrast-enhanced MR angiography with Mn-PyC3A in baboons was compared with an equivalent dose of Gd-DTPA at 3 T using a clinical MRA protocol. The ability of Mn-PyC3A to delineate tumors was evaluated in orthotopic syngeneic mouse models of breast cancer and liver metastasis with comparison to Gd-DOTA and Gd-EOB-DTPA, respectively, at 4.7 T. Blood and urine from the baboon study were analyzed by LC-ICP-MS to quantify pharmacokinetics and metabolism. [^{52}Mn]Mn-PyC3A was administered to male and female rats, urine and feces were collected from metabolic cages, and whole-body biodistribution was performed at 1 and 7 days postinjection with detection of ^{52}Mn by gamma counter. A similar study was performed with Gd-DOTA except using ICP-MS to quantify residual Gd in tissue.

Results: Mn-PyC3A is among the most stable Mn^{2+} complexes reported and contains one inner-sphere water molecule. Relaxivity in plasma is comparable to Gd-DOTA and Gd-DTPA. Mn-PyC3A has an extracellular distribution with similar pharmacokinetics to GBCAs and undergoes a mixed renal/hepatobiliary elimination in mice, baboons, and rats (85% renal in rats). It is excreted unchanged in the urine. Mn-PyC3A provides similar contrast enhancement to Gd-DTPA and Gd-DOTA in angiography and breast cancer models. Because of its partial hepatobiliary elimination, 0.1 mmol/kg Mn-PyC3A provides similar liver-to-tumor contrast compared with 0.025 mmol/kg Gd-EOB-DTPA. Biodistribution studies show that retention of Gd from Gd-DOTA administration is significantly higher than retention of injected Mn from Mn-PyC3A at both 1 day and 7 days postinjection.

Conclusions: Mn-PyC3A possesses a number of favorable properties that present it as a potential alternative to GBCAs. Further development is warranted.

THURSDAY, NOVEMBER 14
SESSION 11: MRI AGENTS: NEW APPLICATIONS

052:

Imaging Therapeutic Cell Transplants in Patients With Avascular Osteonecrosis

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Purpose: Epiphyseal osteonecrosis (ON) is a complication of high dose steroid therapy, which can lead to joint collapse. Orthopedic surgeons treat ON of the hip joints with a decompression surgery and transplantation of mesenchymal stromal cells (MSCs), harvested from the patients' own pelvic bone marrow. This combined procedure yields improved clinical outcomes compared to a decompression procedure alone, with patients showing near-normal joint function after 2 years. The purpose of our study was to track transplanted MSC in ON lesions of patients after decompression surgery.

Methods and Materials: Four patients with 6 ON lesions received an intravenous injection of ferumoxytol at a dose of 5 mg Fe/kg at 24 to 48 hours before decompression surgery. During the surgery, iron-labeled bone marrow cells were aspirated from the iliac crest and injected into the ON. Eight patients with 10 ON lesions did not receive ferumoxytol and were transplanted with unlabeled MSC. All patients received preintervention and postintervention x-rays and MRI scans up to 9 months after the surgery. T2 relaxation times of labeled and unlabeled MSC transplants were compared using a mixed-effects model, clinical outcomes were compared with a Fisher exact test, and times to joint collapse were assessed by a log-rank test.

Results: T2-weighted MR images after injection of iron-labeled MSC showed hypointense (dark) signal in the decompression track and ON. By comparison, control patients who had received unlabeled MSC did not show hypointense signal changes in the access canal. T2* relaxation times were significantly lower for ferumoxytol-labeled MSC than for unlabeled MSC (9.04 ± 0.7 vs 13.7 ± 2.50; $P = 0.02$). Of 6 femoral heads treated with labeled MSC, one (17%) progressed to collapse. Of 10 femoral heads treated with unlabeled MSC, 3 (30%) progressed to collapse. This difference was not significant. In addition, time to progression was also not significantly different between labeled and unlabeled MSC transplants ($P = 0.8$). However, the collective successful outcome of ON treated with core decompression plus MSC transplants was better than previously reported for core decompression alone. In our study, 12 (75%) of 16 femurs showed no collapse within 1 year or more after the intervention, compared with success rates of 53% to 71% for core decompression only, reported previously.

Conclusions: The described immediately clinically applicable imaging test could become a powerful new tool to study the effect of therapeutic cells on bone repair outcomes after corticosteroid-induced ON.

053:

Imaging Zinc Secretion From the Prostate by MRI as a Potential Biomarker of Prostate Cancer

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Purpose: Recently, we demonstrated that the healthy mouse prostate releases zinc ions in response to a glucose bolus and that zinc release is markedly reduced in prostate cancer (PCa), offering a potential technique to distinguish healthy prostate from PCa by MRI. The goal of this work was to correlate the zinc content in prostate tissue as measured by synchrotron radiation x-ray fluorescence (SR-XRF) with glucose-stimulated zinc secretion (GSZS) as detected by MRI. Additional imaging experiments were performed in rats to evaluate the specificity of glucose in stimulating zinc secretion.

Methods and Materials: C57Bl6 mice, transgenic adenocarcinoma of the mouse prostate (TRAMP) mice, and Sprague-Dawley rats were imaged using a 9.4 T Varian/Agilent scanner. Ge3D T1-weighted scans were obtained (TE/TR = 1.69/3.35 milliseconds, average = 4, θ = 20°) before mice received either 0.07 mmol/kg GdL1 (IV) plus 2.2 mmol/kg glucose (IP) or 0.07 mmol/kg GdL1 plus saline. Immediately after MRI (10 minutes postinjection), the prostate was resected, frozen in liq.N2-chilled isopentane, sliced into 50 μm sections, and mounted on XRF-film. The SR-XRF I18 beam line at the Diamond Light Source was used to collect atomic images of Zn and P (8.2 keV) and Gd (11 keV). Rats received 0.1 mmol/kg GdL1 plus glucose or pyruvate.

Results: In Figure 1A, comparison between H&E (top) and SR-XRF (middle) demonstrates that the lateral lobes contain the most zinc in the healthy mouse prostate. After glucose bolus, zinc moves from the central acinar glands (2.4 ± 0.7 mM) to the peripheral gland (2.0 ± 0.5 mM) (Fig. 1B). TRAMP mice evaluated during early disease (11 weeks) and advanced PCa (20 weeks) via MRI demonstrate loss of enhancement in the lateral lobes as disease progressed (Fig. 1A, bottom). SR-XRF quantitative comparison between healthy mice and advanced PCa TRAMP mice (Fig. 1D) reveals a 60% reduction in lateral lobe zinc levels. The MR experiments in rats showed that pyruvate also initiates release of Zn(II) from the prostate.

Conclusions: SR-XRF images demonstrating compartmental movement of Zn(II) ions in response to glucose parallels our previous observations of GSZS by MRI. These data show preferential loss of Zn(II) from the lateral lobe consistent with loss of enhancement (MRI) and zinc content (SR-XRF) in fully developed PCa. This highlights the promising path of the GSZS method for early detection of PCa by MRI. The observation that pyruvate stimulates Zn(II) secretion from rat prostate suggests that the role of glucose in GSZS is to stimulate aerobic metabolism in the TCA cycle.

054:

The Pegylated Superparamagnetic Iron Oxide Nanoparticles (IOPs) Under Clinical Phase II Development for HCC Imaging

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Purpose: Hepatocellular carcinoma (HCC) is the most common primary liver cancer. Imaging is important for establishing a diagnosis of HCC. Dynamic multiphase contrast-enhanced CT or contrast-enhanced MRI is the current standard for imaging diagnosis of HCC. The pegylated superparamagnetic iron oxide nanoparticles, IOP injection, is a suitable contrast agent for liver imaging with good safety and well tolerability in phase 1 clinical study. The objective of the ongoing phase 2 clinical study is to assess the clinical validity, sensitivity, and specificity of IOP-enhanced MRI compared with local hepatic resection result for the detection of HCC.
Methods and Materials: The IOP is a monocrystalline superparamagnetic iron oxide nanoparticle with a mean core-diameter of 10 to 15 nm (TEM) and hydrodynamic diameter around 45 to 70 nm. The magnetic relaxivities (r1 and r2) in water at 37°C and 0.47 T are 33.4 and 170.8 $mM^{-1}s^{-1}$.
Results: Patients with suspected HCCs were injected with single dose of IOP. Both T1 and T2/T* images were taken at various time points postinjection. The signal intensity (SI) change in T1 and T2 images clearly demonstrated the abnormal region, and these data correlate well with the pathological findings
Conclusions: The IOP-enhanced MRI method provides a noninvasive alternative to biopsy for detecting HCC with high sensitivity and specificity. Currently, there were no severe adverse effects found with the IOP injection in both healthy volunteers and HCC patients. The time dependent T1 and T2 images collected so far show the liver lesions well differentiated with normal liver. The image data so far can match with the pathological data. The trial is still ongoing and more patient image data will be collected.

055:

3D Quantitative MRI of Aerosolized Gd-Based Nanoparticles and Contrast Agents in Isolated Ventilated Porcine Lungs

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Purpose: Aerosol therapy represents an attractive and efficient administration route for delivering therapeutic compounds either locally or systemically. Magnetic resonance imaging (MRI) of nebulized MRI contrast agents in the lung of small animal was demonstrated previously. The objective of this work is to evaluate the suitability and performance of UTE (ultra-short echo time) sequences for imaging and quantifying the deposition of nebulized MRI contrast agents in human-sized lungs.
Methods and Materials: Nebulization of clinically used contrast agent (Clariscan, GEHC) and Gd-based nanoparticles (AGuIX, NHTheraguix) was performed using a commercial jet nebulizer (LC Sprint, Pari GmbH) in isolated and ventilated porcine lungs connected to a 3D-printed human upper airways replica. Magnetic resonance images of the lungs were acquired on a 3-T clinical MR scanner (Vantage Galan 3T ZGO, Canon) using 3D UTE sequences. Four different flip angles were used to determine T_1 values and Gd^{3+} concentration in the lungs. ICP-MS (inductively coupled plasma mass spectrometry) was performed to measure the Gd concentration in lung tissue.
Results: Lung images exhibit homogeneous and large MR signal enhancement (above 200%) following nebulization of 6 mL of both types of aerosols. 3D acquisitions with isotropic millimetric resolution were obtained in less than 4 minutes. Deposition of aerosol down to the level of the bronchi of secondary lobules was visualized as shown in the MIP image of figure 1. T_1 values and the concentration of nanoparticles were found to correlate with the amount of nebulized Gd^{3+} ions.
Conclusions: The distribution of aerosolized Gd-based contrast agent or nanoparticles can be visualized and quantified using UTE MRI in large animal ventilated lung model on a clinical MRI scanner. Large signal enhancement of the lung was observed after nebulization of 6-mL solutions containing 1.8 mmol of Gd^{3+}. As a point of comparison, the dose of intravenous administered Gd^{3+} with clinical contrast agent in patient is in the order of 8 mmol. This protocol can be used for assessing and quantifying aerosol regional deposition with high spatial resolution (1 mm 3D isotropic) without ionizing radiation and could be applied in the future for diagnostic or therapeutic applications in patients.

056:

MR Molecular Imaging With MT218 for Imaging and Assessing Therapeutic Response of Breast Cancer

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Purpose: We have developed a targeted macrocyclic GBCA, ZD2-N_3-Gd(HP-DO3A (MT218), specific to EDB fibronectin in tumor extracellular matrix for effective magnetic resonance molecular imaging (MRMI). In this work, we intended to assess the effectiveness of MRMI of EDB-FN with MT218 for detection, characterization, and therapeutic efficacy evaluation of breast cancer. We also wanted to determine the minimal effective dose of MT218 for cancer MRMI. Effective MRMI at a substantial reduced dose of MT218 could minimize dose-dependent adverse effects in clinical use.
Methods and Materials: MT218 was obtained from Molecular Theranostics, LLC (Cleveland, OH). MRI experiments were performed in mouse breast cancer models with a 3-T MRS 3000 scanner (MR Solutions). T1-weighted images were acquired before and after injection of MT218 at doses as low as 20 μmol/kg. A clinical agent ProHance (100 μmol Gd/kg) was used as a nontargeted control. The tumor bearing mice were treated with targeted miR-200c nanoparticles RGD-PEG-ECO/miR-200c once a week for 6 weeks. MR images of the tumors with MT218 were acquired before and after the treatment for assessing tumor response.
Results: MRMI with MT218 produced strong tumor contrast enhancement, an average of 3.5-fold CNR increase, in the aggressive tumors triple negative breast cancer at a dose of 40 μmol/kg as compared with precontrast images. In contrast, the clinical agent ProHance only resulted in approximately 0.5-fold CNR increase. The background noise was also significantly reduced for MT218 at 40 μmol/kg. Significant tumor enhancement was still visible for MT218 at 0.02 μmol/kg, one fifth of the clinical dose for nontargeted agents. The treatment of TNBC tumors with RGD-PEG-ECO/miR-200c significantly suppressed tumor proliferation. MRMI with MT218 revealed that there was a significant reduction of EDB-FN in the tumors treated with the nanoparticles.
Conclusions: MRMI with MT218 is effective for imaging aggressive breast cancer at substantially reduced doses, which will minimize potential dose-dependent adverse effects of GBCAs in clinical practice. MRMI with MT218 also has the promise to provide noninvasive assessment of tumor response to cancer therapy. MRMI with the targeted contrast agent has the potential to be translated into clinical practice for detection, characterization, and therapeutic monitoring of breast cancer.

THURSDAY, NOVEMBER 14
SESSION 12: CEST MRI AGENTS

057:

Development of a paraCEST and Fluorine MRI Contrast Agent

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Purpose: In magnetic resonance imaging, different classes of contrast agents are studied to obtain a better detection of pathologies. Among them, paraCEST contrast agents, based on a saturation exchange between water molecules in the vicinity of a lanthanide complex, are very promising. Indeed, the generated contrast can be turned on at will by the application of a saturation pulse with the advantage that it does not require any preinjection image. ^{19}F-MRI is also promising because there is no background noise, facilitating the detection of the generated contrast. In this study, the combination of a europium-based paraCEST contrast agent with a ^{19}F-MRI contrast agent has been studied. The interaction between the europium complex and the fluorine atoms can indeed reduce the ^{19}F relaxation times, which are usually too long for clinical use.
Methods and Materials: The paraCEST effect has been characterized by recording different Z-spectra at 600MHz, using a continuous wave irradiation of 10 seconds at different saturation frequencies. The samples were prepared in PBS at pH 7.2. The exchange rate constants were measured at 25°C and 37°C by varying the saturation power as described in the literature. The ^{19}F relaxation times have been determined at 500 MHz and 25°C in water.
Results: The europium complex exhibits great CEST properties before the coupling of the fluorine agent. Exchange rate constants of 6415 s^{-1} and 8407 s^{-1} have been determined at 25°C and 37°C, respectively, and the CEST effect remains detectable at concentrations in the millimolar range. After the grafting of the fluorinated agent, we observed an important decrease of the complex solubility as well as an increase of the exchange rate constant, making the detection of the CEST effect more difficult. However, a significant decrease of the ^{19}F relaxation times after complexation of the agent with europium was obtained, with measured T_1 and T_2 of 887 milliseconds and 468 milliseconds, respectively.
Conclusions: The agent shows great paraCEST properties, but the exchange rate constant increases after the grafting of the fluorinated molecule. We nevertheless observe a noticeable decrease of the ^{19}F relaxation times thanks to the interaction of the fluorine atoms with the europium ion. Several perspectives can be envisaged, as the replacement of the europium ion by other paramagnetic ions in order to find the best candidate to have ideal CEST properties and fluorine relaxation times. An increase of the

detection sensibility could also be obtained through the grafting of the agent at the surface of silica nanoparticles.

058:

Nano- and Micro-Sized Systems as Highly Sensitive CEST MRI Probes

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Purpose: CEST agents are useful for designing novel targeting and responsive procedures, but they have low sensitivity. To overcome this drawback, 2 routes have been pursued, that is, (1) increase proton exchange rate or (2) increase the number of exchangeable protons. Important achievements have been reached by exploiting the large pool of water molecules compartimentalized inside liposomes or cells. Three systems have been investigated: LipoCEST, GiantCEST, and Cell-CEST.

Methods and Materials: LipoCESTs were prepared by hydration of thin lipidic film method. GiantCESTs were prepared with gentle swelling method. They were loaded with Ln-shift reagents (SRs) in the inner compartment. CellCESTs were prepared by entrapping SRs through hypotonic swelling or pinocytosis. MRI was carried at 7 T.

Results: The loading of SRs allows the separation of intravesicular/intracellular water proton resonance (δ^{in}) from the bulk water one. In spherical LipoCESTs, the δ^{in} is in the ±4 ppm range (dipolar shift). In not spherical (cigar-like) LipoCESTs, the δ^{in} is shifted up to ±30 ppm (BMS effect), because of particles' alignment in the main magnetic field. The sensitivity threshold is in the pM range. Multicolor LipoCESTs have been developed in which δ^{in} is modified by changing the metal complexes in the cavity and/or in the membrane.

Spherical GiantCESTs show an analogous magnetic behavior but with a sensitivity enhancement of 3-order of magnitude (fM range), because of the 10-times bigger diameter. When subjected to osmotic stress, they display anisotropic shapes with several globe spiral-like membrane invaginations but do not orient in the magnetic field. Importantly, functionalized GiantCESTs agents can be employed in targeting experiments without internalization by endocytosis or sequestering by macrophages.

In Cell-CESTs, the δ^{in} is 3 to 10 ppm depending on (1) the amount, (2) the effective magnetic moment of the Ln(III)-ion, and (3) the compartmentalization of the SRs. The shift is due to the BMS effect because of the not spherical shape of cells or of the asymmetric SR distribution. They display an fM sensitivity, which can be visualized in vivo and used as system for quantifying cells' proliferation.

Conclusions: Nano- and micro-sized CEST systems own a detection threshold in the (sub)picomolar range. Hence, they appear promising for in cellulo/in vivo applications (targeting and responsive experiments) as well as for designing in vitro assays. LipoCESTs, GiantCESTs, or CellCEST allow a number of applications by the proper design of magnetic and chemical characteristics.

059:

Can Iopamidol and CEST MRI Be Used to Characterize Urinary Tract Obstructions?

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Purpose: Urinary tract obstructions are impairments in the flow of urine and can lead to pain, infection, and irreversible kidney damage if left undiagnosed and untreated. Chemical exchange saturation transfer (CEST) is a novel MRI contrast mechanism with one of its promising features sensitivity to environmental changes including changes in pH values. We have developed protocols for measuring perfusion and pH maps using iopamidol to characterize urinary tract obstructions and tested these on a mouse model. Herein we have tested CEST MRI protocols for characterizing urinary tract obstructions.

Methods and Materials: Phantoms

Iopamidol phantomes were prepared by dissolving Seronorm (Sero, United States) in 10 mL of deionized water with 40 mM iopamidol and placed in 5 mm NMR tubes for imaging. Phantom experiments were performed on a Bruker 11.7-T MR scanner at 37°C with images acquired using a RARE sequence with a CW saturation pulse.

Animals

C57Bl/6 mice were employed in these studies. A complete unilateral urinary obstruction was performed, and afterwards, a 100-μL volume of CEST agent (Isovue 370 diluted to 400 mM) was injected via a catheter into the tail vein and in vivo images acquired for 80 minutes by toggling the saturation offsets between 5.5, −4.85, 4.2, and 10 ppm along with 5 M0 images at 40 ppm.

Postprocessing

The preinjection Z-spectra were subtracted from all postinjection images. Ten to 20 images were averaged using a moving average filter to generate the corresponding parameter maps (contrast, FF, pH). The pixel-by-pixel ST = (1-Mz/M0) was calculated to generate ST maps using 4.2, 5.5 ppm. FF was calculated by determining the percentage of kidney pixels with contrast > 20% of the maximum. The pixel by pixel pH was calculated using in vivo ST maps and the in vitro generated calibration curve.

Results: The Z-spectra of iopamidol display 2 well-resolved resonances. Based on this, the mean ST ratio = $[M_{z4.2} \times (M_0 - M_{z5.5})]/[M_{z5.5} \times (M_0 - M_{z4.2})]$ versus pH can be fit to a polynomial to generate pH calibration curves for pH mapping as shown by Longo, Aime, and colleagues. Based on our results, pH values between 7.3 and 5.7 can be readily measured, pH values of 5.5 and below are challenging. As seen in Figure 1, CEST MRI can readily discriminate between control and obstructed kidneys in a mouse model. By 35 hours after obstruction, there is a lower agent uptake in the blocked kidney (Fig. 1B), and the contrast pattern was different for this kidney as a function of time. At 35 hours after obstruction, there was more enhancement around the calyx. As seen in Figure 1C, there was also an increase in variance in pH for the blocked kidney that evolved with time.

Conclusions: We have demonstrated that there are differences seen in CEST contrast maps between unobstructed and obstructed kidneys, which evolve with time after obstruction. This suggests this technology is promising as a diagnostic for characterizing obstructions.

060:

Clinical Plasma Volume Expanders as Potential MRI-CEST Blood-Pool Agents for Tumor Perfusion Imaging

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Purpose: Clinical low-molecular weight Gd-based contrast agents (CAs) are commonly exploited for assessing tumor perfusion and vascular permeability, despite their not optimal properties due to their low relaxivity and passive diffusion. On the other hand, macromolecular CAs, although owing improved contrast efficiency, show reduced blood pool clearance, hence increased risk of Gd accumulation in tissues. Therefore, looking for alternatives to Gd-based CAs is currently deemed of interest. Natural dextrans have been recently exploited as MRI-CEST (chemical exchange saturation transfer) macromolecular probes for assessing tumor perfusion, but their applicability is limited to preclinical studies. In this work, we aimed to investigate 2 Food and Drug Administration–approved agents, currently exploited at clinical level as plasma volume expanders, as novel blood-pool MRI-CEST perfusion agents.

Methods and Materials: In vitro CEST properties were measured in phantoms containing 6% p/v Voluven or Dextran70 in the pH range of 5.5 to 7.9 and with irradiation powers of 1-2-3 μT on a 7-T Bruker MRI scanner at 37°C. In vivo MRI experiments were carried out in a murine breast tumor model (n = 6 for each molecule). CEST spectra were acquired before and after intravenous injection of Voluven/Dextran70 (250 μL). Validation as blood-pool agents was done by sequentially acquiring T1-weighted images in the same tumor region after injection of Gd-AAZTA Madec, a blood-pool CA (dose = 0.03 mmol Gd/kg body weight). Contrast enhancement maps were calculated for both CEST and T1-weighted images, and tumor perfusion was quantified in term of the extravasation fraction, and the similarity of the spatial distribution was evaluated on a pixel-by-pixel basis.

Results: In vitro studies demonstrated comparable size to human serum albumin but different CEST contrast dependency on pH for Voluven and Dextran70. Both agents can generate in vivo similar increase in CEST contrast inside the tumor region reaching a steady state for up to 30 minutes (ΔST, 3%), persisting for longer times than that achievable with small molecular agents (Fig. 1). The observed lower extravasation fraction (30%) in comparison to a small-size CEST perfusion agent (iopamidol, 80%) is due to their reduced extravasation. In mice receiving both blood-pool CEST and T1-weighted agents, voxel-wise analysis shows a better spatial correlation in perfusion maps and higher similarity in contrast-enhanced maps between Voluven and Gd-AAZTA Madec than for Dextran70.

Conclusions: Both plasma volume expanders due to their marked CEST contrast and persisting accumulation in tumors can be considered as potential MRI-CEST blood-pool agents.

061:

TF-BAPTA as a Zinc-Specific ^{19}F-iCEST MRI Contrast Agent for Imaging Prostate Cancer

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Purpose: Of all soft tissues, the normal prostate has the highest mobile zinc content, but this level decreases dramatically during malignant transformation. We aimed to

develop iCEST (ion chemical exchange saturation transfer) ^{19}F-MRI as a noninvasive means to probe zinc depletion in prostate cancer. Although the concept of using BAPTA and TF-BAPTA as metal-specific iCEST MRI contrast agents has been reported previously in vitro, this is the first report of their successful use in vivo.

Methods and Materials: For in vitro validation studies, normal (RWPE1), ZIP1 zinc transporter-downregulated (RWPE2), and malignant (DU145 and LNCaP) human prostate cells were incubated with 75 μM $ZnSO_4$ and induced to secrete zinc using glucose stimulation. Following the addition of 5 mM TF-BAPTA, a ^{19}F-derivative of BAPTA which is a fluorescent dye indicator for zinc, the supernatant was assessed with ^{1}H- and ^{19}F-iCEST MRI at 17.6 T. For in vivo studies, 2×10^6 cancer cells were injected into the prostate of 6 to 8 weeks old immunodeficient NSG mice and allowed to grow for 21 days. As a second in vivo model, a transgenic adenocarcinoma of the mouse prostate (TRAMP) model was used. Immediately after i.p. injection of 80 μL of 20% wt/vol D-glucose and injection of 0.15 g/kg body weight TF-BAPTA into the anterior prostate through a catheter, iCEST MR images were collected at 17.6 T using a modified RARE sequence with an FOV of 2.6 × 2.6 cm, 4 mm slice thickness, and a resolution of 0.8 × 0.8 mm.

Results: In vitro, the strongest iCEST signal was observed for glucose-stimulated RWPE1 cells with a normal zinc transporter level. In normal prostate cells with a downregulated ZIP1 zinc transporter (RWPE2), a weaker iCEST signal was observed. No signal could be observed for DU145 and LNCaP prostate cancer cells. In vivo, the strongest iCEST signal was observed for the normal prostate following i.p. glucose stimulation. Both the normal prostate without glucose stimulation and the 2 orthotopic tumor models with glucose stimulation showed much weaker iCEST signal. For TRAMP mice, a decrease of >300% in iCEST contrast following the transition of normal prostate cells to cancer cells was detected.

Conclusions: Using iCEST MRI, differences in glucose-induced zinc secretion between normal and malignant prostate cells can be readily detected, both in vitro and in vivo. Hence, the use of TF-BAPTA as iCEST MRI contrast agent for serial probing of in vivo zinc content may find applications as a noninvasive imaging biomarker for early detection of prostate cancer.