ABSTRACTS

INVITED LECTURES

1

LIGAND-DIRECTED TARGETING AND MOLECULAR GENETIC IMAGING IN DISEASES WITH AN ANGIOGENESIS COMPONENT. Wadih Arap, Renata Pasqualini. University of Texas M.D. Anderson Cancer Center, TX, USA; contact e-mail: rpasqual@mdanderson.org.

Our group originally developed two broad ligand-directed targeting technology platforms to uncover and exploit functional protein interactions in the context of human disease: combinatorial selection of peptide libraries in patients and hybridoma-free generation of monoclonal antibodies. Essentially, by using these two complementary biotechnologies over the past decade, we have been probing the molecular diversity (for example, of the vascular and lymphatic endothelium or of the humoral immune system) to find unique cell surface addresses—endothelial and otherwise—for delivery to selective cell types or cell populations, vasculature of tissues, and/or organ systems. There are many potential, as yet unrecognized, ligand-receptor interactions that may lead to applications such as targeted drug delivery, vascular-mediated tissue repair. Such a set of ligand-receptor interactions can encompass applications in different organ-specific vascular beds in health and diseased conditions. The aggregate data we have generated thus far indicate that a new targeted pharmacology and its ramifications are now unequivocally at hand. Topics covered in this lecture include—but are not limited to—vascular and lymphatic targeting, molecular-genetic imaging, and other applications of toolkits of scientific and medical value.

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IMAGING THE DEVELOPMENT OF LYMPHOID ORGANS. Dimitris Kioussis,[1] Mark Coles,[1] Henrique Veiga-Fernades,[1] Katie Foster.[1] [1]Department of Molecular Immunology, MRC, National Institute for Medical Research, London, UK; contact e-mail: dkiouss@nimr.mrc.ac.uk.

Utilizing the GFP transgenic and knock-in mice has permitted an analysis of cellular interactions, movement, and function in lymphoid organ development and function. The development of lymphoid organs occurs during embryonic life as a result of interactions between lymph node inducing cells and stromal cells. Utilizing human CD2-GFP transgenic mice we have been able to analyze the molecular requirements for the development of the intestinal immune system. The intestinal immune system consists of Peyer's patches, cryptopatches, intestinal lymphoid follicles, and intraepithelial lymphocytes. The development of Peyer's patches follows the aggregation in the gut wall of Peyer's patch–inducing cells during embryonic development. Utilizing imaging technology we have been able to observe the role of cell movement in the aggregation of Peyer's patches. Utilizing a combination of gene expression analyses of Peyer's patch–inducing cell populations and genetic approaches we have studied molecules that have an important role in Peyer's patch formation.

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TRANSLATIONAL MOLECULAR IMAGING FOR ONCOLOGY. Martin G. Pomper. Johns Hopkins Medical Institutions, Baltimore, MD, USA; e-mail: mpomper@jhmi.edu.

Although most clinical diagnostic imaging studies employ anatomic techniques such as computed tomography (CT) and magnetic resonance (MR) imaging, much of radiology research currently focuses on adapting these conventional methods to physiologic imaging as well as on introducing new techniques and probes for studying processes at the cellular and molecular levels in vivo, ie, molecular imaging. Molecular imaging promises to provide new methods for the early detection of disease and support for personalized therapy. Although molecular imaging has been practiced in various incarnations for over 20 years in the context of nuclear medicine, other imaging modalities have only recently been applied to the noninvasive assessment of physiology and molecular events. Nevertheless, there has been sufficient experience with specifically targeted contrast agents and high-resolution techniques for MR imaging and other modalities that we must begin moving these new technologies from the laboratory to the clinic. Four projects relevant to oncology will be discussed with emphasis on how they were/will be moved from the bench to the clinic.

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BIOLUMINESCENCE IMAGING OF MOUSE MODELS OF BRAIN TUMORS. Eric Holland. Memorial Sloan-Kettering Cancer Center, New York City, NY, USA; contact e-mail: hollande@mskcc.org.

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INTRAOPERATIVE MOLECULAR IMAGING. James P. Basilion. NFCR Center for Molecular Imaging, Case Western Reserve University, Cleveland, OH, USA; contact e-mail: james.basilion@case.edu.

Glioblastoma multiforme (GBM) is the most aggressive and lethal form of brain tumor, making treatment very difficult. There are approximately 15,000 cases reported annually in the United States with a post-diagnosis mean survival time of just 9 to 12 months. The tumors are composed of cells derived from astrocytes that form an initial mass and invade throughout normal brain parenchyma. Complete tumor resection is limited by the availability of accurate surgical technologies to distinguish tumor from healthy tissue at the infiltrative GBM margin. Several reports now conclusively demonstrate that modest improvements in tumor removal as small as 2% significantly increase patients' mean survival times. Since patients' lives could be significantly improved with more complete resections, we are developing a completely novel approach for identifying and aiding in the removal of infiltrating tumor cells. In many cancers, including GBMs, biomarkers such as proteases are differentially regulated, making them a potential target for molecular imaging markers. Near-infrared fluorescence (NIRF) optical imaging is a proven technology for identifying cancers based on specific protease expression. This combined with microscopic surgical techniques is an attractive way to more effectively remove infiltrating tumor cells. We are exploring the use of an activity-based reporter probe (ABP, Blum et al, 2005). Specifically, the ABP is an optically silent small molecule that is able to interact with cathepsins B and L, resulting in covalent modification of the enzyme, inhibition, and activation of probe fluorescence. Thus, this probe can be used to detect and permanently report on the presence of enzymatic activity. We have data to suggest specific cathepsin probe activation in both heterotopic and orthotopic athymic mouse brain tumor models. Probe activation, recorded over time with a MaestroTM In-vivo Imaging System, is seen as early as 5 minutes and remains optically visible at least out to 40 minutes, well within a reasonable operating window. Studies are underway to determine specificity by the use of inhibitors combined with immunocytochemical techniques. This technology can be applied not only to brain cancers but any cancer that has differently regulated cathepsin activity.

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DIAGNOSTIC CHALLENGES IN CARDIOVASCULAR DISEASES—OPPORTUNITY FOR MOLECULAR IMAGING. Michael Schafers. Department of Nuclear Medicine, University Hospital of Munster, Munster, Germany; contact e-mail: schafmi@uni-muenster.de.

Although huge and long-lasting research efforts have been spent on the development of new diagnostic techniques investigating cardiovascular diseases, still fundamental challenges exist, the main challenge being the diagnosis of a suspected or known coronary artery disease or its consequences (myocardial infarction, heart failure, etc.). Beside morphological techniques, functional imaging modalities are available in clinical diagnostic algorithms, whereas molecular cardiovascular imaging techniques are still under development. This review summarizes clinical-diagnostical challenges of modern cardiovascular medicine as well as the potential of new molecular imaging techniques to face these.

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POSITRON EMISSION TOMOGRAPHIC INSTRUMENTATION IN MOLECULAR IMAGING: FROM SINGLE MODALITY TO MULTIMODALITY AND BEYOND. Bernd J. Pichler. Laboratory for Preclinical Imaging and Imaging Technology, Department of Radiology, University of Tuebingen, Tuebingen, Germany; contact e-mail: bernd.pichler@med.uni-tuebingen.de.

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THE ROLE OF ULTRASOUND AND MAGNETIC RESONANCE IMAGING FOR IMAGE-GUIDED LOCAL DRUG DELIVERY. Chrit T.W. Moonen. Laboratory for Molecular and Functional Imaging: From Physiology to Therapy, UMR 5231; CNRS/Université « Victor Segalen » Bordeaux 2, Bordeaux, France.

Local drug delivery has the potential to increase the local therapeutic effect while limiting systemic toxic effects. Either targeted or nontargeted drug-carrying nanoparticles may be used for local drug delivery. The objectives of image guidance are (1) target identification and characterization; (2) temporospatial guidance of actions to release or activate the drugs and/or permeabilize membranes; (3) evaluation of pharmacodistribution; (4) physiological read-outs to evaluate the therapeutic efficacy. Whereas the value of imaging, in particular MRI, is well known in the first and fourth objectives, the emphasis of this review is on the second and third objectives. Special attention is paid to the potential of MRI-guided focused ultrasound. Research in the field of nanoparticles for local drug/gene delivery is very active. Local release may be triggered by natural processes, such as membrane fusion, phagocytosis, and pinocytosis, but also by external physical means, such as ultrasound. Nanoparticles may be designed specifically to enhance ultrasound-induced bioeffects, notably cavitation. Most microbubbles consist of air- or perfluorocarbon-filled microsphere stabilized by an albumin or lipid shell with a size in the range of 1–10 μm. Drugs can be attached to the membrane surrounding the microbubble, be imbedded within the membrane itself, bound noncovalently to the surface of the microbubble, and loaded to the interior of the microbubble, either in an oil or aqueous phase. These microbubbles can be targeted to specific (pathologic) sites using different targeting ligands incorporated into bioconjugates. The concept of using thermosensitive liposomes in combination with local hyperthermia for local drug release was proposed more than 25 years ago by Weinstein et al. Liposomes remain relatively stable in the circulation at temperatures well below the phase transition temperature (T_c) of the liposome membrane. At T_c distinctive structural changes occur in the lipid bilayer, resulting in increased membrane permeability and the accompanying release of the liposomes' content. Liposomes may carry both hydrophilic and hydrophobic drugs in their aqueous interior and lipid bilayer membrane, respectively. The circulation half-life may be increased by incorporating polyethylene glycol (PEG) lipids in the bilayer. The recent developments of measuring and controlling temperature with MRI-guided focused ultrasound should lead to improved control of locally released drugs with temperature-sensitive nanocarriers. Viral-mediated gene transfer is efficient, but safety aspects have limited therapeutic applications. Stem cells and immune cells have a particular advantage as gene delivery systems since they home in to lesions by the action of chemokines. They can be labeled and tracked using imaging methods. A thorough analysis of pharmacodistribution is a mandatory aspect of local drug/gene delivery. Using most of the methods described above, genes are delivered within the vascular system, and the local distribution and its temporal evolution are a function of the local perfusion, uptake by surrounding cells, metabolism, and release. Therefore, such local delivery must be accompanied by evaluation of pharmacodistribution and pharmacokinetics in order to predict outcome. Imaging may provide a noninvasive assessment of such parameters. Similar to the encapsulation of drugs in nanocarriers, contrast agents can be included that report on the local release of drugs and subsequent tissue distribution. Ideally, such agents would be directly linked with the drugs. However, in many cases, co-released MR contrast agents may provide useful data related to pharmacodistribution even when they are not linked. Among the key challenges in gene therapy are the method of gene delivery and the spatial and temporal control of therapeutic (trans)gene expression in the targeted tissue. The ability of high-intensity focused ultrasound (HIFU) to heat tissue deep inside the body can be used to control transgene expression when the gene is placed under control of a heat-sensitive promoter.

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NEAR-INFRARED FLUORESCENCE IMAGING OF SINGLE-WALLED CARBON NANOTUBES IN BIOLOGICAL SYSTEMS. Bruce Weisman. Department of Chemistry, Rice University Houston, TX, USA; contact e-mail: weisman@rice.edu.

Single-walled carbon nanotubes (SWNTs) are a family of artificial tubular nanostructures composed of covalently bonded carbon atoms with diameters near 1 nm and lengths typically of hundreds of nanometers. Although they are hydrophobic, SWNTs can be suspended in aqueous media by noncovalent coating with artificial surfactants, DNA, RNA, or proteins. When excited by visible light, most SWNT structures emit intrinsic fluorescence at well-defined near-IR wavelengths between 900 and 1,600 nm. Because there is very little autofluorescence in this spectral region, SWNTs in biological environments can be detected and imaged with high sensitivity and selectivity. Moreover, SWNTs show

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unsurpassed photostability and an absence of blinking. Recent results will be described in which near-IR fluorescence methods have been used to monitor and track SWNTs in cells, tissues, and organisms. In one project, macrophage cells were grown in the presence of SWNTs. The resulting uptake of nanotubes was quantified by bulk fluorimetry and imaged by near-IR fluorescence microscopy. In an in vivo study, SWNTs fed to *Drosophila* larvae were imaged in the living animals and in dissected tissues. It was possible to observe the location, orientation, and structural identities of individual nanotubes in these tissue specimens. Finally, a mammalian in vivo study explored the pharmacokinetics of SWNTs after intravenous injection into rabbits, using near-IR fluorescence to selectively monitor nanotube concentrations. A circulation half-life of 1.0 hours was determined, and examination of tissue specimens taken 24 hours after exposure revealed SWNTs only in the liver. Future prospects for the use of SWNTs as near-IR fluorophores in biology will be discussed.

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IMAGING RECEPTOR FUNCTION AND PATHWAY ACTIVITY IN THE RODENT BRAIN.
Markus Rudin. Institute for Biomedical Engineering UZH/ETH Zürich and Institute for Pharmacology and Toxicology, UZH, Zürich, Switzerland; contact e-mail: rudin@biomed.ee.ethz.ch.
Due to the inaccessibility of cerebral tissue noninvasive imaging techniques have become central for the study of central nervous system (CNS) pathologies and for monitoring therapeutic interventions. A number of specific imaging approaches are currently being developed that allow annotation of CNS structures with functional and molecular information such as the visualization and quantification of receptor distribution and occupancy, of receptor function, or of the infiltration and migration of labeled cells into the CNS. The focus of the presentation will be the assessment of receptor function using fMRI readouts and molecular imaging tools that target specific signal transduction pathways. fMRI studies involving pharmacological stimulation/inhibition of CNS receptors offer significant opportunities with regard to characterization of CNS disorders such as neurodegeneration or psychiatric diseases, but also in view of evaluating efficacy of therapeutic interventions. The approach assesses functional consequences of the ligand-receptor interaction and complements classical receptor binding studies. While the fMRI-based methods provide an indirect readout on molecular processes via physiological coupling, targeted imaging techniques yield immediate insight in molecular and cellular events in the living organism. Besides direct receptor imaging using a labeled reporter ligand, visualization of the activation of signal transduction pathways constitutes an attractive application. In basic research the use of genetically engineered cells or animals that express a reporter gene upon pathway activation is a commonly used approach for imaging critical pathway molecules or protein-protein interactions. Such reporter gene assays are available for optical, PET, and MR imaging. We are currently developing an assay to assess hypoxia-associated processes.

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MULTICENTER, MULTITRACER, AND HIGH-RESOLUTION POSITRON EMISSION TOMOGRAPHY IN DEMENTIA. Karl Herholz. Wolfson Molecular Imaging Centre, University of Manchester, Manchester, UK; contact e-mail: karl.herholz@manchester.ac.uk.
Brain PET using FDG now is a firmly established technique for demonstration of regional functional impairment in neurodegenerative disease. Most dementing disorders, especially Alzheimer's disease (AD) and frontotemporal dementia (FTD), are associated with typical regional impairment of posterior or anterior cortical association areas that allows very early diagnosis and monitoring of progression and treatment effects. Feasibility of multicenter FDG PET studies in AD has been demonstrated in 1993, showing that with proper study conditions, scanning protocols, and procedures for data analysis controls and AD patients can be discriminated with better than 90% accuracy in typical PET study samples. More recent developments include larger samples (more than 1,000 subjects in the European Network for Efficiency and Standardisation of Dementia Diagnosis – NEST-DD), pooling of autopsy-confirmed cases, voxel-based image analysis, and multivariate methods for discrimination among dementia diseases. Amyloid imaging using the thioflavin analogue 11C-PIB has shown consistently 2- to 3-fold increased tracer retention in patients with AD in many laboratories across the world. Fluorine-18 tracers for easier use in a multicenter setting are under current investigation. Multicenter studies using these tracers will provide in vivo demonstration of amyloid deposition in dementia patients, thus contributing to diagnostic specificity, and are also likely to be useful for monitoring of antiamyloid therapies. The clinical significance of incidental amyloid deposition in cognitively normal subjects is an important current research issue. The potential of other tracers targeting cholinergic and serotoninergic transmitter pathways as well as microglial activation for multicenter studies is being evaluated within the European Network of Excellence on Diagnostic Molecular Imaging (www.dimi-net.org). Parkinson's disease, as the most frequent neurodegenerative movement disorder, is characterized by impaired uptake of 18F-fluorodopa (FDOPA), which—similar to FDG for AD—allows early diagnosis and monitoring of progression and treament effects. PET and SPECT tracers for dopamine reuptake transporters are also being used to study dopaminergic degeneration. This is of clinical interest for diagnosis of dementia with Lewy bodies (DLB), which is characterized by a severe cholinergic and dopaminergic deficit. High-resolution PET using dedicated reserach scanners now can reach isotropic 2.2 mm FWHM, corresponding to a volume of about 10 μL. This opens the possibility to study small structures such as the hippocampus and parahippocampal structures as well forebrain and brainstem nuclei that are potentially impaired in dementia. Currently studies are underway to analyze the interaction between atrophy and functional disturbance in those areas to elucidate the pathophysiology of dementia.

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MERGING STRUCTURAL AND FUNCTIONAL INFORMATION IN NEUROIMAGING: QUANTITATIVE APPROACHES. Mario Quarantelli. Institute of Biostructures and Bioimages, National Research Council, Naples, Italy; e-mail: quarante@unina.it.
Integration of neuroimaging data aims at exploiting the complementary information provided by high-resolution structural images (eg, CT/MRI) and low-resolution functional/metabolic images (eg, PET/SPET or MR spectroscopic images). Merging this information implies a complex process including segmentation of structural images, coregistration of functional and structural data, and correction of functional data for partial volume effects (PVE). In PVE-corrected data the relative contributions of the size and activity of the imaged structure are disentangled, allowing us, for example, to discriminate between the relative contributions of atrophy and hypofunction/hypometabolism in brain pathology, which are otherwise indistinguishable in functional images alone due to PVE. However, despite the fact that several techniques for PVE correction have been proposed, and most neuroimaging studies include today some sort of PVE correction in the processing steps, the impact of these procedures is often unclear, and no consensus has been reached on the best approach to be used when analyzing different functional image sets (eg, when different tracers are used). After reviewing the most diffuse PVE correction approaches, including a discussion on validation results on simulated data, we will present results from different approaches to PVE correction on rCBF, FDG, and neuroreceptor studies in both normal subjects and disease. Particular attention will be paid to the statistical use of the PVE-corrected data, specifically assessing problems related to the application of voxel-based analysis techniques on PVE-corrected images. Presented results will show the importance of correcting PVE and contribute to the choice of the PVC technique to be used.

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IMAGING THE DYNAMICS OF SPREADING DEPRESSION IN THE ISCHEMIC PENUMBRA.
Rudolf Graf,[1] Hajime Nakamura,[1] Anthony J. Strong.[2] [1]MPI for Neurological Research, Cologne, Germany; [2]King's College London, London, UK; contact e-mail: rudolf.graf@nf.mpg.de.
Spreading depression (SD) was first described in 1944 by the Brazilian physiologist Leão as a cortical wave of suppressed electrocorticogram propagating with a velocity of 3–5 mm/min. SD can be elicited by such diverse stimuli as mechanical puncture, high extracellular potassium or glutamate, or electrical high-frequency pulses. Characteristics also include a slow negative direct current potential change associated with major ion redistribution at cellular membranes and increases in tissue lactate. In physiological conditions, SD is normally coupled with a hyperemic vascular response that compensates for the energy needed to reinstall cellular ion homeostasis. Experimental and recent human studies provide evidence that SD occurs spontaneously in pathophysiological conditions such as trauma or hemorrhagic and ischemic stroke. Subsequent to induction of brain injuries, waves of depolarization arise repetitively over prolonged periods. They are most frequent in penumbra zones surrounding infarcts, denominated then as peri-infarct depolarization (PID). Recent dynamic imaging studies of perfusional changes using laser speckle flowmetry show that in peri-infarct regions, SD/PID coupled blood flow alterations may be missing or even hypoemic, so compensatory effects are not achieved. Waves may propagate either in a radial fashion from injured areas outward into peripheral regions, or they may repetitively propagate in a circular fashion around injuries, thereby hitting multiple times border zones of lesions. Resulting stepwise progressive deterioration may finally lead to an inability of the tissue to repolarize and, in consequence, to terminal anoxic depolarization and tissue death.

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QUANTUM DOTS FOR IN VIVO IMAGING. Benoit Dubertret. ESPCI, Laboratoire d'Optique Physique, Paris, France; contact e-mail: benoit.dubertret@espci.fr.
Semiconductor nanoparticles known as quantum dots (QDs) are fluorescent nanometric size nanoparticles with fluorescence emission that can range from 400 nm to 1,500 nm depending on the size and the material used. We will review the different applications of QDs for in vivo imaging stressing the importance of their surface chemistry. We will also discuss the potential use of these nanoparticles as probes for multifunctional imaging.

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INNOVATIVE NANOSIZED MAGNETIC RESONANCE IMAGING PROBES. Enzo Terreno, Daniela Delli Castelli, Silvio Aime. Department of Chemistry, IFM and Molecular Imaging Center, University of Turin, Turin, Italy; contact e-mail: enzo.terreno@unito.it.
The use of nanomaterials in the design of innovative MRI contrast agents has considerably grown up in the last years. The main advantages of these systems include (1) the high payload of contrasting units that can significantly improve the probe sensitivity, (2) the relative easiness to modulate their pharmacokinetic properties and driving their biodistribution (passive and active targeting), (3) the possibility to have probes for different imaging modalities (eg, MRI, optical imaging, PET, SPECT, BCNT) in the same nanosystem, (4) the exploitation of the peculiar properties of the nanomaterials already used in the pharmaceutical field for imaging drug delivery or for the set-up of combined diagnosis and therapy protocols. In this contribution, among the nanosystems considered so far in the design of improved paramagnetic MRI agent, particular attention will be devoted to paramagnetic lanthanide (III)-based liposomes. In addition to the design of innovative Gd-based vesicles as a concentration-independent responsive probe, special emphasis will be given to the recent achievements in the field of LIPOCEST agents and on the use of paramagnetic liposomes as susceptibility T2 agents.

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DETECTING EARLY TUMOR RESPONSES TO THERAPY USING MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY. Kevin Michael Brindle. University of Cambridge and Cancer Research UK, Cambridge, UK; contact e-mail: kmb@mole.bio.cam.ac.uk.
We have been developing noninvasive and clinically applicable magnetic resonance–based methods for detecting the early responses of tumors to therapy. A primary focus has been on the development of methods for detecting tumor cell death since the level of tumor cell death immediately after drug treatment has been shown, in preclinical and clinical studies, to be a good prognostic indicator for treatment outcome. Thus, an oncologist may get an indication of whether a particular drug is working very early during treatment, possibly within 24 to 48 hours, and long before there is any evidence of tumor shrinkage. The primary focus of our work has been the development of a targeted MRI contrast agent that binds to dying cells, and recent progress with this agent will be described. More recently, we have started to work with dynamic nuclear polarization (DNP) of 13C-labeled cell substrates, which offers gains in sensitivity of more than 104-fold, allowing subsecond acquisition of 13C spectral data in vivo. Using DNP MRSI we have studied the metabolism of hyperpolarized [1-13C] pyruvate in an EL-4 lymphoma cells and in implanted EL-4 tumors, before and after treatment with the chemotherapeutic drug etoposide. There was a significant reduction in lactate dehydrogenase–catalyzed exchange of 13C label between pyruvate and lactate in tumors 24 hours after drug treatment. Images of intratumoral 13C pyruvate and 13C lactate showed a marked reduction in intensity in lactate/pyruvate ratiometric images. The decrease in exchange can be explained by a reduction in the lactate concentration in the tumor, a reduction in cellularity, and possibly decreases in intracellular coenzyme (NAD(H)) and lactate dehydrogenase concentrations. The absence of any background 13C signal means that specific images of enzyme activity can be acquired. The lack of ionizing radiation, the use of an endogenous metabolite, and a single imaging modality make DNP 13C MRI an attractive potential tool for imaging the early responses of tumors to treatment in the clinic.

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MULTIPARAMETER IMAGING OF SIGNALING ACROSS THE PLASMA MEMBRANE. Theodorus W.J. Gadella, Joachim Goedhart, Merel Adjobo-Hermans, Gert-Jan Kremers, Laura van Weeren. Molecular Cytology, SILS, University of Amsterdam, Amsterdam, the Netherlands; contact e-mail: gadella@science.uva.nl.
We use fluorescent biosensors that are designed to specifically visualize signalling dynamics across the membrane from G protein–coupled receptors through G proteins, phospholipase C, and protein kinase C in mammalian cells. Within this signaling module, the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P2) into diacylglycerol (DAG) and inositol trisphosphate (IP3) (triggering

intracellular calcium oscillations) is the key step. Our aim is to understand in detail the spatiotemporal aspects of this signaling cascade. Our approach is to systematically label all key molecules and visualize their distribution and activity in living cells with multimode quantitative fluorescence microscopy. To visualize the key signaling enzymes we use visible fluorescent protein (VFP) fusions. We monitor the activity of phospholipase C by the visualization of the signalling lipids employing highly specific lipid-binding protein domains fused to VFPs. The activity of protein kinase C is monitored by using genetic encoded ratiometric FRET sensors reporting on phosphorylation kinetics. The calcium release is imaged using translocating Ca^{2+}-sensitive lipid binding domains. Using multimodal imaging techniques (including total internal reflection [TIRF] microscopy) we are able to perform multiparameter imaging of the activation of this signaling module in live cells. Our results demonstrate complex spatiotemporal behavior, including transient recruitment and activation of effectors at hot spots adjacent to the plasma membrane.

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CANCER AND CANCER-INITIATING CELLS. Rolf Bjerkvig. NorLux, Neuro-Oncology, University of Bergen, Bergen, Norway, and Centre Recherche Public Santè, Luxembourg; contact e-mail: rolf.bjerkvig@pki.uib.no.

The events that lead to the cancer-initiating cell involve critical mutations in genes regulating normal cell growth and differentiation. Cancer stem cells, or cancer-initiating cells, have been described in the context of acute myeloid leukemia, breast, brain, bone, lung, melanoma, and prostate. These cells have been shown to be critical in tumor development and should harbor the mutations needed to initiate a tumor. The origin of the cancer stem cells is not clear. They may be derived from stem cell pools, progenitor cells, or differentiated cells that undergo transdifferentiation processes. It has been suggested that cell fusion and/or horizontal gene transfer events, which may occur in tissue repair processes, also might play an important role in tumor initiation and progression. Fusion between somatic cells that have undergone a set of specific mutations and normal stem cells might explain the extensive chromosomal derangements seen in early tumors. The regulation of the balance between cell renewal and cell death is critical in cancer. Increased knowledge of developmental aspects in relation to self-renewal and differentiation, both under normal and deregulated conditions, can shed light over the mechanisms that lead to tumor initiation and progression and thereby provide the basis for new therapeutic principles. To gain such knowledge, the research group has developed unique animal models to study the growth and progression of cancer stem cells. These models will be discussed in detail.

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UNDERSTANDING STEM CELL–MEDIATED BRAIN REPAIR USING CELLULAR MAGNETIC RESONANCE IMAGING. Michel Modo. Centre for the Cellular Basis of Behaviour, Kings College London, London, UK; contact e-mail: m.modo@iop.kcl.ac.uk.

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MULTIFLUORESCENCE LIVE VISUALIZATION OF VIRUS REPLICATION BY CONFOCAL LASER SCANNING MICROSCOPY. Cornel Fraefel. Institute of Virology, University of Zurich, Zurich, Switzerland; contact e-mail: cornelf@vetvir.unizh.ch.

Autofluorescent proteins are being employed to visualize fundamental cellular processes. When applied to virus research, this strategy allows us to monitor dynamic events in the life cycle of a virus, including adsorption, penetration, intracellular transport, and virus assembly. Herpes simplex virus type 1 (HSV-1) is composed of three different compartments, capsid, tegument, and envelope. We have constructed a recombinant HSV-1 that simultaneously encodes selected structural proteins from all three virion compartments fused with red, yellow, or cyan fluorescent proteins. This triple-fluorecent recombinant HSV-1 is an excellent tool to investigate the dynamics of the virus life cycle. It supports access to more detailed aspects of compartmentalization and interaction among viral proteins in the infected cell. Autofluorescent proteins can also be applied to visualize the spatial and temporal organization of virus genome replication and the competition between replication compartments of different viruses. For example, adeno-associated virus (AAV) is a small, nonpathogenic human parvovirus whose replication depends on the presence of a helper virus, such as herpesviruses and adenoviruses. While the interaction between AAV and adenovirus has been intensively studied, far less is known about the interactions between AAV and HSV-1. We have established live cell visualization assays in order to directly assess the reciprocal interaction between AAV and HSV-1 on the single-cell level. These assays use the binding of autofluorescent repressor proteins (LacI, TetR) with operator sequences (lacO, tetO) cloned into the virus genomes.

ORAL COMMUNICATIONS

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RADIOLABELED G PROTEIN–COUPLED RECEPTOR ANTAGONISTS MAY BE SUPERIOR TO AGONISTS FOR IN VIVO TUMOR TARGETING. Xuejuan Wang,[1] Mihaela Ginj,[1] Hanwen Zhang,[1] Renato Cescato,[2] Damian Wild,[1] Judit Erchegyi,[3] Jean Rivier,[3] Jean Claude Reubi,[2] Helmut R. Maecke.[1] [1]University Hospital Basel, Basel, Switzerland; [2]University of Berne, Berne, Switzerland; [3]Salk Institute, La Jolla, CA, USA; contact e-mail: xuejuanwang31@hotmail.com.

Agonists have been selected exclusively as a radiopharmaceutical for peptide receptor targeting in vivo as they trigger internalization into and accumulation in the cells over time. Recent studies, however, revealed that radiolabeled somatostatin receptor antagonists bind many more sites and thus may be preferable to agonists for in vivo peptide receptor targeting of tumors. On the basis of these findings three potent sst2-selective antagonists, Cpa-c[DCys- Aph(Cbm)-DTrp-Lys-Thr-Cys]-Nal-NH2 (JR3), 4-NO2-Phe-c(DCys-Tyr-DTrp-Lys-Thr-Cys)- DTyr-NH2 (JR4), and 4-NO2-Phe-c(DCys-Tyr-DTrp-Lys-Thr-Cys)-NH2 (JR5), were synthesized and coupled with the macrocyclic chelator 1,4,7,10-tetraazacyclododecane-1,4,7,10- tetraacetic acid (DOTA) for labeling with In-111. In-111/nat-DOTA-JR3, In-111/nat-DOTA-JR4, and 111/natIn-DOTA-JR5, respectively, showed high and selective sst2-binding affinity. Scatchard plots showed that the three antagonists labeled many more sites than the potent sst2-selective agonist, [In-111-DOTA,1-Nal3]-octreotide (In-111-DOTANOC). In-111- DOTA-JR3, In-111-DOTA-JR4, and In-111-DOTA-JR5 were injected intravenously into mice bearing sst2-expressing tumors, and their biodistribution was monitored. In the sst2-expressing tumors, strong accumulation of In-111-DOTA-JR4 was observed, peaking at 4 h with 29.12 $\pm$ 3.90% IA/g and remaining at a high level for $>$ 72 h. The tumor uptake of In-111-DOTA-JR3 was higher than that of In-111-DOTANOC, too. However, at 4 h the uptake of In-111-JR5 into the tumor is quite low (3.56% $\pm$ 0.65%), and almost complete washout at 24 h (1.21% $\pm$ 0.31%) was observed. Excess cold sst2 antagonists blocked the uptake in tumor and normal sst2-expressing organs. Lysine (20 mg) or gelofusine (4 mg) could significantly reduce the renal uptake of In-111-DOTA-JR4. The tumor-to-kidney ratio for In-111-DOTA-JR4 increased from 3.09 to 6.18 (lysine) and 6.01 (gelofusine).

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INTRAINDIVIDUAL COMPARISON OF THE TARGETING AND BIODISTRIBUTION OF EPIDERMAL GROWTH FACTOR RECEPTOR–SPECIFIC NANOBODIES IN MICE USING MICROSPECT-CT. Tony Lahoutte,[1] Olive Gainkam Tchouate,[1] Lieven Huang,[2] Vicky Caveliers,[1] Cindy Peleman,[1] Sophie Hernot,[1] Chris Vanhove,[1] Marleen Keyaerts,[1] Hilde Revets,[3] Patrick De Baetselier,[2] Axel Bossuyt.[1] [1]In Vivo Cellular and Molecular Imaging Laboratory, Vrije Universiteit Brussel, Brussels, Belgium; [2]Department of Molecular and Cellular Interactions, Flanders Interuniversity Institute for Biotechnology, Vrije Universiteit Brussel, Brussels, Belgium; [3]Ablynx N.V., Zwijnaarde, Belgium; contact e-mail: lahoutte@gmail.com.

Nanobodies are a new class of antibody-derived therapeutic proteins. A critical step in the evaluation of new molecules is the assessment of the in vivo targeting efficiency and their biodistribution. In order to reduce the variability of the measurements due to biological differences between the in vivo animal models we compared the new molecules intraindividually using MicroSPECT-CT imaging. **Methods:** We compared two EGFR-targeted nanobodies: 7C12 and 7D12. Nanobodies were labeled via their hexahistidine tail with 99mTc-tricarbonyl (Isolink, Mallinckrodt, the Netherlands). MicroSPECT-CT imaging was performed at 1 h postinjection of on average 80 MBq 99mTc-7C12 or 99mTc-7D12 in A431 mice xenografts (n = 14). Each animal was studied with both tracers on separate days (72 h interval) in a crossover protocol. MicroCT imaging was followed by pinhole SPECT, and both images were fused based on a rigid body transformation using six landmarks. Image quantification was performed using AMIDE. Ellipsoid regions of interest were drawn around tumor, muscle, liver, kidneys, lung, brain, and total body. Tracer uptake is expressed as % injected activity/cm^3 tissue (%IA/cm^3 $\pm$ standard deviation). **Results:** 99mTc-7C12 showed significantly higher kidney uptake (70.9 $\pm$ 13.2 vs 54.2 $\pm$ 10.8 %IA/cm^3, $p < .005$) and lower liver uptake (2.5 $\pm$ 1.0 vs 4.6 $\pm$ 3.2 %IA/cm^3, $p < .05$) compared to 99mTc-7D12. Tumor uptake of 99mTc-7C12 and 99mTc-7D12 was similar: 4.4 $\pm$ 0.9 and 4.6 $\pm$ 1.4 %IA/cm^3, respectively. The tumor to muscle ratio was 26 $\pm$ 14 for 99mTc-7C12 and 23 $\pm$ 11 for 99mTc-7D12. **Conclusions:** 7C12 and 7D12 show high and comparable tumor uptake but different biodistribution patterns within the same animal.

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TRANSIENT MYOCARDIAL AVB3 INTEGRIN EXPRESSION IN A RAT MODEL OF ISCHEMIA AND REPERFUSION EVALUATED BY 18F-GALACTO-RGD POSITRON EMISSION TOMOGRAPHY. Takahiro Higuchi,[1] Frank M. Bengel,[1] Stefan Seidel,[2] Stephan G. Nekolla,[1] Marc C. Huisman,[1] Sybille Reder,[1] Hans J. Wester,[1] Markus Schwaiger.[1] [1]Nuklearmedizinische Klinik und Poliklinik der Technischen Universität München, Klinikum Rechts der Isar, Munich, Germany; [2]Institut für Allgemeine Pathologie und Pathologische Anatomie der Technischen Universität München, Klinikum Rechts der Isar, Munich, Germany; contact e-mail: higuchi@po2.nsknet.or.jp.

$\alpha v\beta 3$ Integrin is thought to play a critical role in migration and adhesion of endothelial cells during myocardial angiogenesis. Using 18F-galacto-RGD, an F-18-labeled glycosylated $\alpha v\beta 3$ integrin antagonist, this study aimed at determining the time course of cardiac integrin expression in a rat model of myocardial ischemia/reperfusion. **Methods and Results:** Rats were subjected to 20 minutes of transient left coronary artery occlusion following by reperfusion. Autoradiographic analysis was used to determine myocardial 18F-galacto-RGD uptake at different time points after the reperfusion. Autoradiography yielded no significant focal myocardial 18F-galacto-RGD uptake in nonoperated controls and 1 day after reperfusion. However, focal accumulation in the risk area started at 3 days (uptake ratio = 1.91 $\pm$ 0.22 vs remote myocardium), peaked between 1 week (3.43 $\pm$ 0.57, $p < .001$ vs 1 day) and 3 weeks (3.43 $\pm$ 0.95, $p < .001$ vs 1 day), and decreased time dependently but was still detectable at 6 months (1.96 $\pm$ 0.40) after the reperfusion. Administration of 18 mg c(RGDfV) per kg weight demonstrated significantly decreased 18F-galacto-RGD uptake in rats at 1 week after reperfusion (1.74 $\pm$ 0.24, n = 6, $p < .001$), indicating the receptor-specific uptake. The time course of focal tracer uptake was correlated with vascular density determined by histology. In vivo imaging using a dedicated small-animal PET system confirmed focal tracer uptake in the myocardium in agreement with autoradiographic findings. **Conclusions:** Regional 18F-galacto-RGD accumulation suggests transient upregulation of $\alpha v\beta 3$ integrin expression in myocardium after coronary occlusion and reperfusion.

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IN VIVO OPTICAL IMAGING OF NEUROGENESIS. Sebastien Couillard-Despres, Sonja Ploetz, Robert Aigner, Ulrich Bogdahn, Ludwig Aigner. Department of Neurology, University of Regensburg, Regensburg, Germany; contact e-mail: sebastien.couillard-despres@klinik.uni-regensburg.de,

Neurogenesis, the generation of new neurons, is a process taking place not only during development but also in specific regions of the adult CNS. Hence, in adult mammalians, including humans, new neurons are continuously generated in the dentate gyrus and in subventricular regions of the lateral ventricle. Neurogenesis is a highly regulated process that can be up- and downregulated by a plethora of factors, from single molecules to environmental factors as well as pathological processes. Moreover, following lesions, newly generated neurons can be targeted to the lesioned tissues. So far no simple tool was available to perform in vivo imaging of neurogenesis. We thus developed an imaging strategy based on doublecortin (DCX), a marker specifically and transiently expressed in neuronal precursors. We could show recently that the level of DCX expression reflected the level of ongoing neurogenesis, although the long-term fate of newly generated neurons cannot be inferred. Taking advantage of the neurogenesis-associated expression pattern of DCX, we generated transgenic mice expressing fluorescent and luminescent reporter genes under the control of the DCX promoter. We demonstrated here that using slice cultures of DCX-fluorescent transgenic mice, newly generated neurons could be observed at the single-cell level and be analyzed in vivo. Moreover, using DCX-luminescent reporter animals, global neurogenesis could be detected in intact animals at different time points. These two new transgenic tools open new avenues for the analysis of neurogenesis in vivo and hence new possibilities to investigate the kinetics of neurogenesis modulation and its functional impact.

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A NEW NONINVASIVE METHOD TO QUANTIFY RENAL FUNCTION IN RATS AND MICE: HIGH-RESOLUTION SMALL-ANIMAL POSITRON EMISSION TOMOGRAPHY WITH FLUORIDE-18. Uta Schnöckel,[1] Stefan Reuter,[2] Klaus Schäfers,[1] Gert Gabriels,[2] Sven Hermann,[1] Otmar Schober,[1] Eberhard Schlatter,[2] Michael Schäfers.[1] [1]Department of Nuclear Medicine, University Hospital Münster, Münster, Germany; [2]Department of Internal Medicine D, University Hospital Münster, Münster, Germany; contact e-mail: uta.schnoeckel@uni-muenster.de.

Renal function can be quantified by laboratory and scintigraphic methods. In the case of small-animal diagnostics, image-based methods are ideal since they work noninvasively (no blood sampling) and can

be repeated. The aim of this study is the validation of a F-18-PET-based method to quantify renal function in rats and the transfer of this new method into mice. Renal function in rats was assessed from a dynamic whole-body acquisition of 60 min length in a small-animal PET scanner following an IV injection of F-18-fluoride. The renal fluoride clearance was calculated by the ratio of the total renal excreted activity and the integral of the blood time activity curve. PET-derived renal function was validated by intraindividual measurements of creatinine and urea clearance ($n = 20$) as well as tubular excretion rate (TER-MAG) ($n = 8$) and split renal function ($n = 10$) after injection of Tc-99m-mercaptotriglycine by blood sampling and scintigraphic imaging. PET-derived renal function was linearly correlated with intraindividual laboratory and imaging measures (PET vs TER-MAG: $r = .81$; PET vs crea: $r = .72$; split function gamma camera vs PET $r = .98$). In conclusion, F-18-PET is able to noninvasively assess renal function in rats and will enable serial studies of renal function in different experimental scenarios. In the future this method should be transferred into mice. First studies showed the principal feasibility of this method with promising results.

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ASSESSMENT OF THE IMAGING CAPABILITY OF THE YAP-(S)PET SMALL-ANIMAL SCANNER IN A RAT MODEL OF ISCHEMIA AND REPERFUSION. Antonietta Bartoli,[1] Vincenzo Lionetti,[2] Paola Anna Erba,[3] Serena Fabbri,[1] Nicola Belcari,[1] Alberto Del Guerra,[1] Fabio Recchia,[4] Giuliano Mariani,[3] Piero Salvadori.[5] [1]Department of Physics "E. Fermi" and Center of Excellence "AmbiSEN," University of Pisa, and INFN, Sezione di Pisa, Pisa, Italy; [2]Scuola Superiore Sant'Anna, Institute of Clinical Physiology-CNR, Pisa, Italy; [3]Nuclear Medicine Section, University of Pisa, Pisa, Italy; [4]Scuola Superiore Sant'Anna, Institute of Clinical Physiology-CNR, Pisa, Italy; Department of Physiology, New York Medical College, Valhalla, NY, USA; [5]Positron Emission Tomography and Radiopharmaceutical Chemistry Department, CNR Institute of Clinical Physiology, Pisa, Italy; contact e-mail: bartoli@df.unipi.it.

Objectives: There is increasing evidence that cell death after myocardial ischemia and reperfusion (IR) may begin as apoptosis rather than necrosis. For the detection of early apoptosis in vivo, we have performed in vivo studies with the YAP-(S)PET scanner in a rat model of IR. The YAP-(S)PET is the only small-animal scanner that combines PET and SPECT techniques on a single gantry and offers good resolution and sensitivity. **Methods:** The IR heart model was realized on male Sprague-Dawley rats (10–12 weeks old). Left thoracotomy at the fifth intercostal space and pericardiotomy were performed, a 6/0 braided silk suture was placed around the proximal portion of the left anterior descending coronary artery, and the coronary artery was occluded for 30 minutes by pulling on the suture. Then the heart was reperfused by releasing the ligature, and the thoracotomy was closed. One hour later, the rats were injected via the tail vein with 10 mCi of 99mTc-Myoview. After 180 minutes of uptake, a static acquisition of 50 minutes was performed. Immediately after, an injection of 8 mCi of 99mTc-annexin V was performed in the tail vein. An acquisition of 1 hour and a half was performed after 90 minutes of uptake. **Results:** The 99mTc-Myoview and 99mTc-annexin V images were recontructed using the EM algorithm with a collimator model and then fused. The 99mTc-annexin V uptake, observed within the area at risk of the heart, confirmed the apoptotic cell death process in vivo. **Conclusion:** The results obtained and the capability of the YAP-(S)PET scanner to perform both PET and SPECT studies produced the willingness to use PET tracers, ie, 13N-NH3, instead of 99mTC-Myoview and to image simultaneously myocardial blood flow and apoptosis process.

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MOLECULAR IMAGING METHODS FOR THE STUDY OF THE PHARMACOKINETICS, DISTRIBUTION, AND IN VIVO ACTIVITY OF SMALL INTERFERING RIBONUCLEIC ACID. Thomas Viel,[1] Raphaël Boisgard,[1] Benoit Jego,[1] Karine Siquier,[1] Françoise Hinnen,[2] Frédéric Dollé,[2] Bertrand Tavitian,[1] Bertrand Kuhnast.[1] [1]CEA, DSV, I2BM, SHFJ, Laboratoire d'Imagerie Moléculaire Expérimentale, Orsay, France; [2]CEA, DSV, I2BM, SHFJ Groupe de Radiochimie et Radio-pharmacie, Orsay, France; contact e-mail: thomas.viel@cea.fr.

RNA interference is a powerful tool to inhibit gene expression. In vivo, the use of oligonucleotides is limited by their bioavailability. Here we used molecular imaging to evaluate different modifications of siRNAs on their biodistribution, pharmacokinetics, and in vivo activity. We prepared chemically modified siRNAs and (1) evaluated their in vitro RNAi efficiency, (2) labeled them with fluorine-18 and analyzed their in vivo biodistribution by positron emission tomography imaging and plasmatic metabolism by HPLC, and (3) evaluated their in vivo RNAi activity by optical imaging. 2′-Fluoro-siRNA showed the same RNAi efficiency as unmodified siRNA. Modification by 2′-O-methyl-nucleotides of both strands, but not of the sense strand only, hampered RNAi activity. We labeled antisense strands with [18F]-fluoropyridine-bromoacetamide, hybridized the two strands of siRNAs, and assessed the intensity of the RNAi effect. In vitro, all radiolabeled siRNAs showed the same interference efficiency as nonconjugated oligonucleotides. The main route of siRNA elimination was the renal system followed by the hepatoenteric route. siRNA kinetics were similar for nonmodified and 2′O-methyl-modified siRNA. For 2′-fluoro-siRNA, the radioactivity peak was reached later and the half-life in plasma was increased threefold in comparison to unmodified siRNA. However, 2′-fluoro-siRNA showed a lower interference effect in xenograft models in mice. To our knowledge this is the first report on the labeling of siRNAs with the short-lived positron emitter fluorine-18. This approach permits parallel and combinatorial preparation of different duplexes and the functionalization of the nonlabeled strand independently of the radiochemistry, therefore allowing us to correlate sequences and chemistries with pharmacodistribution. Indeed, our results show that further in vivo evaluations on the effect of chemical modifications of siRNAs are required in order to understand their consequences on the pharmacology of this new class of regulators of gene expression.

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MULTIMODALITY STUDY OF A RAT MODEL FOR HUNTINGTON DISEASE: CAN WE BRIDGE DIFFERENT TECHNIQUES? Nadja Van Camp,[1] Ines Blockx,[2] Cindy Casteels,[3] Lluisa Camon,[4] Nuria de Vera,[4] Marleen Verhoye,[2] Karolien Goffin,[3] Emili Martinez,[4] Guy Bormans,[5] Anna Planas,[4] Koen Van Laere,[3] Annemie Van der Linden.[2] [1]CEA, DSV, I2BM, SHFJ, Laboratoire d'Imagerie Moléculaire Expérimentale, Orsay, France; [2]Bio-Imaging Lab, University of Antwerp, Antwerp, Belgium; [3]Division of Nuclear Medicine, University Hospital Gasthuisberg and K.U. Leuven, Leuven, Belgium; [4]Institute for Biomedical Research (IIBB-CSIC), IDIBAPS, Barcelona, Spain; [5]Laboratory for Radiopharmacy, K.U. Leuven, Leuven, Belgium; contact e-mail: Nadja.van-camp@cea.fr.

In search of in vivo diagnostic tools for the assessment of disease severity or for the evaluation of putative therapies, imaging techniques such as DTI-MRI and PET are preferred as they provide structural and molecular information noninvasively. In animal studies these in vivo obtained imaging data are very often validated with histology. Nevertheless, to our knowledge the possibilities to correlate multimodality imaging data of MRI-DTI with microPET have not been fully explored yet. In the present study we quantified the lesion in the quinolinic acid (QA) rat model of Huntington's disease ($n = 12$) using (1) the amphetamine challenge rotation test, (2) in vivo DTI-MRI at 7 T, and (3) microPET imaging ([18F]-FDG and [11C]-raclopride). All data were statistically compared with sham animals that were treated similarly ($n = 12$). DTI-MRI revealed significant changes ($p < .05$) in all DTI parameters in both white (internal and external capsule) and gray matter (striatum and cortex) ipsilateral to the lesion, while microPET imaging showed decreased glucose metabolism (-35%, $p < 2.10–12$) and D2-receptor binding (-77%, $p < 2.10–11$) in the affected striatum. Subsequently, correlations between the multimodal imaging parameters measured in striatum and cortex ipsilateral to injection and behavioral data were investigated. The most significant correlations ($p < .05$) were demonstrated in cortex and striatum between different DTI parameters. A weak correlation ($p = .04$) was shown between FDG and axial diffusivity ($\lambda 1$) in the affected cortex of QA animals. Voxel-based statistics are currently in progress, and we expect that this procedure on the coregistered multimodality images might be more sensitive and hence reveal more intermodality correlations.

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IMAGING INDUCIBLE GENE EXPRESSION IN AN INTRACRANIAL GLIOMA MODEL IN VIVO. Alexandra Winkeler,[1] Leonie E.M. Paulis,[2] Yannic Waerzeggers,[1] Miguel Sena-Esteves,[3] Hongfeng Li,[1] Andreas H. Jacobs.[1] [1]Laboratory for Gene Therapy and Molecular Imaging at the Max-Planck Institute for Neurological Research with Klaus-Joachim-Zülch-Laboratories of the Max Planck Society and the Faculty of Medicine of the University of Cologne and Departments of Neurology, University of Cologne and Klinikum Fulda, Cologne, Germany; [2]Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, the Netherlands; [3]Departments of Neurology and Neuroscience, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA, USA; contact e-mail: winkeler@nf.mpg.de.

Objective: To investigate noninvasive assessment of inducible gene expression in an intracranial (IC) tumor model in vivo. **Background:** Noninvasive imaging of gene expression can be performed by positron emission tomography (PET) as well as bioluminescence imaging (BLI). We previously demonstrated that regulated gene expression mediated by inducible HSV-Amplicon vectors (HET6C-tk39/-fluc) can be noninvasively imaged by HSV-1-tk39 (PET) and firefly luciferase (fluc; BLI) in a subcutaneous tumor model in vivo. **Methods:** To noninvasively assess IC regulated gene expression with BLI or PET, we used two doxycycline-regulated HSV-Amplicon vectors controlling gene expression of rfp and fluc or rfp and HSV-1-tk39, respectively. To compare regulation in vivo human gliomas were grown intracranially in nude mice and infected in or ex vivo with both vectors. Subsequently, a series of multimodal imaging was performed with BLI and PET, in the presence or absence of doxycycline. **Results:** Intracranial, HET6C-fluc-regulated gene expression could be noninvasively assessed by BLI in five mice. The maximal ratio of induction after doxycycline treatment was 14.9-fold. In contrast, IC regulated gene expression after in vivo application of HET6C-tk39 could not be imaged with 18F-FHBG-PET. However, recent results with ex vivo infected IC glioma cells showed HET6C-tk39-gene expression in the presence of doxycycline. **Conclusions:** Intracranial induction of exogenous gene expression from in vivo infected cells is imaged with noninvasive BLI whereas visualization of regulation with 18F-FHBG-PET is, up to now, only possible with ex vivo infected cells.

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COLLAGEN BINDING MAGNETIC RESONANCE–DETECTABLE LIPOSOMES. Erik Sanders,[1] Sanne Reulen,[2] Monica Breurken,[2] Willem Mulder,[3] Anita Mol,[4] Maarten Merkx,[2] Gustav Strijkers,[1] Klaas Nicolay.[1] [1]Biomedical NMR, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, the Netherlands; [2]Laboratory of Macromolecular and Organic Chemistry, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, the Netherlands; [3]Department of Radiology, Mount Sinai School of Medicine, New York, New York, USA; [4]Laboratory for Cell and Tissue Engineering, Deparment of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, the Netherlands; contact e-mail: h.m.h.f.sanders@tue.nl.

The extracellular matrix (ECM) plays an important role in normal tissue maturation and in pathological processes, such as atherosclerosis and myocardial infarction. Collagen is the major constituent of the ECM providing strength to tissues, and it plays a key role in all main processes concerning the ECM. It is therefore important to be able to image various ECM components, such as collagen. Magnetic resonance imaging (MRI) has emerged as the potential leading in vivo modality in a number of diagnostic protocols. The goal of this study is to investigate whether it is possible to use MR contrast agents (CAs) to obtain valuable information on collagen. A bimodal liposomal MR-CA, carrying rhodamine functionalized lipids and large amounts of gadolinium-containing lipids, was prepared by lipid film hydration followed by extrusion through 200 nm membranes. The primary amines of CNA-35, a bacterial collagen adhesion protein of *Staphylococcus aureus*, were SATA modified and subsequently conjugated to the distal end of maleimide functionalized PEG lipids. Fluorescence measurements indicated specific CNA-35-mediated binding to rat tail collagen I. A binding experiment indicated a dissociation constant in the nanomolar range. Quantitative MRI measurements clearly showed a significant T1 reduction by targeted liposomes as compared with controls, demonstrating the applicability of this CA for collagen imaging. We infer that this CA might be suitable to follow collagen formation during wound healing, in tissue-engineered constructs, and to monitor the process of atherosclerosis with MRI.

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DEVELOPMENT OF LANTHANIDE LUMINESCENT COMPLEXES FOR CELLULAR IMAGING. Filip Kielar, David Parker. Durham University, Durham, UK; contact e-mail: filip.kielar@dur.ac.uk.

Luminescent lanthanide complexes show potential for use in cellular imaging. Problems associated with the weak light absorption of lanthanide ions, arising from the forbidden nature of the ff transitions, are usually solved by the use of sensitizing chromophores. Chromophores (tetraazatriphenylene, azaxanthone, thiaazaxanthone) containing carboxylic acid groups have recently been incorporated into luminescent lanthanide complexes prepared in our group. The possibility to use these carboxylic groups as linking points was investigated, and a two-step methodology, involving a conversion to an active ester (NHS), was identified as the most versatile. Simple amides of varying chain length derived from these azaxanthone complexes were thus prepared and their cell uptake was investigated. These results are further elaborated. A possibility to use these results in the synthesis of a dual imaging probe (MR-optical) is investigated in cooperation with partners P50 within the DiMI network (Charles University, Prague).

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[18F]FPYKYNE, A FLUOROPYRIDINE-BASED ALKYNE REAGENT FOR THE FLUORINE-18-LABELING OF MACROMOLECULES USING CLICK-CHEMISTRY. Bertrand Kuhnast, Françoise Hinnen, Bertrand Tavitian, Frederic Dolle. CEA/I2BM/SHFJ/LIME, Service Hospitalier Frédéric Joliot, Institut d'Imagerie BioMédicale, Orsay, France.

Introduction: The recent discovery that Cu(I) catalyzes the Huisgen 1,3-dipolar cycloaddition of terminal alkynes and organoazides to form 1,2,3-triazoles, often referred to as "click-chemistry," undoubtedly opens new routes for the prosthetic labeling of macromolecules with the positron emitter fluorine-18. As part of our continuous efforts in the development of novel [18F]fluoropyridine-based reagents (cf, the two recently radiosynthesized thiol-selective reagents [18F]FPyME and [18F]FpyBrA[1,2]) [18F]FPyKYNE (2-[18F]fluoro-3-pent-4-ynyloxypyridine), a novel alkyne reagent for click-[18F]radiochemistry, has been designed. **Methods:** FPyKYNE (as reference) as well as the 2-bromo and 2-nitro analogues (as precursors for labeling with fluorine-18) were synthesized in one step from the appropriated 2-substituted-3-hydroxypyridines and commercially available 5-chloropent-1-yne (K2CO3/KI, DMSO or DMF, 120°C, 15 hrs). [18F]FPyKYNE was synthesized in one single radiochemical step by reaction of K[18F]F-Kryptofix-222 with the bromo or nitro precursor (6 mg, DMSO, 165°C, 5 min) followed by C-18 Sep-Pak cartridge prepurification and finally semipreparative HPLC purification. **Results:** The 2-bromo-, 2-nitro-, and 2-fluoro-pentynyloxypyridines were obtained in 90% yields. Fluorine-18 incorporation gave [18F]FPyKYNE in up to 80% yield (based on radio-TLC). **Conclusion:** [18F]FPyKYNE has been efficiently synthesized in one single radioactive step and could be obtained in less than 1 hour and in 60% nonoptimized, non-decay-corrected yield after HPLC purification.

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USE OF HIGHLY SENSITIVE DUAL PROBES GADOLINIUM-LIPOSOME AND GADOLINIUM-LOADED APOFERRITIN FOR TARGETING TUMOR ANGIOGENESIS FOR MAGNETIC RESONANCE VISUALIZATION AND DRUG DELIVERY. Simonetta Geninatti-Crich, Benedetta Bussolati, Lorenzo Tei, Giovanna Esposito, Cristina Grange, Stefania Lanzardo, Giovanni Camussi, Silvio Aime. University of Torino, Torino, Italy; contact e-mail: simonetta.geninatti@unito.it.

Targeting tumor vessels can be useful for imaging angiogenic blood vessels as a potential predictive marker of antiangiogenic treatment response or as a method to deliver chemiotherapeutic drugs directly to the tumor cells. We recently reported the expression of the neural cell adhesion molecule (NCAM) in immature and tumor endothelial cells (TEC) lining vessels of human carcinomas. Exploiting an in vivo model of human tumor angiogenesis obtained by implantation of TEC in Matrigel in SCID mice, we aimed to image angiogenesis with MRI by detecting the expression of NCAM. For this purpose, we developed new highly efficient probes either by entrapping the T1-contrast agent Gd-HPDO3A (Prohance®) into the apoferritin cavity or by synthesizing liposomes containing a Gd(III) complex in the membrane. Both systems were linked to a specific NCAM binding peptide C3d as targeting vector for TEC and eventually loaded with a chemiotherapeutic drug (doxorubicin) for assessing also the cytotoxicity on the tumor cells. The amplification of the MR signal due to the Gd-loaded apoferritin and to the liposome systems allowed the visualization of TEC both in vitro and in vivo when organized in microvessels connected to the mouse vasculature. The signal enhancement due to the liposome system is higher than in the Gd-loaded apoferritin system because the number of Gd complexes incorporated in the liposome membrane is higher and because the targeting peptide is covalently attached to the liposome and therefore the particles directly recognize the NCAMs without the biotin/straptavidin step. Both imaging probes displayed a good in vivo stability and tolerability.

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MAGNETIC RESONANCE IMAGING REVEALS HEMATOPOIETIC STEM/PROGENITOR CELLS HOMING TO THE BRAIN OF MICE AFFECTED BY METACHROMATIC LEUKODYSTROPHY. Letterio S. Politi,[1] Alessia Capotondo,[2] Angela Gritti,[2] Angelo Quattrini,[1] Giuseppe Scotti,[1] Luigi Naldini,[2] Alessandra Biffi.[2] [1]Neuroradiology Unit, San Raffaele Scientific Institute, Milan, Italy; [2]San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET), San Raffaele Scientific Institute, Milan, Italy; contact e-mail: letterio.politi@hsr.it.

Metachromatic leukodystrophy (MLD) is a demyelinating lysosomal storage disorder resulting from arylsulfatase A (ARSA) deficiency. We showed that transplantation of gene-corrected hematopoietic stem/progenitor cells (HSPCs) in MLD mice corrects disease manifestations. HSPC-derived, gene-corrected microglia constitute the unique source of enzyme in the affected brain. We wanted to unravel whether gene-corrected microglia originates from differentiated cells migrating from the circulation upon engraftment or from progenitors homing to the brain shortly after the transplant. To follow the fate of HSPCs in leukodystrophic animals, we labeled HSPCs from GFP donors with super-paramagnetic contrast agents and monitored their distribution by magnetic resonance imaging (MRI) in vivo upon transplantation. We could detect labeled cells in the brain of transplanted MLD mice shortly after infusion. Prussian blue and GFP stainings confirmed the presence of donor-derived, iron-containing hematopoietic cells in regions highlighted by MRI. Further, bioluminescence coupled to MRI confirmed viability of labeled HSPCs shortly after transplantation upon luciferase gene transfer. Labeled cells were particularly abundant in the hippocampal fimbria and in the corpus callosum of both neonate and 1-month-old MLD mice. Interestingly, in these areas a delay in myelination was observed. Similarly, labeled cells were present in the dentate gyrus of the hippocampus, where we documented an alteration in endogenous neural stem cell proliferation. These data demonstrate homing of hematopoietic progenitors to the brain of MLD mice and suggest that the recruitment of HPSCs might occur preferentially in regions of the brain where pathology is more pronounced.

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IMAGING BONE MARROW–DERIVED TUMOR-INFILTRATING PROGENITOR CELL THERAPY OF GLIOMAS. Hrvoje Miletic,[1] Yvonne Fischer,[2] Sara Litwak,[3] Tsanan Giorglou,[2] Yannic Waerzeggers,[4] Alexandra Winkeler,[4] Hongfeng Li,[4] Uwe Himmelreich,[5] Claudia Lange,[6] Werner Stenzel,[1] Martina Deckert,[1] Harald Neumann,[3] Andreas H. Jacobs,[4] Dorothee von Laer.[2] [1]Abteilung für Neuropathologie, Universität zu Köln, Köln, Germany; [2]Georg-Speyer-Haus, Frankfurt am Main, Germany; [3]Neural Regeneration Unit, Institute of Reconstructive Neurobiology, University of Bonn LIFE & BRAIN Center and Hertie Foundation, Bonn, Germany; [4]Labor für Gentherapie und Molekulares Imaging, Max-Planck-Institut für Neurologische Forschung, Universität zu Köln, Köln, Germany; [5]In-Vivo NMR Laboratory, Max-Planck-Institute for Neurological Research with Klaus-Joachim-Zülch-Laboratories of the Max Planck Society and the Faculty of Medicine of the University of Cologne, Cologne, Germany; [6]Bone Marrow Transplant Center, University Hospital Hamburg-Eppendorf, Hamburg, Germany; contact e-mail: hrvoje_miletic@gmx.de.

Adult stem cells are promising cellular vehicles for therapy of malignant gliomas as they have the ability to migrate into these tumors and even track infiltrating tumor cells. However, their clinical use is limited by a low passaging capacity that impedes large-scale production. In the present study, a bone marrow–derived, highly proliferative subpopulation of mesenchymal stem cells—here termed bone marrow–derived tumor infiltrating cells (BM-TIC)—was genetically modified for the treatment of malignant glioma. Upon injection into the tumor or the vicinity of the tumor, BM-TIC infiltrated solid parts as well as the border of rat 9L glioma. After intratumoral injection, BM-TIC expressing the thymidine kinase of herpes simplex virus (HSV-tk) and eGFP (BM-TIC-tk-GFP) were detected by noninvasive positron emission tomography (PET) using the tracer [18F]FHBG. A therapeutic effect was demonstrated in vitro and in vivo by BM-TIC expressing HSV-tk through bystander-mediated glioma cell killing. Therapeutic efficacy was monitored by PET as well as by magnetic resonance imaging (MRI) and strongly correlated with histological analysis. In conclusion, BM-TIC expressing a suicide gene were highly effective in the treatment of malignant glioma in a rat model and therefore hold great potential for the therapy of malignant brain tumors in humans.

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QUANTITATIVE BIOLUMINESCENCE IMAGING OF THE MIGRATION OF LENTIVIRAL VECTOR-LABELED ENDOGENOUS NEURAL STEM CELLS IN MOUSE BRAIN. Veerle Reumers,[1] Christophe Michel Deroose,[2] Rik Gijsbers,[3] Olga Krylychkina,[3] Martine Geraerts,[3] Luc Mortelmans,[2] Zeger Debyser,[3] Veerle Baekelandt.[1] [1]Laboratory for Neurobiology and Gene Therapy, Katholieke Universiteit Leuven, Leuven, Belgium; [2]Nuclear Medicine, Katholieke Universiteit Leuven, Leuven, Belgium; [3]Laboratory for Molecular Virology and Gene Therapy, Katholieke Universiteit Leuven, Leuven, Belgium; contact e-mail: veerle.reumers@med.kuleuven.be

Objectives: Continuous neurogenesis in the adult rodent brain occurs in the subventricular zone (SVZ). The offspring of the endogenous neural stem cells (eNSCs) in the SVZ migrates to the olfactory bulb (OB). We previously showed stable gene transfer in adult eNSCs in vivo by injection of lentiviral vectors (LV) into the SVZ. We aimed to develop a bioluminescence imaging (BLI) strategy for noninvasive detection and quantification of the migration of neuroblasts to the OB by LV marking of the eNSCs with firefly luciferase (Fluc). **Methods:** LV, encoding both eGFP and Fluc, were stereotactically injected in the SVZ of C57Bl/6 mice. Mice were scanned with an IVIS 100 on day 3 and week 1, 15, and 30 after injection. Mice were sacrificed at different time points and ex vivo BLI was performed. Immunohistochemistry was performed for eGFP followed by stereological quantification of the labeled cells in the OB. **Results:** BLI scans after 3 and 7 days showed signal originating from the site of injection. At 15 weeks a clearly distinct second focus appeared at the OB projection site and this focus was still present at 30 weeks. The OB/SVZ ratio showed an increase at week 15 and week 30 compared with day 3 and 7 ($p < .0005$). Ex vivo BLI of the OB showed an increase of Fluc activity in time. eGFP cell counts in the OB confirmed this result. Both the in vivo BLI signal ($r^2 = .89$) and the ex vivo Fluc activity ($r^2 = .90$) showed a strong linear correlation with the number of eGFP-positive cells. **Conclusions:** We have shown that BLI allows in vivo whole-body imaging and quantification of the migration of LV-labeled eNSCs from the SVZ to the OB.

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DPA COMPOUNDS: PROMISING RADIOLIGANDS FOR NEUROINFLAMMATION IMAGING. Fabien Chauveau,[1] Hervé Boutin,[2] Nadja Van Camp,[1] Cyrille Thominiaux,[1] Roger Fulton,[3] Michelle James,[4] Anna Creelman,[5] Silvia Selleri,[5] Frédéric Dollé,[1] Bertrand Tavitian,[1] Michael Kassiou.[6] [1]CEA, Institut d'Imagerie Biomédicale, Service hospitalier Frédéric Joliot, Laboratoire d'Imagerie Moléculaire Expérimentale, Orsay, France; INSERM, U803, Orsay, France; [2]Faculty of Life Sciences, Neurobiology Group, University of Manchester, Manchester, UK; [3]Department of PET and Nuclear Medicine, RPAH, NSW, Australia; [4]Department of Pharmacology, University of Sydney, NSW, Australia; [5]Dipartimento di Scienze Farmaceutiche, Università di Firenze, Firenze, Italy; [6]School of Medical Radiation Sciences, University of Sydney, NSW, Australia; School of Chemistry, University of Sydney, NSW, Australia; Ramaciotti Centre for Brain Imaging, Brain and Mind Research Institute, University of Sydney, NSW, Australia; contact e-mail: fabien.chauveau@cea.fr.

Neuroinflammation has been identified in several degenerative disorders and in stroke. The peripheral benzodiazepine receptor, also named translocator protein, is mainly expressed by activated microglia in the brain and is therefore considered a reliable marker of neuroinflammation. [11C]PK11195 is the reference radiotracer for this target, but its use is hampered by high nonspecific signal and subsequent quantification difficulty. Several 'DPA' compounds based upon a common pyrazolopyrimidine moiety have been recently radiolabeled as an alternative to the isoquinoline-based PK11195. The potential of [11C]DPA-713, [18F]DPA-714, [11C]DPA-715 has been evaluated in vivo in healthy baboons and in rats with unilateral lesion of the striatum. This allows direct comparison of the three compounds, in relation to various in vitro data. Imaging of the baboon brain reveals poor uptake of [11C]DPA-715 in contrast to high uptake and slow washout of [11C]DPA-713 and [18F]DPA-714. The binding of those two molecules can be blocked by pretreatment with an excess of PK11195. Imaging of the operated rats shows a higher uptake in the lesioned area than in the symmetrical area in the intact contralateral hemisphere for all radioligands. [11C]DPA-715 gives a lower contrast than [11C]PK11195 due to poor entry in the brain, whereas [11C]DPA-713 and [18F]DPA-714 give a higher contrast, which can be totally abolished by an excess of PK11195. This study highlights the great potential of [11C]DPA-713 and [18F]DPA-714 for increasing the sensitivity of neuroinflammation detection. More generally, it supports the efficiency of animal PET imaging in screening and identifying radiotracer candidates for clinical development.

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FOLLOWING THE SHAPING OF THE PERIPHERAL T CELL REPERTOIRE IN NEWBORN MICE BY FLUORESCENCE MOLECULAR TOMOGRAPHY. Anikitos Garofalakis,[1] Ana Sarasa,[1] Giannis Zacharakis,[1] Clio Mamalaki,[2] Jorge Ripoll.[1] [1]FORTH-IESL, Heraklion, Crete, Greece; [2]FORTH-IMBB, Heraklion, Crete, Greece; contact e-mail: agarof@iesl.forth.gr.

Fluorescence molecular tomography (FMT) is a volumetric imaging technique that can achieve several centimeters of tissue penetration, as well as detecting very low levels of fluorochromes in whole living animals. With this system, 3D tomographic images of small animal models are reconstructed by collection at multiple projections of photons propagated throughout tissue. We intend to monitor, by combining this optical imaging technique with molecular immunology procedures, specific events crucial for T cell development and function, as is the shaping of the peripheral T cell repertoire early in life. To this aim we use murine transgenic lines where all T cells are tagged with fluorescent proteins, namely GFP or DsRed. Cervical lymph nodes of neonates and young mice were analyzed by FMT at different time points. The animals were then sacrificed, their lymph nodes were dissected under a fluorescence stereoscope, and the corresponding number of lymphocytes expressing the fluorescent protein was quantified by flow cytometry. There is a linear correlation between the reconstructed data and the number of fluorescently tagged cells collected by flow cytometry. Repetitive measurements to monitor fluorescence in vivo were taken, and cervical lymph nodes were shown to grow in terms of the number of T cells during the first days of life. We are currently trying to follow the migration rates of T

cells expressing a fluorescent protein. We will furthermore determine the rates of apoptotic death by annexin V staining previous to flow cytometry.

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TRANSLATIONAL RESEARCH FROM MICE TO MAN: MOLECULAR IMAGING OF INTESTINAL GRAFT-VERSUS-HOST-DISEASE ACTIVITY WITH POSITRON EMISSION TOMOGRAPHY.
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Gastrointestinal graft-versus-host disease (GI-GvHD) is a common and potentially life-threatening complication after allogeneic hematopoietic stem cell transplantation (HSCT). Detection of disease activity is pivotal for diagnosis and therapeutic management. Intestinal F-18-FDG uptake was serially assessed in an experimental murine model of acute GvHD over 3 weeks. Mice were scanned 1 hour after intravenous injection of 10 MBq fluorodeoxyglucose (FDG) using a dedicated small-animal PET scanner (quadHIDAC). Animal studies demonstrated an enhanced FDG uptake in the colon of animals with GvHD compared with no FDG uptake in the control group, which was proven by in vivo/ex vivo studies with eGFP- (fluorescence) positive donor lymphocytes, radiotracer distribution, and corresponding histology. The findings of the mouse studies were directly translated to the clinical scenario in patients with suspected gastrointestinal GvHD (n = 22). Twelve patients showed a significant FDG uptake of the gut, again predominantly in the colon. In all of these patients, GvHD responded to immunosuppressive treatment, and reevaluation with FDG-PET showed markedly decreased FDG uptake in 6 of 6 patients. None of the 10 patients with normal FDG-PET findings developed GvHD of the gut. The findings indicate that diagnostic imaging using FDG-PET is a sensitive noninvasive procedure to assess localization and activity of GI-GvHD with the potential for widespread clinical use following allogeneic HSCT.

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IMAGING BONE MORPHOGENETIC PROTEIN 7–INDUCED CELL CYCLE ARREST IN EXPERIMENTAL GLIOMAS.
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Bone morphogenetic proteins (BMPs) belong to the super family of TGFβ-like cytokines. Both TGFβ and BMPs can act as tumor suppressors as well as tumor promotors. In thyroid and prostate cancer, it has already been shown that BMPs block tumor progression. Our investigations focus on analyzing the effects of BMP-7 treatment during glioma cell proliferation in vitro and in vivo. First, we investigated the mRNA expression of BMP-7 and TGFβ1–3 as well as the corresponding type 1 and type 2 receptors and downstream signaling molecules by RT-PCR after isolation of total cellular RNA from different human glioblastoma-derived cell lines (Gli36wt, Gli36ΔEGFR, Gli36ΔEGFR-LITG, U87wt, U87ΔEGFR, U251, G55T2, A172). BMP-7 treatment decreased the proliferation of Gli36ΔEGFR-LITG cells, which were stably transfected with a retroviral construct coding for firefly luciferase-IRES-tk-gfp (LITG) up to 50%, which was not due to increased apoptosis. In Gli36ΔEGFR-LITG cells BMP-7 clearly induced a cell cycle arrest in G1 phase, which was further elucidated by analyzing the expression and activity of potential targets, such as cyclin-dependent kinases and its inhibitors. Furthermore, antiproliferative effects of human recombinant BMP-7 were imaged in experimental gliomas by optical imaging of luciferase activity (LUC-OI) in vivo. Gli36ΔEGFR-LITG cells were implanted intracranially into nude mice, which received recombinant BMP-7 (100 μg/kg/day) daily by IV injection. LUC-OI was performed at several time points after initiation of BMP-7 treatment monitoring the remarkable antiproliferative effect of the cytokine, which points out new therapeutic strategies for clinical applications of malignant gliomas in future.
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REFLECTANCE SPECTROPHOTOMETRY AS INTRAOPERATIVE ASSESSMENT OF MUCOSAL PERFUSION IN ESOPHAGEAL ANASTOMOSIS (RESPECT STUDY).
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Following esophageal resection the most important complication and cause of death is anastomotic leakage. In many cases, reinterventions are needed with drainage of the anastomosis and mediastinal space. Patients experiencing this complication are confronted with a significantly longer hospital stay. Ischemia might be an important underlying cause of anastomotic leakage as the esophagus is recontructed with a conduit constructed from the stomach removed from its anatomical position. Furthermore, the circulation is compromised by resection of the affected part of the esophagus. In this study the technical feasibility of a new intraoperative technique to measure tissue ischemia by reflectance spectrophotometry is assessed. A protocol to determine tissue saturation of the esophageal stump and the gastric conduit was developed, measuring at operative stages that might compromise circulation. Ten patients underwent esophageal resections performed between November 2005 and April 2006 in the University Medical Center Groningen and the Martini Hospital Groningen. No technical problems were encountered in the measuring procedure. The standard deviation of the saturated hemoglobin value (StO2) was less variable in serosal recordings (3.6–10.5%). Two patients showed anastomotic leakage in the postoperative course. In these patients a saturation drop of 18.3% in the gastric conduit was measured compared with 1.7% in nonanastomotic leakage. **Conclusion:** Reflectance spectrometry measurements are easy and safe to perform during esophageal resections and show a small variability in measurements. In this feasibility study a decrease in gastric serosal saturation was demonstrated in patients with an anastomotic leakage. Further multicenter studies are in progress to validate the value of VLS and StO2 as a predictor of anastomotic leakage.

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PERSISTENT LUMINESCENT NANOPARTICLES: A NEW TOOL FOR BIOLOGISTS?
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Due to growing demands of imaging tools for biomedical research, existing imaging systems have been rapidly improved and new imaging techniques have been developed during the past decades. Nowadays, magnetic resonance imaging (MRI), microcomputed tomography (micro-CT), ultrasound, positron emission tomography (PET), and other major imaging systems are available to scientists. Each technique has advantages and disadvantages, thus making them complementary. Optical imaging is a rapidly expanding field with direct applications in pharmacology and in the development of tools for diagnostics and research in molecular and cellular biology. Despite the increasing use of fluorescence for in vivo imaging, this technique presents several limitations, especially due to tissue autofluorescence under external illumination and weak tissue penetration of low wavelength excitation light. These drawbacks can limit the ability to detect fluorescent probes from background signal in deep tissues imaging. We present here an alternative optical imaging system using near-infrared persistent luminescent (commonly called phosphorescent) nanoparticles suitable for small-animal imaging. The main advantage of this technique resides in the absence of autofluorescence as the nanoparticles continue to emit light in the animal without the need for any kind of excitation. Using these probes in small-animal imaging, we demonstrate that nanoparticles can be excited prior to injection to follow their in vivo distribution for more than 1 hour without any external illumination source. Chemical modification of nanoparticle surface led to lung or liver targeting or to long-lasting blood circulation. Tumor mass was also identified on a mouse model.

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INTEGRIM: A VISUALIZATION, MATCHING, AND INTERPRETATION TOOL FOR FUSED MICRO-COMPUTED TOMOGRAPHY AND MULTIVIEW BIOLUMINESCENCE IMAGING.
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Background: BLI is a sensitive imaging technique enabling monitoring in vivo molecular processes overtime. The fusion with micro-CT data allows the combination of functional and anatomical data. A problem in longitudinal studies is the difference animal pose. **Aim:** To develop an image processing tool for mapping of small animals to a standardized template to allow detailed studies of the dynamics of the BLI sources over time combined with the anatomical details. **Material and Methods:** INTEGRIM enables the comparison between different micro-CT data and multi-angle BLI through registration of multiview BLI data with micro-CT by selecting a small set of characteristic 3D landmarks indicated in the CT data and the multi-angle BLI data; a coarse, back-projection-based 3D localization for superficial BLI hot spots, estimating the outer source envelopes of the hot spots; automated registration of the whole-body 3D data of mice including BLI data to a standardized template using articulated registration using the bone structure from the micro-CT data. The data were down-sampled, and the coarse structure of all bones that are included in the registration process was retained. **Results:** The platform has been tested on data from 12 mice with different poses. The method gave accurate automatic alignment of the skeletal structures in all cases. Also, the corresponding 3D BLI sources could be reconstructed and mapped. Due to subsampling, the ribs were removed, but this did not affect the registration procedure. **Conclusion:** INTEGRIM enables intuitive registration, standardization, visualization, and interpretation of fused multiview BLI and micro-CT.

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AN FDOT INSTRUMENT FOR IN VIVO MICE LUNG TUMOR FOLLOW-UP.
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This paper presents a fluorescence diffuse optical tomographer dedicated for in vivo small animal studies. fDOT allows 3D reconstruction of the fluorescence yield obtained after injection of specific markers to the animal. Our system has two main specificities. First, it does not require animal immersion in an index matching medium, which facilitates the animal inspection protocol. Second, it allows inspection of highly attenuating regions, which enables evaluation of drug efficiency in regions such as lungs. Our continuous-wave instrument consists of a laser source (690 nm 26 mW) coupled to two motorized translation stages, filters, and a cooled CCD camera. The bench is equipped with a heating holder that maintains the animal between two glass plates to limit its movements. A typical acquisition is done in two steps. First, a diode illumination by the sides of the animal delimits the areas in contact with the upper glass plate, so as to determine the relevant detectors. Then the animal is scanned by the laser thanks to the motorized stages. For each source position, the CCD camera records the transmitted (excitation) images; then after insertion of a Schott high pass RG9 filter, it records the fluorescence (emission) images. Our bench is also equipped with an FRI Fluorescence Reflectance Imaging system based on LEDs (660 nm). The efficiency of the system is illustrated by a longitudinal study of mice lungs at different stages of tumor development; the results have been compared to FRI and fDOT benefits have been pointed out.

POSTERS
Animal Models

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MAGNETIC RESONANCE IMAGING OF INFLAMMATION SITES WITH INJECTED, SPIO-LABELED MONOCYTES.
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Tracking of individual cells by MRI requires the intracellular accumulation of contrast agent. In vitro labeling of cells permits incorporation of large amounts of iron oxide and consequently high detection sensitivity, but it remains controversial whether labeled cells would respond normally to stimuli. This question was addressed in an established experimental system for acute inflammatory processes, the murine air pouch model. In this system an air pouch is generated on the back of mice. Within 6 days

this causes the formation of a layer of resident cells lining the cavity, creating a new, isolated compartment that can be easily manipulated. By flushing the compartment with buffer, large numbers of infiltrated cells can be collected and analyzed. Bone marrow–derived macrophages (BMDMs) were differentiated in vitro, labeled with Endorem (SPIO), and unlabeled cells were eliminated by magnetic enrichment. Purified and enriched BMDM were injected intravenously into the tail vein of isogenic mice presenting a carrageenan-induced inflammation in the air pouch. Endorem-labeled macrophages were detected by fluorescent microscopy as well as by MR imaging ex vivo in the cell populations eluted from carrageenan pouches of mice injected IV with in vitro labeled BMDMs. This strongly suggests that Endorem-labeled macrophages can still respond to chemokine gradients. Labeled BMDMs were also injected into mice carrying a cryolesion in one of the brain hemispheres to evaluate the potential of injected, labeled BMDMs for in vivo MR imaging of inflammatory diseases.

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A NEW MOUSE MODEL TO MONITOR P53 ACTIVATION IN VIVO: EFFECT OF DOXORUBICIN. Arnaud Briat,[1] Andrew Merron,[1] Georges Vassaux.[2] [1]Queen Mary's University of London, London, UK; [2]INSERM, Nantes, FRANCE; contact e-mail: arnaud.briat@cancer.org.uk.

Monitoring p53 transcriptional activity to identify genotoxic damages induced by drugs has been proposed and validated in vitro. However, this methodology is limited to one cell line and cannot account for the tissue-specific toxicities that may be encountered in vivo. In the present study, we have fully validated a luciferase-based p53-reporter system in vitro and in vivo. We used this system to generate a mouse transgenic line (p53RE-Luc) to image noninvasively p53 activation in response to chemically induced DNA damage. To validate this model, doxorubicin was injected intraperitoneally and bioluminescence imaging on the whole animal was used to detect the expression of the reporter gene. In female transgenic mice, no signal was detected in response to the drug. By contrast, in males, luciferase activity was detected in the lower abdominal region. Bioluminescence imaging of various organs obtained after cull and dissection of the male transgenic animals revealed that the luciferase activity was generated from the testis. Immunohistological analysis demonstrated that the entire cell population of the seminiferous tubule was luciferase positive. Considering that doxorubicin has already been demonstrated to activate p53 as well as apoptosis in male germ line cells in rodent models and that this drug is known to cause sterility in male cancer patients, we advocate that the p53RE-Luc transgenic mice could be a very powerful tool to predict, map, and characterize at the cellular level the toxicity of compounds in humans and to help in the design of new therapeutic agents.

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PROTOCOL DEVELOPMENT OF BOWEL IMAGING IN RATS WITH MAGNETIC RESONANCE IMAGING. Sandra T. van Tiel,[1] Piotr A. Wielopolski,[1] Ingrid B. Renes,[2] Ernst J. Kuipers,[3] Gavin C. Houston,[4] Klaas Nicolay,[5] Gerben A. Koning,[6] Gabriel P. Krestin,[1] Monique R. Bernsen.[1] [1]Radiology, Erasmus MC, Rotterdam, the Netherlands; [2]Neonatology, Erasmus MC, Rotterdam, the Netherlands; [3]Gastroenterology, Erasmus MC, Rotterdam, the Netherlands; [4]GE Healthcare, Global Applied Science Lab, Den Bosch, the Netherlands; [5]University of Technology, Eindhoven, the Netherlands; [6]Experimental Surgery, Erasmus MC, Rotterdam, the Netherlands; contact e-mail: s.vantiel@erasmusmc.nl.

Background: For diagnosis of early-stage inflammatory bowel disease (IBD) an MRI protocol and liposomes for local contrast enhancement have to be developed. **Methods:** To scan the bowels of a rat various steps have to be taken for image quality improvement. To reduce motion of the bowels antispasmodic agents have been used. For local contrast enhancement at inflammatory sites, we incorporated gadolinium (Gd) in long-circulating liposomes. Such liposomes are expected to extravasate at the inflammatory sites, resulting in a local hyperintense signal. For optimal detection of local contrast enhancement by Gd-liposomes the contrast between the bowel wall and the bowel lumen has to be increased, ie, a hypointense bowel lumen. For this purpose, we tested different agents (water, Endorem, Lumirem) at different concentrations. Fibers in the food disturb the MR image. We replaced the standard food with powder food (less fibers), which is made into a paste by mixing it with Lumirem. **Results:** For reduction of bowel movement 30–50 μL IM. Buscopan gives the best results. Optimal contrast between bowel lumen and bowel wall is best achieved by oral administration of 1:3 diluted Lumirem hours prior to scanning, which is further improved by feeding with a low-fiber diet. With this protocol, contrast enhancement in the bowel wall is seen after administration of Gd-liposomes in rats with IBD. **Conclusion:** Rat bowels can be scanned with MRI after administration of a spasmolytic agent was given and an oral contrast agent. Local contrast enhancement of inflammatory lesions in IBD may be feasible through the use of Gd-liposomes.

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IN VIVO PINHOLE SINGLE-PHOTON EMISSION COMPUTED TOMOGRAPHIC IMAGING OF FEMOROTIBIAL CARTILAGE IN MICE WITH 99MTC-NTP 15-5. Elisabeth Miot-Noirault, Aurélien Vidal, Jean-Claude Madelmont, Nicole Moins. UMR 484 INSERM, Clermont Ferrand Cédex, France; contact e-mail: noirault@inserm484.u-clermont1.fr.

Background: Our lab develops the "cartilage targeting imaging strategy" with a tracer (99mTc-NTP 15-5) that selectively binds to the proteoglycans of cartilage. **Methods:** Cartilage imaging was performed using the gammaImager (10 cm FOV, Biospace) equipped with a parallel-hole collimator for planar acquisition and a 1 mm pinhole for SPECT. After IV injection of 99mTc-NTP 15-5 (37 MBq and 185 MBq/mouse for planar and SPECT, respectively) dynamic planar imaging (2 h duration) and pinhole SPECT acquisitions (90-minute duration) were performed in five mice. Mice were then sacrificed for tracer uptake quantitation. Tomographic reconstructions were performed using a 3D-OSEM algorithm with slices (0.25 mm thick) being reconstructed in the three axes of the animals. **Results:** After IV administration, 99mTc-NTP15-5 rapidly accumulated in the joint (around 6% ID/g within knee cartilage at 15 min) and maintained for 2 h. Since bone and muscle did not show any accumulation of the tracer (< 0.1%ID/g) a highly contrasted cartilage imaging was obtained. When pinhole SPECT acquisition was focused on the knee, both the 3D volume and coronal slices enabled an exact localization of the tracer within the medial and lateral compartments of both femoral condyle and tibial plateau (around 2 mm in diameter in the mouse) that were highly delineated. **Conclusion:** The combination of both the strength of pinhole SPECT imaging with the specificity of 99mTc-NTP 15-5 tracer for proteoglycans allows in vivo cartilage imaging in the mouse, therefore providing a unique opportunity to assess the pathophysiological pathways of osteoarthritis in small-animal experimental models.

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ACCURATE ANATOMICAL MAPPING OF LIVER AND SPLEEN USING CONTRAST-ENHANCED MICRO-COMPUTED TOMOGRAPHY: A STUDY ON THE TIME COURSE OF CONTRAST ENHANCEMENT IN HEALTHY MICE. Inneke Willekens,[1] Kristof Gentens,[2] Tony Lahoutte,[3] Cindy Peleman,[3] Chris Vanhove,[3] Rudi Deklerck,[2] Axel Bossuyt,[3] Johan de Mey.[4] [1]In Vivo Cellular and Molecular Imaging–ICMI-Vrije Universiteit Brussel, Department of Radiology, UZ Brussel; [2]Electronics and Informatics-ETRO-IRIS-Vrije Universiteit Brussel; [3]In Vivo Cellular and Molecular Imaging–ICMI-Vrije Universiteit Brussel; [4]Department of Radiology, UZ Brussel; contact e-mail: inneke.willekens@vub.ac.be.

Background: Micro-CT allows high-resolution volumetric imaging of the inner anatomy of living animals. There is too little contrast between the abdominal organs for a correct delineation. This problem can be solved using CT contrast agents. **Aim:** The purpose of this study was to evaluate the time course of contrast enhancement of liver and spleen using Fenestra VC in healthy mice. **Methods:** Healthy C57bl/6 mice ($n = 12$) were used in this study. For anesthesia isoflurane 2% was used. Fenestra VC (Alerion Biomedical, San Diego, CA, USA) was administered intravenously at a dose of 0.1 mL/20 g ($n = 6$) or 0.2 mL/20 g ($n = 6$). Imaging was performed using micro-CT (Skyscan 1178) at a resolution of 83 μm. Each animal underwent a micro-CT scan before contrast injection (baseline) and immediately after contrast injection. Additional scans were performed at 1 h, 2 h, 3 h, 4 h, 24 h, and 48 h after contrast. Images were reconstructed using filtered backprojection (NRecon, Skyscan) and analyzed using Amide (Loening et al). Regions of interest (ROIs) were drawn in liver, spleen, lung, heart, brain, bladder, fat, bone, and muscle tissue. Signal to noise ratio (SNR) and contrast to noise (CNR) ratio were measured using water as a reference. **Results and Conclusion:** The Fenestra VC contrast enhancement of the liver and spleen reaches a maximum at 24 hours. CNR of the spleen is higher compared with the liver. The contrast enhancement allows volumetric analysis of the spleen at a dose of 0.1 mL/20 g, whereas for the liver 0.2 mL/20 g is required.

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DYNAMIC BIOLUMINESCENCE IMAGING OF FLUC+ TUMOR-BEARING MICE: COMPARISON OF INTRAPERITONEAL AND INTRAVENOUS ADMINISTRATION OF D-LUCIFERIN. Marleen Keyaerts,[1] Jacob Verschueren,[1] Tomas Bos,[2] Cindy Peleman,[1] Gainkam Olive Tchouate,[1] Karine Breckpot,[3] Vicky Caveliers,[1] Sophie Hernot,[1] Kris Thielemans,[3] Axel Bossuyt,[1] Tony Lahoutte.[1] [1]In Vivo Cellular and Molecular Imaging Laboratory, Vrije Universiteit Brussel (VUB), Brussels, Belgium; [2]Department of Haematology and Immunology, Vrije Universiteit Brussel (VUB), Brussels, Belgium; [3]Laboratory of Molecular and Cellular Therapy, Department of Physiology and Immunology, Medical School of the Vrije Universiteit Brussel (VUB), Brussels, Belgium; contact e-mail: mkeyaert@vub.ac.be.

In vivo reporter gene imaging using firefly luciferase (Fluc) allows a sensitive and noninvasive assessment of tumor burden. This method is used for testing the effect of new therapeutics on tumor growth. However, measurements can show substantial variability. **Aim:** To compare the intensity, kinetic profile, and variability of light output (LO) after IV and IP administration of D-luciferin using dynamic bioluminescence imaging (D-BLI). **Methods:** 1 × 10E5 Fluc+ rhabdomyosarcoma cells were subcutaneously inoculated in 12 nu/nu-mice. Daily dynamic bioluminescence imaging (D-BLI) of LO was performed after IP/IV administration of D-luciferin (30 mg/kg) using a photo imager (Biospace). Results are expressed as the average ± SE. **Results:** LO was on average 7× higher for IV compared to IP. The kinetic profile of the LO was significantly different: time to peak was 4.2 ± 0.4 minutes for IV and 9.6 ± 0.5 minutes for IP ($p < .0001$). The mean relative increase in LO over 2 days was similar ($p = .26$) for IV and IP: IV: 2.10 ± 0.61 versus IP: 1.54 ± 1.14 (CI 95%: 1.59–2.61 for IV versus 0.59–2.50 for IP). However, the variance for IP is significantly higher than for IV ($F < .10$). Our data show that in a therapeutic experiment, a mean decrease in LO of 24% is significant ($p < .05$) for IV whereas for IP a decrease of 61% is required using this animal model. **Conclusion:** LO after IV injection of D-luciferin is more intense and less variable when compared with IP injection. Our results suggest that IV administration is a more sensitive method for the follow-up of tumor burden using D-BLI.

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PRELIMINARY RESULTS ON THE BRAIN METABOLISM OF A TRANSGENIC RAT MODEL OF SCA17. Nadja Van Camp,[1] Fabien Chauveau,[1] Huu Phuc Nguyen,[2] Stephan Von Hörsten,[3] Olaf Riess,[2] Bertrand Tavitian.[1] [1]CEA, DSV, I2BM, SHFJ, Laboratoire d'Imagerie Moléculaire Expérimentale, Orsay, France, INSERM U803, Orsay, France; [2]Department of Medical Genetics, University of Tuebingen, Tuebingen, Germany; [3]Experimental Therapy, Friedrich-Alexander-University of Erlangen-Nürnberg, Erlangen, Germany; contact e-mail: nadja.vancamp@ua.ac.be.

Autosomal dominant spinocerebellar ataxias (SCAs) are a heterogeneous group of neurodegenerative disorders, comprising more than 25 subtypes characterized by progressive ataxia and various other features. One genetic subform (SCA17) is caused by the expansion of a CAG-repeat encoding for polyglutamine stretches in the TATA-binding protein (TBP). Previous PET studies demonstrated a reduced glucose metabolism in the putamen (Minnerop et al. Ann Neurol 2005) and reduction of the dopamine transporter in human patients, which were correlated with clinical severity (Salvatore et al. Mov Disord 2006). We recently generated a transgenic rat model of SCA17 expressing mutant TBP with 64 expanded CAG-repeats. In order to validate this rat model with the human pathology, imaging studies similar to those performed in patients are essential in addition to behavioral and physiological phenotyping. Here we present the first preliminary [18F]-FDG data on young (8 months) transgenic ($n = 8$) and age-matched wild-type ($n = 8$) SCA17 animals. After intravenous injection of 65.1 ± 22.Mbq [18F]-FDG in awake restrained rats, animals were returned to their cages and placed in a dark quiet room during 1 hour. A μPET scan of 30 min was performed under isoflurane anesthesia on a Concorde Focus PET camera. Surprisingly and in contrast to clinical studies, we observed a significantly ($p < .05$) increased FDG accumulation (expressed as %ID/cc) in caudate-putamen, cerebellum, cortex, and total brain of transgenic rats as compared to wild types. Follow-up studies are necessary to confirm whether this increased brain metabolism could reflect a hypercompensation for neuronal dysfunction that may later be followed by decreased glucose metabolism at advanced stages of the disease.

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APPLICATION OF BIOLUMINESCENCE IN STEM CELL–BASED BONE FORMATION. Karien E. de Rooij,[1] Geertje van der Horst,[1] Hetty C.M. Sips,[1] Ivo Que,[1] Lianne J.A. van der Wee-Pals,[1] Jakomijn Hoogendam,[2] Eric L. Kaijzel,[1] Marcel B.J. Karperien,[1] Clemens W.G.M. Löwik.[1] [1]Department of Endocrinology, LUMC, the Netherlands; [2]Department of Pediatrics, LUMC, the Netherlands; contact e-mail: k.e.de_rooij@lumc.nl.

Mesenchymal stem cells (MSCs) have great potential for application in tissue engineering of bone and cartilage. However, differentiation of MSCs in vitro or in vivo as well as interactions with other cells or

biomaterials in vivo is poorly understood. Therefore, we have created a stem cell model that, in combination with bioluminescent imaging, allows us to study these processes in intact mice over time. The murine MSC-like KS483 cell line was genetically modified, enabling efficient generation of isogenic stable cell clones by Flp-mediated recombination by integrating one copy of an FRT-target site into the genome. The FRT site was used to insert a luciferase-2 gene containing a His-tag enabling us to follow cell fate in vivo by bioluminescence after implantation in nude mice and detection of cells ex vivo by immunohistochemistry. KS-Frt-HisLuc2 cells were used in a bone marrow ablation assay. Cell fate was followed by noninvasive bioluminescent imaging. At several time points, mice were sacrificed and tibias were isolated for analysis. In addition, to study their fate outside the bone-forming environment and to study possible adverse effects, cells were also transplanted subcutaneously. We have followed cell fate noninvasively for 20 weeks and afterwards isolated luciferase-expressing tissue for immunohistochemical analysis. In conclusion, KS-Frt cells provide a simple and fast model to study MSC function. In combination with bioluminescent imaging, we will use this model to evaluate the effects of biomaterials on stem cell function in vitro and in vivo as a first step toward bone replacement therapy for osteoporosis.

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CONTRAST-ENHANCED MAGNETIC RESONANCE IMAGING OF ATHEROSCLEROSIS IN MICE USING PARAMAGNETIC LIPOSOMES AND MICELLES. Glenda van Bochove,[1] Leonie Paulis,[1] Dolf Segers,[2] Willem Mulder,[3] Gustav Strijkers,[1] Rob Krams,[4] Klaas Nicolay.[1] [1]Eindhoven University of Technology, Eindhoven, the Netherlands; [2]Erasmus University, Rotterdam, the Netherlands; [3]Mount Sinai School of Medicine, New York, NY, USA; [4]VUMC, Amsterdam, the Netherlands; contact e-mail: g.s.v.bochove@tue.nl.

Introduction: Contrast-enhanced imaging of atherosclerotic plaques using Gd3+-containing T1-lowering contrast agents has promising potential for the detection and characterization of atherosclerosis. Contrast generated in the plaque by intravenous injection of paramagnetic Gd3+ liposomes and Gd3+ micelles was investigated to obtain insight in the permeability of different lesions, which provides essential information for studies with targeted liposomes and micelles. **Materials and Methods:** 12 ApoE$^{-/-}$ mice were put on a high-cholesterol diet. After 3 weeks a cast was surgically placed around the right carotid artery to induce both vulnerable and stabilized plaque (Cheng et al. Circulation 2006). For imaging the mice were separated into four groups: 6 weeks after surgery receiving liposomes ($n = 2$) or micelles ($n = 4$), 9 weeks after surgery receiving liposomes ($n = 2$) or micelles ($n = 4$). T1-weighted spin-echo images were acquired before, 15 min after, 60 min after, and 24 hours after injection of contrast agent in all groups. **Results:** Very little or no signal enhancement was observed for mice injected with liposomes. For the 6 weeks group injected with micelles the lesions showed an increase in signal intensity after 24 hours of 44% ± 14%, while for the 9 weeks group a few lesion signal intensities were increased. **Conclusions:** These lesions are not very permeable for liposomes, which leads to a beneficially low background for subsequent targeted studies. Therefore, liposomes are a good contrast agent when targeting endothelial markers. Micelles do enter the lesions and therefore will be used for targeting factors inside the atherosclerotic plaque.

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EVALUATION OF ATHEROMA IN A RABBIT MODEL: EX VIVO VERSUS IN VIVO POSITRON EMISSION TOMOGRAPHY IMAGING. David Izquierdo,[1] John R. Davies,[2] Tim D. Fryer,[1] James H.F. Rudd,[2] Katrin Probst,[1] Hugh K. Richards,[3] Franklin I. Aigbirhio,[1] John C. Clark,[1] Peter L. Weissberg,[2] Elizabeth L. Warburton.[4] [1]Wolfson Brain Imaging Centre; [2]Cardiovascular Medicine Division; [3]Neurology Unit; [4]Department of Clinical Neurosciences; contact e-mail: di219@wbic.cam.ac.uk.

Introduction and Methods: The presence of activated macrophages is a very important predictor of plaque rupture within vessels. In this study, our aim was to determine the accuracy of 18F-FDG microPET for quantifying plaque macrophage content in a rabbit model. Rabbits were divided into two groups: a high-cholesterol and a low-cholesterol diet group. Both in vivo and ex vivo microPET as well as histological quantification of the atherosclerotic aortas was subsequently carried out on both groups. A discrepancy between the in vivo and ex vivo results was observed due to partial volume effects (PVE), as a consequence of the limited spatial resolution of the microPET scanner. Errors before and after partial volume correction (PVC) in vivo have been quantified with a vessel phantom. **Results and Conclusions:** Plaque macrophage density (% of plaque area) was greater in the high-cholesterol group than in the low-cholesterol group (7.79 ± 2.42 vs 1.09 ± 1.17, $p < .00012$). Across both groups there was a close correlation between histologically determined macrophage content and plaque FDG uptake derived from ex vivo microPET ($r^2 = .89$, $p < .000005$). However, the correlation between in vivo microPET and plaque macrophage density was poor ($r^2 = .04$, $p < .46$). As expected, PVE significantly modifies the in vivo results. Errors before and after PVC on the vessel phantom show a dramatic effect: reduction from 77% to 12% error after PVC has been applied. This study confirms that the PET tracer 18F-FDG can be used to quantify plaque macrophage content. However, improvement in scanner resolution and methods for dealing with the PVE are required for accurate in vivo quantification.

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DYNAMIC IMAGING OF ARTHRITIC INFLAMMATION IN MOUSE MODELS USING HIGH-RESOLUTION MULTIPINHOLE SINGLE-PHOTON EMISSION COMPUTED TOMOGRAPHY AND RGD. A. Wirrwar,[1] D. Buchholz,[1] O. Gottschalk,[2] F.W. Schwaiger,[3] S.O. Viehöver,[1] N.U. Schramm,[2] H.W. Müller,[1] M. Funk,[4] [1]Department of Nuclear Medicine, Heinrich-Heine University Düsseldorf, Düsseldorf, Germany; [2]Central Electronics Laboratory, Research Center Jülich, Jülich, Germany; [3]Aurigon Life Science GmbH, Tutzing, Germany; [4]MediGene AG, Martinsried, Germany; contact e-mail: wirrwar@uni-duesseldorf.de.

MPH-SPECT measurements were performed under short general anesthesia in two collagen-induced arthritis (CIA) mice (17.8 g and 21.1 g, 6 weeks) that had arthritis in the front and back paws and were independently scored macroscopically before starting the studies. Two control mice (24.4 g and 23.8 g, 6 weeks) were compared with the MPH analysis of arthritis mouse models. SPECT data were acquired with a conventional gamma camera (PRISM 2000XP, Philips), which was outfitted with a new constructed MPH collimator (12 pinholes with a diameter of 1.5 mm). After the injection of 35.6 ± 14.2 MBq In-111-labeled RGD peptide, a dynamic measurement between 0 and 60 minutes p.i. and frame lengths of 2 and 5 minutes was performed. By using MPH-SPECT, all the arthritic lesions could be detected, which were quantitatively analyzed. The regional evaluation showed that after 6 minutes, the percentual accumulation in the inflamed ankles was 0.34% and remained stable until 1 hour p.i while in healthy control mice an uptake of 0.09% was measured. There was no significant uptake difference observed in the knees between healthy and arthritic mice. Compared with traditional standardized scoring methods for the assessment of arthritis in animal studies, this new method provides an accurate and quantitatively precise measurement of joint inflammation. The results of our studies strongly suggest that the multipinhole SPECT technique in combination with labeled RGD peptide can be used as a diagnostic instrument for monitoring therapy studies and imaging joint pathology in arthritis mouse models.

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SCREENING OF ACTIVE PARAMAGNETIC MOLECULES IN ZEBRAFISH EMBRYOS BY MAGNETIC RESONANCE IMAGING. Laurence Canaple,[1,2] Olivier Beuf,[3] Mircea Armenean,[2,3] Jens Hasserodt,[4] Marc Janier,[2] Jacques Samarut.[1] [1]Institut de Génomique Fonctionnelle, UMR 5242, Ecole Normale Supérieure, Lyon, France; [2]Plateforme ANIMAGE, Rhône Alpes Genopole, Bâtiment CERMEP, Bron, France; [3]Creatis-LRMN, CNRS UMR 5220, Université Lyon 1, INSA-Lyon, Villeurbanne, France; [4]CNRS UMR 5182, Université de Lyon, IFR 128 BioSciences Lyon-Gerland, Laboratoire de Chimie, Ecole Normale Supérieure,Lyon, France; contact e-mail: Laurence.Canaple@ens-lyon.fr.

The zebrafish combines the relevance of a vertebrate with the scalability of an invertebrate and constitutes a powerful model in many fields of modern experimental biology. We investigated the potential of the MR imaging approach on the zebrafish embryo model. MRI of the zebrafish embryo required the development of a coil dedicated to its miniature size (2–4 mm long), the design of an experimental set-up suitable with an aquatic life, and the definition of a specific MRI protocol adapted to 7 T magnetic field strength used. Herein, we arrayed a large number of living embryos, which were microinjected at very early stages of development with different contrast agents. We showed that the MRI signal intensity is correlated to the gadolinium concentration injected in the embryos. This allowed us to validate the zebrafish embryos as a promising model platform for the exploration of the MR fundamental aspects and as an intermediate vertebrate model, to screen and to track active MR molecules over time before to go to more complex living systems. Designing a specific 5 mm inner diameter surface coil, we also obtained high spatial resolution images of living zebrafish embryos with a 47 µm isotropic voxel size for an acquisition time of 39 minutes. This work can serve as a new way to explore development and disease in the fish, visualize and decipher gene expression in living embryos, identify drug potency, and select innovative imaging approaches for numerous diseases.

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EVALUATION OF CLINDE AS A POTENT PERIPHERAL-TYPE BENZODIAZEPINE RECEPTOR TRACER IN A RAT MODEL OF MICROGLIAL ACTIVATION. Nicolas Arlicot,[1] Denis Guilloteau,[1] Andrew Katsifis,[2] Filomena Mattner,[2] Sylvie Chalon.[1] [1]INSERM U619, Tours, France; [2]ANSTO, Sydney, Australia; contact e-mail: arlicot.n@voila.fr.

The peripheral-type benzodiazepine receptors (PBRs) are localized in mitochondria of glial cells and are very low expressed in normal brain. Their expression rises after microglial activation consecutive to brain injury. Accordingly, PBRs are potential targets to evaluate neuroinflammatory changes in a variety of CNS disorders. To date no effective tool is available to explore PBR by SPECT. We characterized here 6-chloro-2-(4′iodophenyl)-3-(N,N-diethyl)-imidazo[1,2-a]pyridine-3-acetamide or CLINDE in a rat model of excitotoxic lesion. Excitotoxicity was induced in male Wistar rats by unilateral intrastriatal injection of different amounts of quinolinic acid (QA: 75, 150, or 300 nmol). One week later, two groups of rats (n = 5–6/group) were IV injected with [125I]-CLINDE (0.4 MBq), one group being preinjected with PK11195 (5 mg/kg). Brains were removed 30 minutes after tracer injection and the radioactivity of cerebral areas was measured. Complementary ex vivo autoradiography and immunohistochemical studies (OX42) were performed on brain sections. In the control group, [125I]-CLINDE binding was significantly higher ($p < .001$) in lesioned than that in the intact side (striatum: 0.552 ± 0.109 vs 0.123 ± 0.012% I.D./g tissue; cortex: 0.385 ± 0.126 vs 0.131 ± 0.007% with 300 nmol QA). This binding disappeared in rats pretreated with PK11195 ($p < .001$), showing specific binding of CLINDE to PBRs. Ex vivo autoradiography and immunohistochemistry were consistent with this, revealing a spatial correspondence between radioactivity signal and activated microglia. Regression analysis yielded a significant correlation ($p < .001$) between the ligand binding and the dose of QA. These results demonstrate that CLINDE is suitable for PBR in vivo SPECT imaging to explore their involvement in neurodegenerative disorders associated with microglial activation.

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IN VIVO NEAR-INFRARED FLUORESCENT TUMOR IMAGING USING FLUOROCHROME-LABELED ANTIBODIES AGAINST CARCINOEMBRYONIC ANTIGEN. Marcus-Rene Lisy,[1] Annika Goermar,[1] Claudia Thomas,[1] Jutta Pauli,[2] Ute Resch-Genger,[2] Werner A. Kaiser,[1] Ingrid Hilger.[1] [1]Institute for Diagnostic and Interventional Radiology, Friedrich-Schiller-University, Jena, Germany; [2]Federal Institute for Materials Research and Testing (BAM), Working Group Optical Spectroscopy, Berlin, Germany; contact e-mail: marcus.lisy@med.uni-jena.de.

Purpose: To noninvasively detect carcinoembryonic antigen (CEA)-expressing tumors with a novel high-affinity probe consisting of a near-infrared fluorochrome and the clinically used anti-CEA antibody arcitumomab. **Materials and Methods:** A bio-optical high-affinity fluorescent probe (antiCEA-DY676) was designed by coupling the near-infrared fluorescent (NIRF) cyanine dye DY-676 (Dyomics, Jena, Germany) to arcitumomab. The spectroscopic properties of AbCEA-DY676 were investigated in comparison to a low-affinity control FabIgG-DY676. Both probes were tested for NIRF imaging in vitro on CEA-positive LS-174T cells and CEA-negative A-375 cells using a bio-optical near-infrared small-animal imager and confocal laser scanning microscopy (CLSM). In vivo data of xenografted tumors LS-174T and A-375 in mice ($n = 10$) were recorded and analyzed statistically at different time points (2–24 h) after IV probe injection (2 mg per kg body weight). Imaging results were verified by immunohistological investigation of tumor sections. **Results:** In vitro investigations of CEA-positive LS-174T cells clearly showed higher fluorescence intensities after incubation with antiCEA-DY676 as compared with CEA-negative A-375 cells or than after incubation with FabIgG-DY676. In vivo, LS-174T tumors xenografted in mice could be clearly distinguished from A-375 tumors by application of the antiCEA-DY676 but not by the FabIgG-DY676 after IV. injection of the probes. Semiquantitative analysis of in vivo images revealed maximal fluorescence signals of antiCEA-DY676 in CEA-expressing tumors about 8 h after probe injection. Differences between CEA-expressing and nonexpressing tumors were statistically significant 24 h after probe injection ($p < .005$). Immunohistology of tumor sections revealed very high CEA expression levels in LS-174T tumors whereas no CEA could be detected in A-375 tumors. **Conclusions:** Our study indicates the potential of antiCEA-DY676 as a high-affinity probe for specific NIRF in vivo tumor diagnosis.

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HIGH-FREQUENCY ULTRASOUND EVALUATION OF MURINE THYROID. Adelaide Greco,[1] Marcello Mancini,[2] Giuliana Salvatore,[3] Paolo Salerno,[3] Emilia Vergara,[1] Andrea Affuso,[4] Arturo Brunetti,[1] Marco Salvatore.[1] [1]Department of Biomorphologic and Functional Science, Medicine Faculty, Naples, Italy; [2]IBB, CNR, Naples, Italy; [3]Endocrinology and Oncology Institute, CNR, Naples, Italy; [4]Biogem, s.c.a.r.l., Ariano Irpino (AV); contact e-mail: adegreco@unina.it.

Purpose: Animal models are valuable in the studies of the pathogenesis of human thyroid diseases. We used high-frequency ultrasound (HFUS) for thyroid evaluation of normal mice and murine models of

thyroid diseases. **Methods and Materials:** Thyroid ultrasound was performed with a high-frequency ultrasound system (Vevo 770, Visualsonics) using a 40 MHz transducer, in 10 normal mice (N), 8 mice with hyperplasia induced by postnatal exposure to propylthiouracil (PTU), and 22 transgenic mice models of thyroid carcinoma. HFUS was performed under general anesthesia using 2% of isoflurane vaporized in oxygen. Normal mice were examined prior to autopsy at the age of 8 months, PTU subjects were examined weekly during the 4-week period of treatment and were sacrificed at the age of 6 months, and transgenic mice were imaged every 6 months until 18 months. **Results:** Diffuse thyroid enlargement was found in PTU (volume N: 3.44 ± 1.08 μL, PTU: 6.49 ± 1.67 μL; $p < .005$), and in two cases nodules were seen. In all transgenic subjects HFUS was able to detect and measure thyroid tumors (size range 0.4 mm to 8.38 mm) subsequently confirmed at histology. In 22 transgenic mice HFUS identified a single nodule process in 2 cases and multiple nodules in 15 cases. Repeat examinations were carried out to determine the grow rate. **Conclusion:** The thyroid gland can be successfully studied with HFUS in mice. Small thyroid tumors can be detected and monitored over time and mice can be safely repetitively imaged. HFUS can be a useful tool for the study of murine models of thyroid pathology.

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COMPARISON OF DIFFERENT IN VIVO MOLECULAR IMAGING MODALITIES FOR THE DETECTION OF MULTIPLE ENDOCRINE NEOPLASIA TYPE 2A IN A TRANSGENIC MICE MODEL. Carine Pestourie,[1] Karine Gombert,[1] Stéphane Le Helleix,[2] Benoît Thézé,[1] Frédéric Dollé,[2] Bertrand Tavitian,[1] Frédéric Ducongé.[1] [1]CEA, DSV, I2BM, SHFJ, Laboratoire d'Imagerie Moléculaire Expérimentale; INSERM U803, Orsay, France; [2]CEA, DSV, I2BM, SHFJ, Laboratoire D'imagerie Moléculaire Expérimentale, Groupe de Radiochimie et Radio-pharmacie, Orsay, France; contact e-mail: carine.pestourie@cea.fr.

Multiple endocrine neoplasia type 2 (MEN-2) is a dominantly inherited cancer syndrome that comprises three clinical subtypes: MEN type 2A (MEN-2A), MEN type 2B (MEN-2B), and familial medullary thyroid carcinoma (FMTC). Medullary thyroid carcinoma (MTC) is characterized by a malignant tumor arising from calcitonin-secreting thyroid C cells. Mutations of the receptor tyrosine kinase Ret are implicated in these pathologies. A transgenic model of mice has been created that expresses the human mutated form of Ret (RetC634Y) implicated in MEN-2A.[1] These mice displayed first overt bilateral C cell hyperplasia and subsequently, after more than 1 year, developed multifocal and bilateral MTC, which are morphologically and biologically similar to human MEN-2A MTC. We decided to evaluate different imaging modalities in this transgenic model. We particularly focus on PET imaging because it is a quantitative technique that is more efficient to use during drug development. We have evaluated two PET tracers already used for human diagnosis of neuroendocrine tumors: [18F]-FDG and [18F]-DOPA. Our first results suggest that [18F]-DOPA is able to detect the appearance of the pathology earlier than [18F]-FDG, the latter being hampered by unspecific labeling of the carotids. Actually, we are comparing these results with SPECT tracers ([123I] MIBG and [111In] pentetreotide). Imaging of MEN-2A using PET in this transgenic mouse could be useful to drug evaluation, such as tyrosine kinase inhibitors. That is what we planned to do with the aptamer D4, which has been identified as a Ret inhibitor.[2]

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IN VIVO IMAGING METHODOLOGIES APPLIED TO THE MURINE XENOGRAFTS OF HUMAN BREAST CANCER: A PRECLINICAL H-PRUNE ANIMAL MODEL. Massimo Zollo,[1] Natascia Marino,[2] Adelaide Greco,[3] Cristin Roma,[2] Livia Garzia,[2] Pietro Carotenuto,[2] Emilio Cusanelli,[4] Angela Papaccioli,[5] Jvana Sommella,[5] Arturo Brunetti.[3] [1]CEINGE, Advanced Biotechnology, Naples, Italy; Biochemistry and Biotechnology Department, University of Naples ''Federico II,'' Naples, Italy; [2]CEINGE, Advanced Biotechnology, Naples, Italy; [3]Department of Biomorphologic and Functional Science, Medicine Faculty, Naples, Italy; [4]CEINGE, Advanced Biotechnology, Naples, SEMM European School of Molecolar Medicine, Naples, Italy; [5]IBB, CNR, Naples, Italy; contact e-mail: zollo@ceinge.unina.it.

Purpose: The aim of our study was to create an animal model for preclinical studies on drug inhibition. It is reported that human Prune (h-prune) binds and inhibits NM23-H1 inducing cell migration. This process is correlated with advanced stage and metastasis in breast cancer. MDA-MB-231 LUC human breast carcinoma cell line stable with luciferase gene was used to produce clones that overexpress or knock-down H-prune gene expression. We analyzed over 24 weeks breast tumor h-prune-induced in an orthotopic murine model by high-frequency ultrasound (US), Bioluminescence imaging (BLI), and positron emission tomography (PET). **Materials and Methods:** MDA LUC Prune and MDA LUC Prune-interference cells were implanted into the mammary fat pad of 20 female nude mice. US (Vevo 770, Visualsonics, 40 MHz) was performed to assess tumor size and blood flow. With BLI (Xenogen's IVIS 200), we evaluated the increase in the luminescent cell number in tumor growth. PET (YAP(S)PET, ISE s.r.l.), FDG, and FLT studies were performed to visualize radiotracer uptake in tumors. **Results:** At US at 20 weeks of observation in h-prune-injected animals tumors (mean size ± SD = 848.22 ± 89.06 mm^3) were detected. The tumor growth observed weekly was 118.25 ± 40.16 mm^3. The orthopic tumor was detected both by FDG and FLT scan. **Conclusions:** US, BLI, and PET permit noninvasive analysis of our mouse model of breast cancer. This animal model could be used to assay new drugs to inhibit h-prune procancer and metastases formation in vivo.

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PHENOTYPING OF MOUSE MODELS OF ALZHEIMER'S DISEASE BY POSITRON EMISSION TOMOGRAPHY. Alexandra Winkeler,[1] Norbert Galldiks,[1] Yannic Waerzeggers,[1] Uwe Himmelreich,[2] Andreas H. Jacobs.[1] [1]Laboratory for Gene Therapy and Molecular Imaging at the Max-Planck-Institute for Neurological Research with Klaus-Joachim-Zülch-Laboratories of the Max-Planck Society and the Faculty of Medicine of the University of Cologne, Center for Molecular Medicine and Department of Neurology at the University of Cologne, Cologne, Germany; [2]In-vivo NMR Laboratory, Max-Planck-Institute for Neurological Research with Klaus-Joachim-Zülch-Laboratories of the Max Planck Society and the Faculty of Medicine of the University of Cologne, Cologne, Germany; contact e-mail: winkeler@nf.mpg.de.

Objective: To characterize newly developed and existing mouse models of neurodegenerative diseases (ND) in vivo by positron emission tomography (PET). **Background:** Noninvasive imaging by PET has been shown to be a useful tool in diagnostics of patients with neurodegenerative diseases. Mouse models of Alzheimer's disease (AD) and newly developed mouse models with direct interest in this topic may be of specific value for the analysis, understanding, and eventually therapy of this disease. Noninvasive imaging of mouse models of neurodegenerative disease will help us to characterize and phenotype these models in more detail and may be of great interest in monitoring early events, disease progression, and potential treatment strategies. **Methods:** Different mouse models of AD or mouse models investigating cross-connections to neurodegenerative diseases have been monitored by multitracer PET, with special focus on 18F-FDG-PET uptake but also [11C]FMZ binding, and [11C]MP4A trapping has been assessed by microPET in vivo. In collaboration PET imaging results have been compared with the molecular analysis of mouse brain as well as behavioral analysis. **Results:** The different mouse models do not show significant differences in [18F]FDG brain-to-background ratios. Only APP23 mice with locus ceruleus (LC) degeneration leading to noradrenergic depletion show a significant alteration in cerebral glucose metabolism, neuronal integrity, as well as acetylcholine esterase activity as assessed by multitracer microPET in vivo. **Conclusions:** Noninvasive imaging via PET seems to be a useful tool to characterize and phenotype animal models of neurodegenerative disease.

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DETECTION OF SELECTIN EXPRESSION IN AN EXPERIMENTAL TRAUMATIC BRAIN INJURY. Laurent Lemaire,[1] Catherine Chapon,[1] Franck Lacoeuille,[1] Florence Franconi,[2] François Hindre,[1] Jean-Pierre Benoit[1], Jean-Jacques Le Jeune.[1] [1]INSERM U646; [2]Université Angers; contact e-mail: laurent.lemaire@univ-angers.fr.

Introduction: Traumatic brain injury (TBI) is known to induce brain edema, perfusional deficit, and major metabolic perturbations. In this study we explored the expression of endothelial selectin in an experimental animal model of TBI using a 99mTc chelating peptide. **Subjects and Methods:** TBI was induced in rats after a 2.5 mm craniotomy via a fitting tube connected to the fluid percussion device. Injection of CIELLQAR peptide derived to chelate 99mTc was injected via the tail vein. Fixation was evaluated by beta-counting. **Results:** The surgical procedure induces a traumatism of the parietal muscle ipsilateral to the craniotomy leading to the fixation of the 99mTc chelating peptide. I/C (ratio ipsilateral/contalateral) increases from 0.99 ± 0.07 in normal rats to 3.9 ± 0.2 in rats traumatized 1 h prior to injection. Specific brain fixation was, however, null in this case (I/C = 1.01 ± 0.10). In traumatized rats, a specific fixation was observed 1 h, 4 h, 24 h, or 72 h post-trauma with an averaged I/C = 1.68 ± 0.17. However, I/C calculated from a 2 mm slice directly in regard to the craniotomy is larger (I/C = 2.42 ± 0.36). At the 72 h point, the entire traumatized hemisphere presents a massive specific fixation (I/C > 4). **Conclusion:** CIELLQAR peptide derived to chelate 99mTc allows the targeting of selectin expression. The fixation appears to be located to the region of impact. At the late time point where the BBB is permeable (Pasco et al. J Neurotrauma 2007), peptide accumulation may be overestimated by passive diffusion in the lesion.

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NONINVASIVE IN VIVO IMAGING OF PROTEIN KINASE A ACTIVITY. Jan Oivind Moskaug,[1] Harald Carlsen,[2] Rune Blomhoff.[1] [1]University of Oslo, Oslo, Norway; [2]Cgene and University of Oslo, Oslo, Norway; contact e-mail: j.o.moskaug@medisin.uio.no.

Protein kinases play pivotal roles in almost all cellular signaling pathways, and modulation of their activity is desirable both in disease treatment and in studies of their function. For these reasons protein kinase inhibitors have been studied extensively in vitro. We now describe an animal model where protein kinase A (PKA) and its activity can be monitored noninvasively in vivo. The model utilizes luciferase, which has been mutated to contain a target sequence of PKA, RRFS, thus making luminescence from the enzyme dependent on its state of phosphorylation. The PKA-sensitive luciferase was incorporated into the mouse genome, and several transgenic animals produced exhibited beta-adrenergic responsive luminescence from various organs as shown by treatment with isoproterenol, ie, reduced luminescence. Beta-adrenergic positive tissues such as pancreas, muscle, liver, stomach, and fat responded to isoproterenol by reduction in luminescence as expected. Localized administration of isoproterenol in skeletal muscle gave a 70% reduction in luminescence, and general anesthetics known to involve beta-adrenergic receptors also reduced luminescence from the abdominal region of the animals. To our knowledge this is the first model where intracellular protein kinase activity can be measured in intact living animals, thus facilitating screening of protein kinase inhibitors in highly relevant physiological conditions.

Cancer

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MAGNETIC RESONANCE IMAGING AND MAGNETIC RESONANCE SPECTROSCOPY IN PATIENTS WITH AN ELEVATED PROSTATE-SPECIFIC ANTIGEN LEVEL AND A PREVIOUS NEGATIVE BIOPSY. Stefano Cirillo,[1] Massimo Petracchini,[1] Patrizia Dellamonica,[1] Teresa Gallo,[1] Laura Martincich,[1] Cinzia Ortega,[2] Eligio Vestita,[3] Ugo Ferrando,[4] Daniele Regge.[1] [1]Institute for Cancer Research and Treatment, Radiology, Candiolo, Torino, Italy; [2]Institute for Cancer Research and Treatment, Oncology, Candiolo, Torino, Italy; [3]Ivrea Hospital, Urology, Ivrea, Torino, Italy; [4]San Giovanni Battista Hospital, Urology, Torino, Italy; contact e-mail: stefano.cirillo@ircc.it.

Purpose: Frequently, urologists are faced with the dilemma of managing patients with prostate-specific antigen (PSA) suspicious levels but one or even more negative biopsies. The aim of the present study was to assess the usefulness of combined endorectal magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) examinations for these patients. **Patients and Methods:** Fifty-four men with a total PSA > 4 ng/mL and a former negative biopsy underwent endorectal MR examinations (Signa 1.5 T, GE). Axial, coronal, sagittal T2-w FSE and axial T1-w FSE sequences were performed. Three-dimensional MRS data were acquired by 3D-CSI. A peripheral prostatic area was classified suspicious either if present low-intensity signals on T2-weighted images or if choline + creatine/citrate was > 0.86. In all the patients, a 10 cores following peripheral biopsy scheme was done to which were added supplementary samples targeted by MRI or MRS indications. **Results:** Histological findings were positive for prostate cancer in 17 of the 54 patients. Thirty of 54 MRI scans were classified as suspicious (55.6%); histological findings confirmed cancer in 17 (56.7%). Twenty-six of 54 patients were classified as suspicious at MRS (48.1%); cancer was confirmed by histology in 15 (57.7%). Combined MRI-MRS evaluation identified 35 suspicious patients; histology confirmed cancer in 17 patients (48.6%). **Conclusions:** In our study combined MRI-MRS evaluation is able to select negative patients. In addition, the cancer detection rate is higher with MR-guided biopsy in comparison with the standard core biopsy procedure. Combined MRI-MRS improving cancer localization might help the urologist in the management of patients who are at high risk of having prostate cancer.

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MOLECULAR IMAGING OF ONCOLYTIC ADENOVIRUS IN VIVO USING THE NA/I SYMPORTER AS A REPORTER GENE. Andrew Merron,[1] Inge Peerlinck,[2] Arnaud Briat,[1] Georges Vassaux.[3] [1]Cancer Research UK; [2]Cancer Research UK, MRC; [3]INSERM, Nantes, France; contact e-mail: andrew.merron@cancer.org.uk.

Oncolytic adenoviruses have shown some promise in cancer gene therapy. However, their efficacy in clinical trials is often limited, and additional therapeutic interventions have been proposed to increase their efficacies. In this context, molecular imaging of viral spread in tumors could provide unique information to rationalize the timing of these combinations. Here, we describe the use of the human sodium iodide symporter (hNIS) as a reporter gene in wild-type and replication-selective adenoviruses. By design, hNIS cDNA was positioned in the E3 region in a wild-type adenovirus type 5 (AdIP1) and in an adenovirus in which a promoter from the human telomerase gene (RNA component) drives E1 expression (AdAM6). Viruses showed functional hNIS expression and replication in vitro and the kinetics of spread of the different viruses in tumor xenografts were visualized in vivo using a dedicated small-animal SPECT/CT camera. The time required to reach maximal spread was 48 hours for AdIP1 and 72 hours for AdAM6, suggesting that genetic engineering of adenoviruses can affect their kinetics of spread in tumors. Considering that this methodology is potentially clinically applicable, we conclude that hNIS-mediated imaging of viral spread in tumors may be an important tool for combined anticancer therapies involving replicating adenoviruses.

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SYNTHESIS OF AN 11C-LABELED CYCLOOXYGENASE-2 INHIBITOR AND POSITRON EMISSION TOMOGRAPHY IMAGING IN HT-29 AND FADU TUMOR-BEARING MICE. Torsten Kniess, Ralf Bergmann, Frank Wuest. FZ-Dresden/Rossendorf Institute of Radiopharmacy; contact e-mail: kniess@fzd.de.

Cyclooxygenase-2 (COX-2) is an enzyme induced during inflammation by various stimuli, but overexpression of COX-2 has been observed also in oncogenesis in a variety of tumors. Although several COX-2 inhibitors have recently been radiolabeled with isotopes for positron emission tomography (PET), their potential for tumor imaging has not been explored extensively. Herein we report the synthesis and radiopharmacological evaluation of 1-(4-[11C]methoxyphenyl)-2-(4-methylsulfonylphenyl)-1-cyclopentene as a novel 11C-labeled radiotracer for PET imaging of COX-2. **Methods:** The radiolabeling was performed via a 11C-methylation reaction of the corresponding desmethyl precursor using [11C]MeI in a TRACERLab FXC module. Biodistribution studies were performed in normal Wistar rats. Small-animal PET studies were performed using xenografted HT-29 and FaDu tumors in mice using a micro-PET P4 scanner. **Results:** The radiolabeling was achieved by the reaction of 1-(4-hydroxyphenyl)-2-(4-methylsulfonylphenyl)-1-cyclopentene at 60°C in DMF/aqueous NaOH with [11C]CH3I. After semipreparative HPLC purification and solid phase extraction the 11C-labeled COX-2 inhibitor was obtained in 12 to 14% decay-corrected radiochemical yield at a specific activity of 30 GBq/μmol at the end of synthesis. The radiochemical purity exceeded 97%. Biodistribution in normal rats showed high radioactivity accumulation in the liver, adrenals, and brown adipose tissue, reaching 0.6 ± 0.1, 0.7 ± 0.2, and 0.8 ± 0.1 %ID/g 1 hour postinjection, respectively. Tumor-to-muscle ratios of 1.7 ± 0.2 and 2.1 ± 0.3, respectively, were determined with small-animal PET studies of xenografted HT-29 and FaDu tumors in mice 1 hour postinjection.

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IMAGING OF TUMOR CELLS VIA TRANSFERRIN RECEPTOR TARGETED BY TF-PIN. Soraya Benderbous,[1] Souad Ammar,[2] Pierre-Antoine Eliat,[3] François Chau,[4] Anne Bessard,[5] Georges Baffet.[6] [1]LEPG, FRE-CNRS 2969, Université de Tours, Tours, France; [2]ITODYS, Université Paris VII, Paris, France; [3]PRISM-IMAGIVEC-UPRES-EA 3890 Université Rennes 1, Rennes, France; [4]ITODYS, Université Paris VII, Paris, France; [5]INSERM U522, Université de Rennes 1, Rennes, France; [6]INSERM U522, IFR 97; contact e-mail: benderbous@univ-tours.fr.

Purpose/Introduction: The early detection of cancerous cells is a key point in studying cell proliferation mechanisms. Cells that are magnetically labeled with superparamagnetic iron oxide particles (SPIOs) can be observed in vivo using MRI. We propose to use particles conjugated with human transferrin molecules to increase tumor cell labeling. **Material and Methods:** Superparamagnetic iron oxide nanocrystals (PIN) have been prepared by forced hydrolysis in polyol and functionalized with transferrin. F1 biliary epithelial cells were incubated 48 hours with functionalized (PIN-Tf) and nonfunctionalized (PIN). Prussian blue staining and transmission electron microscopy (TEM) were used to study the presence of iron oxide nanoparticles inside the cells. FLASH T1w and RARE T2w MR images of a suspension of labeled and nonlabeled cells in agar were acquired on 4.7 T horizontal imaging system. T2 of labeled and nonlabeled cells was calculated based on a multi-spin-echo sequence. **Results:** Prussian blue staining and TEM confirmed the presence of PIN and PIN-Tf inside the F1 cells. Transferrin improved the uptake of nanoparticles in the cells. When compared with nonlabeled control cells, tumor cells labeled with PIN-Tf showed significantly decreased T2 (39%–53%). **Discussion/Conclusion:** The magnetic nanocrystals are well defined with multiple capabilities, such as small and uniform size, strong magnetization, and high biocompatibility. PIN-Tf shows active functionality for desired receptors. Intracellular cytoplasm uptake was demonstrated with electron microscopy and was visualized at 4.7 T. In conclusion, tumor cells can be efficiently labeled and tracked with PIN-Tf contrast agent. This opens to the use of PIN-Tf for MRI in vivo monitoring of cancerous implanted cells in the liver.

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TARGETING TUMORS WITH MAGNETIC RESONANCE IMAGING PROBES BASED ON AN ULTRASMALL PARTICLE IRON OXIDE–ANTIBIOTIN SYSTEM. Gabriella Baio,[1] Marina Fabbi,[2] Daniela de Totero,[2] Silvano Ferrini,[2] Lorenzo E. Derchi,[1] Carlo E. Neumaier.[3] [1]DICMI, Radiologia, University of Genoa, Genoa, Italy; [2]Laboratory of Immunological Therapy, National Cancer Institute, IST, Genoa, Italy; [3]Department of Radiology, National Cancer Institute, IST, Genoa, Italy; contact e-mail: gabriellabaio@yahoo.it.

Purpose: To test ultrasmall particle iron oxide-antibiotin antibody (USPIO-antibiotin) associated with a biotinylated antibody, for in vivo tumor labeling in a murine xenotransplant model using MR at 1.5 T. **Materials and Method:** Human anaplastic B cell lymphomas D430B expressing the CD70 surface antigen were inoculated subcutaneously in NOD/SCID mice. In vivo examination was performed by MR at 1.5 T using T2*- sequences. Subsequently, 40 μg per mouse of the biotin-conjugated anti-CD70 monoclonal antibody LD6 was injected into the tail vein, followed, after 4 hours, by injection of USPIO-antibiotin (Miltenyi Biotech) at the dose of 14 μmol Fe/Kg per mouse. Twenty-four hours later MRI was performed. SE and FFE sequences with multiple TRs and TEs to calculate R1 and R2 relaxation rates were also performed. As a reference product we used ferumoxides and USPIO-antibiotin alone. The presence and location of the iron particles in the tumor were evaluated quantitatively and correlated with blue Prussia stain. **Results:** Twenty-four hours after the administration of biotinylated antibody, tumors showed an inhomogeneous decrease in SI on T2* images .The image analysis was in accordance with the signal intensity decrease (35% ± 5) after USPIO-antibiotin administration and correlated well with histopathology. In both control groups, no appreciable differences in SI of the tumor were observed. **Conclusion:** Superparamagnetic antibiotin particles associated with biotinylated antibodies are able to target tumors. This ''indirect'' approach allows the potential targeting of any cell surface antigen by its specific biotin-labeled antibody, revealed by the USPIO-antibiotin as common secondary reagents. This combination may become a useful tool in oncological imaging.

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DIFFERENTIAL PHYSIOLOGICAL RESPONSE TO CARBOGEN OF TWO DIVERSE PROSTATE TUMOR LINES DETECTED BY TISSUE WATER 1H MAGNETIC RESONANCE IMAGING. Jesús Pacheco-Torres,[1] Dawen Zhao,[2] Jennifer McAnally,[2] Ralph P. Mason.[2] [1]Instituto de Investigaciones Biomédicas ''Alberto Sols,'' CSIC, Madrid, Spain; [2]University of Texas Southwestern Medical Center, Dallas, TX, USA; contact e-mail: jpacheco@iib.uam.es.

Tumor oxygenation plays an important role in cancer malignancy. Recently, studies have suggested a possibility of assessing tissue oxygenation based on the shortening of the tissue water T1 due to oxygen. Here, we are investigating differences in T1- and T2*-weighted signal intensity, as well as maps of R1, R2, and R2* in response to carbogen between two Dunning prostate R3327 rat tumor sublines: AT1 (anaplastic and poorly vascularized) and HI (moderately well differentiated and vascularized). In response to carbogen breathing, significantly increased signal intensity in both T1- and T2*-weighted images was found in both tumor lines. Much higher enhancement in both T1- and T2*-weighted signals was observed in HI 0.7 in 2.2 vs 5.4 ± compared with AT1 tumors (mean maximum ΔSI (%) = 8.6 ± T1-weighted; 23.9 ± 8 vs 9.8 ± 1.8 in T2*-weighted). R1 maps revealed that carbogen induced significantly increased R1 values in both the periphery and center of the HI tumors (mean ΔR1 = 0.012 (periphery) vs 0.006 s-1 (center); $p < .01$), while no significant increase was seen in the AT1 tumors. Similarly, reduction in R2* values in response to carbogen was found in the HI tumor but not the AT1 tumors. These results are in line with previous studies in these two tumor lines. While SI of T1-weighted image increased, T1 values were not shortened in the AT1 tumors with carbogen inhalation. This may be attributed to an increase in blood flow associated with carbogen. Since this approach is totally noninvasive it appears worthy of further investigations for characterizing tumors and response to adjuvant interventions.

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INTEREST OF OPTICAL IMAGING FOR THE DESIGN AND IMPROVEMENT OF TARGETED-DRUG DELIVERY SYSTEMS IN ONCOLOGY. Veronique Josserand, Jean Luc Coll. Institut Albert Bonniot, INSERM U823, Cibles diagnostiques ou thérapeutiques et vectorisation de drogues dans le cancer du poumon; contact e-mail: veronique.josserand@ujf-grenoble.fr.

Lung cancer is a major problem of public health, before breast, colon, and prostate cancers all together. Non–small cell lung cancers (NSCLC), which account for 75% of these tumors, are curable by surgery in association or not with an adjuvant radiochemotherapy in only 10% of the cases if they are localized. In the metastatic forms, chemotherapy has a very limited impact on survival. There is thus a very strong rationale for developing new targeted therapies. Nonetheless, the weak effectiveness of vectorization systems is still a major issue. In this context, we generate new targeting vectors for drugs or biomolecule delivery (DNA, RNAi, peptides). To undertake this work, we also developed a platform of optical imaging allowing the follow-up and evaluation of our vectorization systems. Based on our recent work I will present a general overview of how optical imaging can help for the development of targeted therapeutics and image-guided surgery in oncology.

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IN VIVO PINHOLE SINGLE-PHOTON EMISSION COMPUTED TOMOGRAPHY IMAGING OF MELANOMA PULMONARY METASTASES IN MICE WITH THE TM TRACER LABELED WITH 125 IODINE. Elisabeth Miot-Noirault, Janine Papon, Jean-Michel Chezal, Françoise Degoul, Jean-Claude Madelmont, Nicole Moins. UMR 484 INSERM, Clermont Ferrand Cédex, France; contact e-mail: noirault@inserm484.u-clermont1.fr.

Background and Objective: Our group develops melanoma-specific imaging agents for early diagnosis of melanoma and its metastases. Among all benzamide analogues studied, a derivative (called TM) was selected on the basis of its biodistribution properties. This study aimed to assess the potential of ^{125}I-labeled TM for in vivo longitudinal monitoring of pulmonary B16F0 melanoma metastases in mice. **Materials and Methods:** Pulmonary uptake of ^{125}I-labeled TM was studied in mice at 11, 15, 20, 25, and 30 days after B16F0 cells IV injection and compared with control mice. Pinhole SPECT (90-minute duration) was performed 24 hours after tracer injection (9.25 MBq/mouse) with a small-animal gamma camera (10 cm FOV, gammaImager, Biospace) equipped with a 1 mm pinhole collimator. At study ending (day 30), mice were sacrificed for autoradiography. Tomographic reconstructions were performed using a 3D-OSEM algorithm and coronal slices were reconstructed. **Results:** In healthy animals, ^{125}I-labeled TM was not detectable in lungs, with uptake being 0.3% ID/g. From day 11, all mice with melanoma-pulmonary lesions exhibited a significant lung uptake of tracer, uptake increasing with pathology progression. Both the 3D volume and coronal slices enabled an exact localization of the tracer within the pulmonary areas with melanoma nodules being highly delineated. Coronal slices reconstructed from in vivo imaging could be superimposed to autoradiographic images and anatomical slices. **Conclusion:** The combination of the strength of pinhole SPECT with the high specificity of the ^{125}I-labeled TM tracer allows melanoma pulmonary metastases detection, thus offering a way to the pathophysiological study of dissemination.

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EPITHELIAL AND MESENCHYMAL TUMOR HISTOTYPES EXHIBIT A COMPLEMENTARY PATTERN OF VASCULAR PERFUSION AND GLUCOSE METABOLISM. Mirco Galiè,[1] Paolo Farace,[1] Cristina Nanni,[2] Antonello Spinelli,[3] Elena Nicolato,[1] Federico Boschi,[1] Paolo Magnani,[1] Flavia Merigo,[1] Andrea Sbarbati,[1] Francesco Osculati,[1] Pasquina Marzola.[1] [1]Dip. Scienze Morfologico-Biomediche, sez. Anatomia ed Istologia, Università di Verona, Verona, Italy; [2]UO Medicina Nucleare, Azienda Ospedaliero-Universitaria di Bologna Policlinico S.Orsola-Malpighi, Bologna, Italy; [3]Servizio di Fisica Sanitaria, Azienda Ospedaliero-Universitaria di Bologna Policlinico S.Orsola-Malpighi, Bologna, Italy; contact e-mail: mirco@anatomy.univr.it.

Cancer cells have an inherent tendency to anaerobic glycolysis. This is on the basis of the increased glucose consumption and lactate extrusion that are typical of tumors and are considered diagnostic indexes of malignancy. However, it has been recently shown that tumor-associated stromal cells are capable of aerobic metabolism with low glucose consumption, and it has been proposed that they could clear and recycle the lactate produced by the anaerobic metabolism of cancer cells. This reciprocity could be theoretically linked to the vascular difference between epithelial (low vascularization) and stromal (high vascularization) tumor compartments, which provides these compartments with

differential levels of oxygenation. In order to investigate the link between glucose consumption, vascular perfusion, and tumor histotype we compared the maps of dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) and [F-18]-fluorodeoxyglucose (FDG) positron emission tomography (PET) in carcinoma and mesenchymal tumor models. We observed that (1) inside both the carcinoma and mesenchymal tumors, vascular perfusion and FDG-uptake maps appeared roughly reciprocal; (2) this reciprocity was more conspicuous in carcinomas than in mesenchymal tumors; (3) in carcinomas, regions with a high vascular/low FDG-uptake pattern roughly matched stromal capsula and intratumoral large connectival septa; (4) mesenchymal tumors exhibited a higher vascular perfusion and a lower FDG uptake than carcinomas. We concluded that (1) glucose consumption has a reversed relation with vascular supply; (2) glucose consumption is lower and vascular supply is higher in stromal than in epithelial compartments of the same carcinoma; (3) glucose consumption is lower and vascular supply is higher in mesenchymal tumors than in carcinomas.

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VASCULARIZATION ANALYSIS BY IN VIVO OPTICAL IMAGING OF TRANSGENIC INDUCED MAMMARY CANCER. Stéphanie Guillermet, Jean-Louis Alberini, Benoît Thézé, Benoit Jego, Karine Siquier-Pernet, Bertrand Tavitian, Raphaël Boisgard. CEA, DSV, I2BM, SHFJ, Laboratoire d'Imagerie Moléculaire Expérimentale, Orsay, France; INSERM U803, Orsay, France; contact e-mail: stephanie.guillermet@cea.fr.

The aim of this study was to follow, by optical imaging, changes in breast tumor vascularization during tumoral development and chemo-induced regression, in a transgenic mouse (PyMT) model expressing the polyoma middle T oncoprotein in mammary epithelial cells. Changes in the vascular density, diameter, and morphology of blood vessels were monitored by fluorescence imaging after intravenous injection of dextran-FITC, using the CellVizio (Mauna Kea Technologies), a fibered confocal fluorescence microscope that can monitor these parameters in vivo with a cellular resolution. The vascular permeability was assessed after intravenous injection of SuperhanceTM680, a vascular contrast agent (Visen Medical) with a whole-body optical imaging camera (Biospace Mesures). Comparison of the vascularization of normal and tumoral mammary glands showed an increase in the vascular density and of the blood vessel diameter in tumors. Moreover, tumoral blood vessels appeared dramatically more tortuous than normal ones. A longitudinal analysis of the PyMT mice was performed. Images obtained with the CellVizio showed an enhancement in vascular density during tumoral development. Preliminary results obtained with Superhance680 showed an increased vascular permeability of the tumors during tumoral development. The effect of chemotherapy treatment on tumor angiogenesis was evaluated after three administrations of paclitaxel (10 mg/kg/week) between 9 and 11 weeks of age. At the end of treatment, the vascular network was found to have returned to a similar morphology as that observed in normal mammary glands. In conclusion, optical imaging illustrates modifications of the vascular network in PyMT mice during tumoral development and under chemotherapy and can be useful for evaluation of the efficiency of new antiangiogenic therapies.

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MULTIMODALITY IMAGING OF TUMOR PROGRESSION AND OSTEOLYTIC BONE METASTASES IN MOUSE MODELS OF CANCER. Eric Kaijzel,[1] Ivo Que,[1] Gabri Van der Pluijm,[1] Peter Kok,[2] Boudewijn Lelieveldt,[2] Jouke Dijkstra,[2] Clemens Lowik.[1] [1]Department of Endocrinology, Leiden University Medical Center, Leiden, the Netherlands; [2]Department of Radiology, Leiden University Medical Center, Leiden, the Netherlands; contact e-mail: e.l.kaijzel@lumc.nl.

Breast and prostate cancer metastasize preferentially to bone and often lead to osteolytic or osteosclerotic lesions, respectively. The development of novel anticancer strategies requires more sensitive and less invasive methods to detect and monitor in vivo tumor progression, metastasis, and minimal residual disease in cancer models. Whole-body bioluminescence imaging (BLI) enables high sensitivity for monitoring in vivo molecular processes, though it provides little structural detail. Fusion with micro-CT or MR is desired to complement imaging sensitivity with anatomical detail. In the present work we have combined visualization and analysis of multi-angle BLI (Xenogen IVIS 3D) and 3D micro-CT (Skyscan 1178). Luciferase-expressing human renal cell carcinoma cells (RC21-luc) were injected under the renal capsule of nude mice and scanned after 3 weeks. 3D reconstructions using the Xenogen Living Image Software 3D Analysis Package showed clear clusters of bioluminescence with different intensities at the site of transplantation but also at the end of the descending blood vessels from the kidney, which is in the capillary beds. Mice intracardially injected with MDA-231-luc cells and imaged after 40 days show bone metastases in the scapula and vertebrae of the spinal cord. Osteolytic lesions are clearly visible from the fast CT scans. 3D reconstructions were performed using an in-house developed software platform (INTEGRIM) enabling fusion of micro-CT and multi-angle BLI.

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IN VIVO DETECTION OF STRESS TUMOR RESPONSE TO GEFITINIB MEDIATED BY THE INTERACTION OF PHOSPHORYLATED BCL-2 AND INOSITOL TRISPHOSPHATE RECEPTOR TYPE III. Antonella Zannetti,[1] Francesca Iommelli,[1] Angela Papaccioli,[1] Jvana Sommella,[1] Arturo Brunetti,[2] Marco Salvatore,[2] Silvana Del Vecchio.[2] [1]Istituto di Biostrutture e Bioimmagini, CNR; [2]University of Naples "Federico II," Naples, Italy; contact e-mail: antonella.zannetti@ibb.cnr.it.

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Targeted inhibition of epidermal growth factor receptor (EGFR) is one of the currently adopted strategy in treatment of human solid tumors. Gefitinib is a small molecular weight compound that inhibits EGFR receptor autophosphorylation and downstream signaling pathways. The aim of our study was to detect early tumor response to gefitinib using microSPECT imaging with 99mTc-sestamibi and to investigate the molecular events underlying such a response. Our previous studies showed indeed an increase in 99mTc-sestamibi uptake in Bcl-2-overexpressing and parental breast cancer cells upon exposure to staurosporine, a general kinase inhibitor. In the present study MCF-7, MDA-MB231, and T47D (wild type and Bcl-2 transfected) breast cancer cells; A549 and SKLU-1 lung cancer cells; and A431 epidermoid cancer cells were incubated with increasing concentration of gefitinib ranging between 0.5 and 20 μM for 1 hour. Then cells were tested for 99mTc-sestamibi uptake, and levels of EGFR, P-EGFR, Bcl-2, P-Bcl-2, and inositol trisphosphate receptor type I (IP3R1) and III (IP3R3) were assessed in whole-cell lysates, subcellular fractions, and immunoprecipitated samples. Furthermore, imaging studies by microSPECT were performed before and after gefitinib treatment in nude mice bearing control and Bcl-2-overexpressing breast carcinomas. We found that gefitinib treatment causes an increase in phosphorylated Bcl-2 levels in the endoplasmic reticulum (ER) and enhances its physical interaction with inositol trisphosphate receptor type III. These molecular events could be traced and visualized in vivo by 99mTc-sestamibi. A high tumor uptake was detected in post-treatment imaging studies in all tumor xenografts while no tumor uptake could be observed in the baseline studies independently of tumor size. Our findings indicate that gefitinib treatment causes an ER-mediated stress response involving the interaction of phosphorylated Bcl-2 and IP3R3 that can be visualized both in vitro and in vivo by 99mTc-sestamibi.

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EVALUATION OF NOVEL TARGETED TUMOR THERAPIES BY NONINVASIVE NEAR-INFRARED IMAGING. Joanna Napp,[1] Friedemann Müller,[2] Andres van de Locht,[2] Torsten Steinmetzer,[2] Christian Dullin,[3] Sarah Kimmina,[4] Marta Zientkowska.[1] [1]Department of Haematology/Oncology, University of Goettingen, Goettingen, Germany; [2]CURACYTE, Leibzig, Germany; [3]Department of Diagnostic Radiology, University of Goettingen, Goettingen, Germany; [4]Department of Laboratory Animal Science, University of Goettingen, Goettingen, Germany; contact e-mail: jnowako1@gwdg.de.

Proteolytic enzymes expressed on the surface of tumor cells are a novel group of targets for anticancer and/or antimetastatic therapies. In our study we noninvasively monitored expression and activity of matriptase, a trypsin-like membrane-bound serine protease, highly expressed in pancreatic tumors and involved in processes of tumor progression. For this purpose near-infrared imaging technique (eXplore Optix, General Electrics) was applied in an orthotopic pancreatic tumor model either in combination with a fluorescent labeled antibody or with a fluorescent labeled substrate to evaluate in vivo expression and activity of matriptase, respectively. Human pancreatic cancer AsPC-1 cells were orthotopically implanted into nude mice. Prior to scanning with the eXplore Optix system a Cy5.5-labeled matriptase antibody or the fluorescent substrate was injected intravenously. Binding of the antibody and thereby matriptase expression was detected at sites of primary tumors as well as in lymph nodes and distant metastases by in vivo measurement of fluorophore concentration, fluorescence lifetime, and location. Matriptase activity could be determined in tumor-bearing mice and in response to therapy by monitoring changes in fluorescent properties of the substrate upon enzymatic cleavage. At the same time animals were scanned by flat panel volume computed tomography (fpVCT; General Electrics) to determine tumor progression and thereby correlating fluorescent signals to anatomical structures. In summary, near-infrared imaging allows analyzing expression and activity of matriptase in vivo. Monitoring enzyme activity in response to matriptase inhibitors leads preclinically to a better understanding and management of this cancer targeted therapy.

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NONINVASIVE ASSESSMENT OF E2F-1-MEDIATED TRANSCRIPTIONAL REGULATION IN VIVO. Parisa Monfared,[1] Alexandra Winkeler,[1] Markus Klein,[1] Anke Klose,[1] Hongfeng Li,[1] Sigrun Korsching,[2] Yannic Waerzeggers,[1] Andreas H. Jacobs.[1] [1]Laboratory for Gene Therapy and Molecular Imaging at the Max-Planck Institute for Neurological Research with Klaus-Joachim-Zülch-Laboratories of the Max Planck Society and the Faculty of Medicine of the University of Cologne and Departments of Neurology at the University of Cologne and Klinikum Fulda, Cologne, Germany; [2]Institute of Genetics, University of Cologne, Cologne, Germany; contact e-mail: parisa.monfared@nf.mpg.de.

There is strong evidence supporting the theory that deregulation of the E2F-1 transcription factor via alteration of the p16-cyclinD-Rb pathway is a key event in the malignant progression of most human gliomas. Moreover, E2F-1 has properties as a tumor suppressor. Recent data indicate that the E2F-1 protein level is increased in response to DNA damage. In this study we demonstrate that the Cis-E2F-LUC-IRES-TKGFP reporter system is sufficiently sensitive to monitor the transcriptional regulation of E2F-1 in the Rb/E2F signal transduction pathway and DNA damage–induced upregulation of E2F-1 transcriptional activity using molecular imaging technology. Our noninvasive imaging results were confirmed by independent measures of E2F-1 activity and could be correlated to altered expression levels of p53 and p21. We believe that noninvasive imaging of E2F-1 as a common downstream factor in cell cycle progression by using the Cis-E2F-LUC-IRES-TKGFP reporter system may be useful in assessing novel therapeutic approaches in glioma therapy.

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COMPARATIVE PRECLINICAL STUDIES OF DTPA-, DOTA-, AND HYNIC-EXENDIN-4 CONJUGATES. Damian Wild,[1] Andreas Wicki,[2] Rosalba Mansi,[3] Martin Behe,[4] Jean C. Reubi,[5] Gerhard Christofori,[2] Helmut R Maecke.[3] [1]Clinic and Institute of Nuclear Medicine, University Hospital, Basel, Switzerland; [2]Institute of Biochemistry and Genetics, Medical School University of Basel, Basel, Switzerland; [3]Division of Radiological Chemistry, University Hospital, Basel, Switzerland; [4]Division of Nuclear Medicine, University Hospital, Marburg, Germany; [5]Institute of Pathology, University of Bern, Bern, Switzerland; contact e-mail: dwild@uhbs.ch.

The main rationale to develop a radiopeptide for the targeting of the GLP-1 receptor is based on the need to develop tools for imaging and localization of insulinomas by SPECT, PET, or use of a surgical probe for intraoperative localization. In this preclinical study we compared the three different conjugates Lys40(Ahx-DTPA-In-111)-exendin-4, Lys40(Ahx-DOTA-In-111)-exendin-4, and Lys40(Ahx-HYNIC-Tc-99m)-exendin-4 for detecting GLP-1 receptor–expressing tumors. The conjugates were synthesized on solid phase using the Fmoc strategy. Exendin-4 was modified C-terminally with Lys40-NH2, whereby the lysine side chain was conjugated with the Ahx-chelator. The conjugates were labeled with In-111 and Tc-99m. For SPECT/CT imaging and biodistribution studies in insulinoma-bearing transgenic mice (Rip1Tag2) were used. These mice develop tumors from pancreatic beta-cells. Beta-tumor cells were established from beta-cell tumors and used in internalization and externalization assays. Biodistibution studies 4 h after injection of [Lys40(Ahx-DTPA-In-111)NH2]exendin-4 and the corresponding DOTA-derivative showed a very high (> 280% IA/g) and specific tumor uptake, whereas the uptake of [Lys40(Ahx-HYNIC-Tc-99m)NH2]exendin-4 still was high showing about 100% IA/g tumor but distinctly lower than the ^{111}In-labeled radiopeptides. The difference in tumor uptake corresponds to a factor of 2 lower internalization rate. As found for other radiopeptides the exendin-4 derivatives show high kidney uptake amounting to tumor:kidney uptake ratios between 1.3 and 1.5. SPECT/CT and SPECT/MRI showed excellent tumor visualization. The high GLP-1 receptor density and the high specific uptake of these conjugates encourage further studies for pre- and intraoperative localization of insulinomas in patients and for therapeutic applications.

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[18F]FLUORODEOXYGLUCOSE AND [11C]CHOLINE POSITRON EMISSION TOMOGRAPHY FOR THE ASSESSMENT OF CANCER DEVELOPMENT AND PROGRESSION IN PRECLINICAL MOUSE MODELS OF HORMONE-DEPENDENT AND -INDEPENDENT PROSTATE CANCER. Sara Belloli,[1] Rosa Maria Moresco,[1] Elena Jacchetti,[2] Elisa Coradeschi,[1] Pasquale Simonelli,[1] Maria Grazia Minotti,[1] Massimo Danna,[1] Matteo Bellone,[2] Ferruccio Fazio.[1] [1]IBFM-CNR, Nuclear Medicine Department, San Raffaele Scientific Institute, University of Milan-Bicocca, Milan, Italy; [2]Cancer Immunotherapy and Gene Therapy Program, San Raffaele Scientific Institute, Milan, Italy; contact e-mail: sara.belloli@hsr.it.

Transgenic mouse prostate (TRAMP) model is a valuable model for spontaneous development of hormone-dependent and -independent PC that closely resemble the human pathology. However,

abdomen palpation as a tool to measure PC development in living animals is moderately quantitative. The aim of the study was to validate PET imaging as a reliable tool for the assessment of tumor development and progression as well as treatment efficacy in TRAMP mice. Experimental conditions were set up on a xenograft mouse model based on the subcutaneously injection of TRAMP-C1 cells, derived from a hormone-independent TRAMP lesion. In the xenograft model in vivo and ex vivo studies showed a clear uptake of [18F]FDG and [11C]choline that was definitely higher for [18F]FDG. The addition of paclitaxel or doxorubicin to TRAMP-C1 cell cultures significantly reduced proliferation index and [18F]FDG uptake (54.7% and 43.4%, respectively; $p < .05$). On the contrary, in the xenograft models, both drugs failed to modify lesion volume and [18F]FDG uptake even if single-subject responses were observed. As for transgenic mice, [18F]FDG and [11C]choline were able to detect adenocarcinoma as well as neuronendocrine as indicated by imaging and postmortem tissue histology. PET represents a state-of-the-art tool for in vivo monitoring of tumor progression in transgenic TRAMP mice and may be of particular help for screening of newly developed therapeutic approaches for PC both in the xenograft and transgenic TRAMP model.

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A WHOLE-CELL SELEX FOR IN VITRO SELECTION OF NUCLEIC ACID–BASED APTAMERS AGAINST GLIOBLASTOMA CELLS. Laura Cerchia,[1] Carla Lucia Esposito,[1] Frédéric Duconge,[2] Bertrand Tavitian,[2] Vittorio de Franciscis.[1] [1]Istituto per l'Endocrinologia e Oncologia Molecolare ''G. Salvatore''; [2]CEA-DSV-I2BM-SHFJ-LIME INSERM U803; contact e-mail: cerchia@unina.it.

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Malignant gliomas are the leading cause of CNS tumor-related death, and patients with glioblastoma have a life expectancy of less than 1 year despite surgery and chemo- and radiotherapy. Growth and dissemination of gliomas will reproduce the complex cellular heterogeneity of these tumors. Thus, there is a need for a rapid increase of understanding the biological pathways leading to these diseases in order to find new therapeutic and diagnostic modalities. We have developed a whole-cell SELEX technology to isolate RNA-based aptamers against malignant glioblastoma cell lines with the aim of selecting ligands capable of detecting tumor-specific epitopes as a class of new potential diagnostic and/or therapeutic agents. Following 14 rounds of selection on malignant U87MG cells, preceded by counterselection steps on a nontumorigenic T98G cell line, we obtained a sevenfold enrichment of the original library for aptamers able to discriminate between malignant and nonmalignant phenotypes. We have isolated 70 sequences that are in course of analysis for sequence comparison and secondary structure prediction. Binding experiments on U87MG cells and on T98G cells will be presented.

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COMPUTED TOMOGRAPHY AND MAGNETIC RESONANCE–BASED QUANTIFICATION OF THE TRANSPORT OF NANO-ENCAPSULATED CONTRAST AGENTS THROUGH ABNORMAL VASCULATURE IN TUMORS. Jinzi Zheng,[1] Christine Allen,[2] David A. Jaffray.[1] [1]University of Toronto/Princess Margaret Hospital, Toronto, ON, Canada; [2]University of Toronto, Toronto, ON, Canada; contact e-mail: jinzi.zheng@rmp.uhn.on.ca.

The angiogenic vascular network found in tumors has been shown to allow preferential leakage and subsequent retention of macromolecules of specific molecular weights and sizes in the tumor interstitium. This phenomenon is known as the enhanced permeation and retention effect, often referred to as passive targeting. Our research group has previously developed a multimodal CT and MR contrast agent through the co-encapsulation of iohexol (Omnipaque, GE Healthcare, USA) and gadoteridol (Prohance, Bracco Diagnostics, Italy) inside unilamellar liposomes of ≈80 nm in diameter. This macromolecular contrast agent has been previously shown to retain within healthy blood vessels following intravenous injection, exhibiting a vascular circulation half-life of ≈18 hours in mice and ≈100 hours in rabbits. When administered to VX2 sarcoma-bearing New Zealand White rabbits (2.7–3.0 kg), the liposomal agent preferentially accumulated in the tumor interstitium (with no measurable signal increase in the muscle or fat) with significant CT and MR enhancement lasting more than 7 days. The intratumoral accumulation pattern (both spatial and temporal) of the liposomal agent was heterogeneous. 2D and 3D region of interest (ROI)-based techniques were used to measure the spatial and temporal changes in the concentrations of the multimodal agent in selected subregions of the tumor after registering the time course CT and MR images using Pinnacle v7.6c (Philips Radiation Oncology Systems). The successful optimization of reliable methods to quantify the intratumoral distribution of this liposome contrast agent will lead to improved understanding and characterization of macromolecule transport through abnormal vasculature found in diseases.

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99MTC-NTP 15-5 IMAGING FOR THE EARLY AND SPECIFIC DIAGNOSIS OF CHONDROSARCOMA: EXPERIMENTAL PROOF OF PRINCIPLE IN RATS. Elisabeth Miot-Noirault,[1] Aurélien Vidal,[1] Maryse Rapp,[1] François Gouin,[2] Michèle Borel,[1] Jean-Claude Madelmont,[1] Dominique Heymann,[2] Françoise Redini,[2] Nicole Moins.[1] [1]UMR 484 INSERM, Clermont Ferrand Cedex, France; [2]INSERM EA 3822 INSERM ERI7, Nantes Cedex 1, France; contact e-mail: noirault@inserm484.u-clermont1.fr.

Background and Objective: Our lab develops the ''cartilage targeting imaging strategy'' with 99mTc-NTP 15-5 that selectively binds to proteoglycans. This study performed in chondrosarcoma-bearing rats aimed to assess the relevance of 99mTc-NTP 15-5 imaging for the early diagnosis and follow-up of chondrosarcoma. **Methods:** 99mTc-NTP 15-5 longitudinal imaging of Swarm chondrosarcoma-bearing rats was performed at 10, 20, 25, 35, and 45 days after paratibial orthotopic implantation of the tumor in the right paw, the left paw being used as control. Scintigraphic ratios were calculated (tumor/vertebra and tumor/muscle uptake) and their time course followed. Tumor volume was measured with a caliper. At study ending (day 50), each animal underwent bone scintigraphy with 99mTc-MDP, currently used for chondrosarcoma radionuclide diagnosis in patients. **Results:** From day 10, all rats ($n = 6$) exhibited a significant tracer tumor uptake that increased with pathology progression. At the early stage day 10, a positive chondrosarcoma imaging was obtained with 99mTc-NTP 15-5 tracer, while no palpable and measurable tumor could be assessed. (Tumor became palpable only at day 20.) The time course of scintigraphic ratios appeared to be closely related to chondrosarcoma development. It should be mentionned that 99mTc-MDP imaging was negative at the later stage of tumor development (day 50). **Conclusion:** These results underlined the potential of 99mTc-NTP 15-5 as a tracer for the identification of chondrosarcoma and early detection, in a clinical context where the differential diagnosis from osteosarcoma is of real need.

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11C-MET AND 18F-FLT-POSITRON EMISSION TOMOGRAPHY AS TRACERS FOR TUMOR ACTIVITY IN COMPARISON TO PERFUSION AND DIFFUSION MAGNETIC RESONANCE IMAGING. Roland Ullrich, Lutz Kracht, Kristina Kesper, Jan Sobesky, Alexander Schuster, Andreas Jacobs. Max-Planck Institute for Neurological Research with Klaus-Joachim-Zülch-Laboratories, Department of Neurology, University of Cologne, and Klinikum Fulda, Cologne, Germany; contact e-mail: ullrich@nf.mpg.de.

Introduction: In the differentiation of malignant brain tumors the apparent diffusion coefficient (ADC) and the relative cerebral blood volume (rCBV) obtained by MRI have been evaluated.[1] The aim of this study was to investigate the relation between 11C-MET and 18F-FLT as amino acids for the assessment of proliferation in PET to rCBV respectively to the ADC. **Material and Methods:** In total 19 patients with primary glioma were included. All patients underwent 11C-MET PET investigation and 10 patients 11C-MET and 18F-FLT PET. DW and PW images were performed in 17 of 19 patients. The rCBV, the ADC, 11C-MET uptake, and 18F-FLT uptake were determined by using a region of interest (ROI) approach. **Results:** Maximal rCBV ratios within the tumor are strongly related to the highest 11C-MET and 18F-FLT uptake ratios (MET: $r = .89$, $p < .001$; FLT: $r = .68$, $p = .05$). After coregistration in the tumor with the highest rCBV increased 11C-MET uptake/18F-FLT ratios were also found (MET: $r = .66$, $p = .004$; FLT: $r = .77$, $p = .016$). An inverse correlation was observed between the ADC ratio and the uptake ratios of 11C-MET and 18F-FLT (11C-MET: $r = -.76$, $p = .001$; 18F-FLT: $r = -.77$, $p = .73$). **Conclusion:** The strong relation between high metabolism of 11C-MET and 18F-FLT and rCBV ratios, respectively, ADC ratios indicates that high proliferating parts of the tumor as depicted by PET are associated with a high perfusion and a high cellular density detected by MR.

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IN VIVO IMAGING OF ALPHA(V)BETA(3) INTEGRIN EXPRESSION WITH A RADIOLABELED CHIMERIC RGD PEPTIDE. Antonella Zannetti,[1] Silvana Del Vecchio,[2] Maria Rosaria Panico,[1] Antonio Speranza,[1] Francesca Iommelli,[1] Angela Papaccioli,[1] Jvana Sommella,[1] Michele Saviano,[1] Laura Zaccaro,[1] Annarita Del Gatto,[1] Carlo Pedone,[1] Marco Salvatore.[2] [1]Istituto di Biostrutture e Bioimmagini, CNR; [2]University of Naples ''Federico II,'' Naples, Italy; contact e-mail: antonella.zannetti@ibb.cnr.it.

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The alpha(v)beta(3) integrin is a cell adhesion receptor involved in angiogenesis, tumor cell migration, and metastatic dissemination. The tripeptide sequence RGD binds to alpha(v)beta(3) but also interacts with other integrins. We developed a novel cyclized RGD pentapeptide covalently linked by a spacer to an echistatin domain that showed a high selectivity for alpha(v)beta(3) integrin (J Med Chem 2006;49:3416–20). In the present study we characterized this chimeric RGD peptide (RGDechi) in human erythroleukemia K562 cells, stably cotransfected with cDNA of alpha(v) or alpha(IIb) and beta(3) or beta(5) subunits by adhesion assays, competitive binding assays, and cross-linking experiments. RGDechi was then conjugated with DTPA and labeled with ^{111}In for SPECT imaging whereas a one-step procedure was used for labeling the chimeric peptide with 18F for PET imaging. K562 cells overexpressing alpha(v)beta(3) or alpha(v)beta(5) were subcutaneously injected into opposite flanks of individual nude mice and allowed to grow up to 0.5 cm in size. Alternatively, U87MG human glioblastoma cells and A431 human epidermoid cells endogenously expressing high levels of alpha(v)beta(3) and alpha(v)beta(5), respectively, were used to develop xenografts in nude mice. Imaging studies were then performed using both SPECT and microPET. Adhesion assays showed that the chimeric RGDechi was able to inhibit adhesion of alpha(v)beta(3)-overexpressing cells but not alpha(IIb)beta(3)- and alpha(v)beta(5)-overexpressing clones to native ligands. Competitive binding assays and cross-linking experiments confirmed the selectivity of binding to alpha(v)beta(3). Nude mice bearing K562 and U87MG tumor xenografts, both overexpressing alpha(v)beta(3), showed a high tumor uptake of ^{111}In-labeled and ^{18}F-labeled RGDechi assessed by SPECT and microPET, respectively. No tumor uptake of radiolabeled RGDechi could be observed in K562 and A431 tumor xenografts overexpressing alpha(v)beta(5). Our findings indicate that chimeric RGDechi is suitable for in vivo selective alpha(v)beta(3) receptor imaging.

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MULTIMODAL IMAGING OF A NOVEL IN VIVO ANIMAL MODEL FOR HUMAN MULTIPLE MYELOMA. Henk Rozemuller,[1] Mieke C. Zwart,[1] Andries Bloem,[2] Clemens W.G.M. Lowik,[3] Ivo Que,[3] Nora DeClerck,[4] Andrei A. Postnov,[4] Anton C. Martens.[1] [1]Department of Immunology, Experimental Hematology Lab, University Medical Centre Utrecht, Utrecht, the Netherlands; [2]Department of Hematology, University Medical Centre Utrecht, Utrecht, the Netherlands; [3]Department of Endocrinology and Metabolic Diseases, Leiden University Medical Centre, Leiden, the Netherlands; [4]Department of Biomedical Sciences and Physics, University of Antwerpen, Antwerp, Belgium; contact e-mail: h.rozemuller@umcutrecht.nl.

Preclinical testing of new therapeutic strategies for the treatment of multiple myeloma (MM) requires animal models that closely resemble human disease. We developed a novel in vivo MM model by engraftment with GFP-luciferase gene-marked U266 or RPMI-8226/S cells, both of human origin, into RAG2common gamma double knockout mice (RAG2common gamma) and applying real-time bioluminescence imaging (BLI) for measuring the initial growth of the MM cells. Within 2 weeks after intravenous injection significant BLI signals were detectable, predominantly in the skeletal bones such as the pelvic region, skull, limbs, ribs, sternum, and vertebrae. Infiltration in soft tissues was not observed. Mice that were imaged weekly showed a good correlation between the BLI signal and the growth of MM, validated by the free Ig light chain secretion in the urine of the mice. The BLI signals could postmortem be confirmed by flow cytometry and immunohistology of GFP-, CD45-, CD138-, and CD38-positive cells in affected bones. To determine the exact localization of the MM growth we combined BLI with micro-CT. This confirmed the skeletal location of the MM cells. The CT scan also showed several aberrations in the bone structure such as demineralization and trabecular bone loss. The latter was confirmed with fluorescence molecular tomography by the presence of ProsenseTM and OsteosenseTM activity in the areas where BLI revealed the location of MM cells. With these different imaging modalities we show the resemblance of this MM mouse model with human myeloma, making it suited for quantitative evaluation of experimental treatment and for studying bone remodeling.

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MAGNETICALLY BASED ENHANCEMENT OF NANOPARTICLE UPTAKE IN TUMOR CELLS: COMBINATION OF MAGNETIC LABELING AND MAGNETIC HEATING. Melanie Kettering,[1] Jörn Winter,[1] Matthias Zeisberger,[2] Sibylle Bremer-Streck,[3] Hartmut Oehring,[4] Christian Bergemann,[5] Christoph Alexiou,[6] Rudolf Hergt,[2] Karl-Jürgen Halbhuber,[4] Werner A. Kaiser,[1] Ingrid Hilger.[1] [1]Institute of Diagnostic and Interventional Radiology, University Hospital, Jena, Germany; [2]Institute for Physical High Technology, Jena, Germany; [3]Institute of Clinical Chemistry and Laboratory Medicine, University Hospital, Jena, Germany; [4]Institute of Anatomy II, University Hospital Jena, Jena, Germany; [5]Chemicell GmbH, Berlin, Germany; [6]Department of Otorhinolaryngology, Head/Neck Surgery, University Erlangen-Nürnberg, Erlangen, Germany; contact e-mail: melanie.kettering@med.uni-jena.de.

Background: Magnetic nanoparticles (MNPs) are known to be versatile tools in diagnostic and interventional radiology.[1–6] **Aim:** The motivation of this study was to assess if MNPs could be used for multiple purposes: an increased MNP loading of tumor cells in vitro piloted by an external magnetic field (magnetic targeting) in combination with cell inactivation by exposure to an alternating magnetic field in order to generate heating (magnetic heating). **Methods:** Human adenocarcinoma cells (0.5 to 5 $\times$ 10^7 BT-474 cells) were incubated with different amounts of MNPs (10 to 30 mg) while exposed to an external magnetic field gradient (1 to 24 hrs; 56 mT [magnet A] and 83 mT [magnet B] labeling, respectively; controls: cells without magnetic labeling). The combination effects of both magnetically based labeling and magnetic heating were assessed by the determination of the temperature increase (frequency, 400 kHz; amplitude, 24.6 kA/m). The amount and localization of intracellular iron were determined by atomic absorption spectrometry and electron microscopy. **Results:** An increased MNP cell uptake (eg, 122 $\pm$ 7 pg Fe/cell, 0.32 mg Fe/mL, 24 hrs, 5 $\times$ 10^6 cells during magnetic heating) due to 83 mT labeling was observed over time and cell concentration as compared with controls (eg, 100 $\pm$ 11 pg Fe/cell), showing a selective MNP accumulation within numerous tremendous endosomes in the cells. Magnetic heating of 83 mT labeled cells revealed distinct temperature elevations (eg, 28.2 $\pm$ 0.4 K) compared with controls (eg, 19.4 $\pm$ 1.4 K). Furthermore, our data showed that the magnetic induction of magnet A was insufficient to enrich a distinctly higher iron concentration within tumor cells and therefore to obtain a higher temperature rise in comparison with control B. **Conclusions:** An additive, cytotoxic effect on tumor cells in vitro in dependence of MNP concentration, incubation time, and cell number was observed due to the combination of magnetic labeling and magnetic heating. Therefore, MNPs are shown to be valuable tools for the combination of magnetically based therapy modalities in the long term.

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MULTIMODALITY IMAGING WITH 18F-FDG-PET/CT, 99MTC-MIBI, AND MAGNETIC RESONANCE IMAGING OF MULTIPLE MYELOMA. Rosa Fonti,[1] Barbara Salvatore,[2] Mario Quarantelli,[1] Cesare Sirignano,[1] Sabrina Segreto,[2] Fara Petruzziello,[2] Lucio Catalano,[2] Bruno Rotoli,[2] Silvana Del Vecchio,[2] Leonardo Pace,[2] Marco Salvatore.[2] [1]CNR-Istituto di Biostrutture e Bioimmagini; [2]University ''Federico II,'' Naples, Italy; fontir@tin.it.

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Multimodality imaging provides a significant contribution to early diagnosis, staging, follow-up, and evaluation of therapeutic response of patients with neoplastic diseases. In multiple myeloma (MM) x-ray and CT are the techniques of choice to detect osteolytic lesions and more recently the use of MRI allows direct visualization of bone marrow with high spatial resolution. In addition 18F-FDG-PET is able to detect and monitor metabolic activity of infiltrating plasma cells while 99mTc-MIBI bone marrow uptake is directly and significantly correlated with percentage of infiltrating plasma cells, as shown by our previous studies. Therefore, the aim of the present study was to compare whole-body 18F-FDG-PET/CT, whole-body 99mTc-MIBI, and MRI of the spine and pelvis in a multimodality evaluation of patients with MM in order to assess the relative contribution of each imaging technique to the staging of this neoplastic disease. Thirty-three newly diagnosed patients with MM were studied. Diagnosis and staging of patients were made according to standard criteria. All patients underwent whole-body 99mTc-MIBI, whole-body 18F-FDG-PET/CT, and MRI of the spine and pelvis within 10 days and the results of these imaging studies were compared. 18F-FDG-PET/CT was positive in 32 patients: 3 had diffuse uptake and 29 showed focal lesions in the presence (13 patients) or absence (16) of diffuse uptake. Whole-body 99mTc-MIBI resulted positive in 30 patients: 11 presented diffuse uptake and 19 focal uptake with (13) or without (6) association of diffuse uptake. MRI of the spine and pelvis was positive in 27 patients: 13 had a diffuse pattern and 14 a focal pattern in combination with a diffuse pattern (8) or alone (6). 18F-FDG-PET/CT showed a total of 196 focal lesions (178 in the bones and 18 in the soft tissues) of which 121 were in districts other than the spine and pelvis whereas 99mTc-MIBI visualized 63 focal lesions (60 in the bones and 3 in the soft tissues) of which 53 were in districts other than the spine and pelvis. In the spine and pelvis, 18F-FDG-PET/CT detected 75 focal lesions (35 in the spine and 40 in the pelvis), 99mTc-MIBI visualized 10 focal lesions (1 in the spine and 9 in the pelvis), and MRI detected 51 focal lesions (40 in the spine and 11 in the pelvis). In conclusion 18F-FDG-PET/CT proved to be more sensitive than 99mTc-MIBI and MRI in the detection of focal lesions. Although 18F-FDG-PET/CT and 99mTc-MIBI were more panoramic than MRI, MRI should be preferred for the detection of focal lesions in the spine.

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COMPARISON OF CARCINOEMBRYONIC ANTIGEN AND FLUORODEOXYGLUCOSE-18 POSITRON EMISSION TOMOGRAPHY/COMPUTED TOMOGRAPHY IN THE POSTOPERATIVE EVALUATION OF LOCAL AND DISTANT METASTASIS IN COLORECTAL CANCER. Kazuo Itoh,[1] Takaya Kusumi,[2] Masao Hosokawa.[2] [1]Radiological Imaging Center, Keiyu-kai Sapporo Hospital, Sapporo, Japan; [2]Department of Gastroenterological Surgery, Keiyu-kai Sapporo Hospital, Sapporo, Japan; contact e-mail: kaito55@keiyukaisapporo.or.jp.

Early and correct diagnosis of local and distant metastasis after the curative operation of colorectal cancer is very important in the treatment of a patient. To evaluate the diagnostic accuracy of CEA and FDG-18 PET/CT, 97 patients who were followed with known or suspected metastasis after the operation were enrolled in this study. 125 MBq to 345 MBq (median = 235 MBq) of commercially available FDG-18 (Nihon Medi-Physics Co., Japan) was administered to a patient fasted for at least 4 hours. Attenuation-corrected PET/CT (Gemini GXL, Philips Co.) imaging was performed in 3-D acquisition mode with 2 minutes per bed from the top of the skull to the upper thighs 60 minutes after injection of the tracer. Increased focal accumulation was evaluated as positive. The final diagnosis of local and distant metastasis was established by histopathological findings and clinical follow-up longer than 6 months by CT and MRI. CEA less than 5 ng/mL is evaluated as normal. PET/CT findings in nine cases were not concordant with clinical diagnosis at the time of the test. There were three false positives (early stage after partial hepatectomy in two, surgical scar in one, and pneumonia in one), four true positives (lymph node metastasis in three and local recurrence in one), and one true negative (multiple small lung nodules). Sensitivity and specificity of CEA and PET/CT were 72.4% and 100%, and 64.1% and 89.7%, respectively. FDG-18 PET/CT was significantly superior to CEA in the evaluation of local and distant metastasis after the curative operation.

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MULTIMODALITY IMAGING TO STUDY THE ROLE OF MT4 MEMBRANE-TYPE MATRIX METALLOPROTEINASE IN BREAST METASTASIS. Stephanie Ricaud,[1] Carine Pestourie,[1] Karine Gombert,[1] Chabottaux Vincent,[2] Agnès Noël,[2] Bertrand Tavitian,[1] Frédéric Ducongé.[1] [1]CEA, DSV, I2BM, SHFJ, Laboratoire d'Imagerie Moléculaire Expérimentale, INSERM U803, Orsay, France; [2]Laboratory of Tumor and Development Biology, Centre de Recherche en Cancérologie Expérimentale, Center for Biomedical Integrative Genoproteomics, University of Liège, Liège, Belgique; contact e-mail: stephanie.ricaud@cea.fr.

Human breast epithelial cancer expresses high levels of MT4-MMP (membrane-type matrix metalloproteinase) protein in comparison with normal breast tissue. In vitro studies showed that overexpression of MT4-MMP does not affect cell proliferation or angiogenesis of the MDA-MB-231 breast cancer cells. However, the stable expression of MT4-MMP promotes tumor growth and lung metastasis when this cell line is xenografted to nude mice. To elucidate this discrepancy between in vitro and in vivo results we followed tumor progression using in vivo multimodality imaging (nuclear and optical). Using ^{18}fluorodeoxyglucose PET imaging, we followed the growth of MDA-MB-231 xenograft. Due to high uptake in the kidneys and heart we decided to monitor metastasis by optical imaging. We stably transfected MDA-MB-231 cells that overexpress or not MT4-MMP with eGFP and luciferase gene reporters. Cell invasion in lung and lymph nodes was imaged using eGFP- and luciferase-expressing cells with fibered confocal fluorescence microscopy and bioluminescence imaging, respectively. Lymph node invasion could be observed for both MT4-MMP-expressing cells and control, whereas only MT4-MMP-expressing cells had metastasized to the lung 53 days after cell transplantation in mice. These preliminary results show that MT4-MMP overexpression in breast cancer cells has no impact in lymph node invasion but influences lung metastasis. Moreover, MT4-MMP-expressing cells induced a strong difference in vasculature architecture in vivo using fibered confocal fluorescence microscopy after injection of FITC-dextran. Altogether this work will help to better understand the crosstalk between metastasis and MT4 expression.

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IN VIVO BIODISTRIBUTION OF D4 APTAMER IN NUDE MICE BEARING RETC634Y-EXPRESSING TUMOR XENOGRAFTS USING MULTIMODALITY OPTICAL IMAGING. Carine Pestourie,[1] Karine Gombert,[1] Benoît Theze,[1] Laura Cerchia,[2] Vittorio De Franscicis,[2] Bertrand Tavitian,[1] Frédéric Ducongé.[3] [1]CEA, DSV, I2BM, SHFJ, Laboratoire d'imagerie moléculaire expérimentale; INSERM U803, Orsay, France; [2]Instituto per l'Endocrinologia e l'Oncologia Sperimentale del Consiglio Nazionale delle Ricerche G. Salvatore, Naples, Italy; [3]CEA, DSV, I2BM, SHFJ, Laboratoire d'imagerie moléculaire expérimentale; INSERM U803, Orsay, France; contact e-mail: carine.pestourie@cea.fr.

Aptamers are nucleic acid ligands selected by an iterative selection procedure named SELEX. We validated a whole-living cell SELEX protocol to target the transmembrane receptor tyrosine kinase (RTK) RET (REarranged during Transfection) in its natural environment. RET is mutated in multiple endocrine neoplasia type 2A and 2B syndromes and in familial medullary thyroid carcinoma. The C634Y mutation in the extracellular domain causes constitutive activation of the receptor. We isolated one aptamer, named D4, which binds specifically to RET and blocks RET dimerization-dependent signaling pathways induced either by GDNF or by the C634Y activating mutation. We are now evaluating the aptamer D4 and some derivatives for in vivo molecular imaging of tumors expressing RET. We used nude mice bearing RETC634Y-expressing tumor xenograft of mouse fibroblast NIH3T3 cell lines: NIH3T3/MEN2A. As a first approach, we labeled the D4 aptamer and a scramble sequence control with Alexafluor680 and measured biodistribution using the whole-body fluorescence camera PhotoImager. First experiments demonstrate that a low but specific tumor uptake could be observed for D4 whereas no signal could be observed for the control. Using fibered confocal fluorescence microscopy (CellVizio488), we could see that this uptake is restricted to some specific cell types inside the tumor. We hope that this aptamer could serve as a platform for molecular derivation that could be a test to improve the pharmacokinetic parameters of aptamers.

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FLUORODEOXYGLUCOSE-POSITRON EMISSION TOMOGRAPHY/COMPUTED TOMOGRAPHY: DOES THE SEMIQUANTITATIVE PARAMETER SUVMAX HAVE SIGNIFICANCE AS AN OUTCOME PREDICTOR IN PATIENTS WITH NEWLY DIAGNOSED STAGE I–II NON–SMALL CELL LUNG CANCER? Vincenzo Arena,[1] Silvia Novello,[2] Andrea Skanjeti,[1] Giovanni Selvaggi,[2] Anastasios Douroukas,[1] Andrea Bille',[2] Matteo Giaj Levra,[2] Francesca Giunta,[1] Marina Longo,[2] Giorgio Scagliotti,[2] Ettore Pelosi.[1] [1]Centro PET IRMET, Torino, Italy; [2]Università di Torino, Dipartimento di Scienze Cliniche e Biologiche, ASO S. Luigi, Orbassano, Torino, Italy; contact e-mail: v.arena@irmet.com.

Purpose: Malignant tumors are characterized by an increase in glucose metabolism, a cellular aspect that is detected and can be measured with FDG-PET. The aim of our study was to evaluate the prognostic value of SUVmax and diameter of the primary pulmonary lesion in a patient with newly diagnosed stage I–II NSCLC. **Methods:** Forty-three patients (mean age: 67.3) with newly diagnosed NSCLC were included in this study. All patients underwent an FDG-PET/CT scan as part of their initial staging work-up. SUVmax and diameter of the primitive lesion were calculated for all patients; patients were followed up for at least 9 months. For statistical analysis, we divided the patients in two major groups depending on the presence of disease recurrence.

The relationship between SUVmax values and diameter versus disease recurrence in the two groups was sought using the Student *t*-test. **Results:** Thirty-one patients were disease free after a mean follow-up period of 16 months, while 13 presented a disease recurrence (disease-free survival time, mean 10.6 months). SUVmax mean values in the two groups were 8.96 (SD: 5.26) and 12.8 (SD: 5,3), respectively (p = .038, Student *t*-test). Lesion dimension mean values were 31.27 (SD: 18.65) and 40.5 (SD: 9.4) (p = .11, NS, Student *t*-test). **Conclusions:** In this population of patients with stage I–II NSCLC, we found a statistically significant relationship between SUVmax values and disease recurrence.

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EARLY DETECTION OF THERAPEUTIC RESPONSE BY 1H MAGNETIC RESONANCE SPECTROSCOPY/MAGNETIC RESONANCE IMAGING IN MOUSE XENOGRAFTS OF DIFFUSE LARGE B-CELL LYMPHOMA TREATED WITH CHOP CHEMOTHERAPY. Ming Q. Huang, Seung-Cheol Leeper, David S. Nelson, Steven Pickup, Rong Zhou, Harish Poptani, E. James Delikatny, Jerry D. Glickson. Molecular Imaging Laboratory, Department of Radiology, University of Pennsylvania, Philadelphia, PA, USA; contact e-mail: huangmq@mail.med.upenn.edu.

Introduction: Our laboratory has been exploring the use of a number of 1H MRS and MRI indices of response in xenograft models and in the clinic. **Methods:** Human diffuse, large B-cell lymphoma cells were implanted in female SCID mice that were treated with CHOP (three cycles) or CHOP plus bryostatin 1 (CHOPB; four cycles). Bryostatin inhibits multidrug resistance (mdr1 gene). NMR experiments were performed with 9.4 T (MRS) and 4.7 T (MRI) Varian spectrometers utilizing STEAM to localize choline and lipid, selective multiple-quantum coherence transfer to edit lactate, ISIS to localize 31P MRS, and conventional diffusion-weighted and T2-weighted MRI. **Results:** Tumor volumes in the CHOPB-treated animals decreased to about 60% of the pretreatment values; tumor volumes of CHOP-treated animals stabilized, and those of controls increased monotonically. The lactate and TCho levels (1H MRS) and PME/bNTP ratios (31P MRS) of CHOPB-treated animals decreased significantly after the first cycle of chemotherapy (1 week); corresponding parameters of untreated control tumors increased slightly. CHOP produced a significant decrease only in lactate after one cycle of therapy, which correlated with a decrease in Ki-67 assay of tumor proliferation. CHOPB treatment produced significant decreases in average ADC and T2 after one and two cycles, respectively. Most importantly, there was clear evidence that response was localized in a well-defined region of the tumor. **Conclusions:** We have demonstrated that lactate and tCho can be used as early 1H MRS response indicators. The MRI methods detect regional response and could be used to guide multimodal therapy.

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[68]GA-DOTA-PEPTIDES TARGETING MATRIX METALLOPROTEINASE 9 IN MELANOMA XENOGRAFTS. Tiina Anneli Pöyhönen,[1] Merja Huttunen,[2] Anu Autio,[1] Pauliina Virsu,[1] Satu Salomäki,[3] Ian Wilson,[4] Mathias Bergman,[2] Anne Roivainen.[1] [1]Turku PET Centre, Turku University Hospital, Turku, Finland; [2]Karyon-CTT Ltd, Helsinki, Finland; [3]Turku PET Centre, Turku University Hospital, and Department of Chemistry, University of Turku, Turku, Finland; [4]Turku Imanet, Itäinen Pitkäkatu 4B, Turku, Finland; contact e-mail: tiina.poyhonen@utu.fi.

Aim: Since matrix metalloproteinase 9 (MMP-9) is overexpressed in most tumor types and is associated with tumor growth, metastasis, and angiogenesis, it may be a suitable target for the development of tumor-specific imaging agents. **Materials and Methods:** MMP-9 targeting peptides [68]Ga-DOTA-TCTP-1(cystine bridge), [68]Ga-DOTA-lactam-TCTP-1 (lactam bridge), and linear control [68]Ga-DOTA-lin-TCTP-1 were preclinically evaluated. Stability and protein binding were studied in vitro. Human melanoma cells SC implanted into athymic mice and rats were allowed to develop tumors for 1 to 4 weeks. Biodistribution and biokinetics of IV administered [68]Ga-DOTA-peptides were evaluated by ex vivo radioactivity measurements of excised organs at different time points and in vivo by PET imaging. **Results:** The half-lives of [68]Ga-DOTA-TCTP-1, [68]Ga-DOTA-lactam-TCTP-1, and [68]Ga-DOTA-lin-TCTP-1 in human plasma were 2.5 h, $>$ 4 h, and 1 h, respectively. Approximately 35% of [68]Ga-DOTA-peptides bound to plasma proteins. Ex vivo biodistribution studies in rats showed tumor-to-muscle ratio of 5.5 $\pm$ 1.3 (mean $\pm$ SD, $n = 3$) for [68]Ga-DOTA-TCTP-1, 3.2 $\pm$ 0.2 for [68]Ga-DOTA-lactam-TCTP-1, 3.2 $\pm$ 0.6 for [68]Ga-DOTA-lin-TCTP-1 at 2 h after injection. In mice the ratios were 6.2 $\pm$ 3.2 for [68]Ga-DOTA-TCTP-1 and 2.3 $\pm$ 1.7 for [68]Ga-DOTA-lin-TCTP-1 at 2 h. The most promising was [68]Ga-DOTA-TCTP-1; PET imaging of melanoma xenografts in rats showed prolonged presence in the tumors up to 2 h. All [68]Ga-DOTA-TCTP peptides were rapidly cleared via the liver and the kidneys. **Conclusions:** [68]Ga-DOTA-TCTP-1 is suitable for imaging of tumors overexpressing MMP-9, like melanoma. Due to lower accumulation of the lactam compound in tumors further optimization has to be done on in vivo half-life versus affinity.

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IMAGING GENE THERAPY OF GLIOMA WITH LENTIVIRAL VECTORS. Hrvoje Miletic,[1] Yvonne Fischer,[2] Tsanan Giroglou,[2] Maria Adele Rueger,[3] Alexandra Winkeler,[3] Hongfeng Li,[3] Uwe Himmelreich,[4] Werner Stenzel,[5] Andreas H. Jacobs,[3] Dorothee von Laer.[2] [1]Department of Biomedicine, University of Bergen, Bergen, Norway; [2]Georg-Speyer-Haus, Frankfurt am Main, Germany; [3]Labor für Gentherapie und Molekulares Imaging, Max-Planck-Institut für Neurologische Forschung, Universität zu Köln, Köln, Germany; [4]In-vivo NMR Laboratory, Max-Planck-Institute for Neurological Research with Klaus-Joachim-Zülch-Laboratories of the Max Planck Society and the Faculty of Medicine of the University of Cologne, Cologne, Germany; [5]Abteilung für Neuropathologie, Universität zu Köln, Köln, Germany; contact e-mail: hrvoje_miletic@gmx.de.

Lentiviral vectors pseudotyped with glycoproteins of the lymphocytic choriomeningitis virus (LCMV-GP) are promising candidates for gene therapy of malignant glioma as they specifically and efficiently transduce glioma cells in vitro and in vivo. Here, we tested the therapeutic efficacy of LCMV-GP pseudotyped lentiviral vectors for DsRed-modified (9LDsRed) gliomas using the suicide gene thymidine kinase of the herpes simplex virus type 1 (HSV-1-tk). LCMV-GP pseudotypes mediated a successful eradication of 9LDsred tumors with 100% of long-term survivors. Before initiation of ganciclovir (GC) treatment, a strong HSV-1-tk expression within the tumor was detected by noninvasive positron emission tomography (PET) using the tracer [18F]FHBG. Therapeutic outcome was monitored by MRI and PET imaging and correlated with the histopathological data. In conclusion, suicide gene transfer using pseudotyped lentiviral vectors was very effective in the treatment of rat glioma and is therefore an attractive therapeutic strategy also in human glioblastoma especially in conjunction with an imaging-guided approach.

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SENSITIZATION OF HUMAN MELANOMAS TO CHEMOTHERAPY BY SELECTIVE METABOLIC ACIDIFICATION. Rong Zhou,[1] Lin Z. Li,[1] David S. Nelson,[1] Dennis B. Leeper,[2] Jerry D. Glickson.[1] [1]Molecular Imaging Laboratory, Department of Radiology, University of Pennsylvania, Philadelphia, PA, USA; [2]Department of Radiation Oncology, Thomas Jefferson University, Philadelphia, PA, USA; contact e-mail: rongzhou@mail.med.upenn.edu.

Introduction: There is currently no effective systemic chemotherapy for melanoma. We have recently demonstrated that melanoma xenografts in SCID mice can be selectively acidified by hyperglycemia, blockage of oxidative phosphorylation with meta-iodobenzlguanidine (MIBG), and inhibition of the monocarboxylic transporter (MCT) with 1-cyano-4-hydroxycinnamic acid (CNCn) (Zhou. Cancer Res 2000). We now use this procedure to selectively sensitize these tumors to cisplatin, an agent that is activated by acidification. **Methods:** Age- and weight-matched athymic nude mice ($\approx$25 g) were implanted with 10**6 early passage human DB1 melanoma cells SC in the flank and studied when tumor volumes reached 100 to 300 µL. Cohorts were either sham-treated with saline and DMSO, cisplatin (7.5 mg/kg, IV), metabolic acidification, or metabolic acidification plus cisplatin. Metabolic acidification was achieved by continuous infusion of glucose to maintain plasma glucose at 26 $\pm$ 1 mM and treatment with MIBG (22.5 mg/kg IP) and CNCn (150 mg/kg, IP). Anesthesia was maintained with ketamine-acepromazine (55-5 mg/kg, IP). **Results:** Cohorts 1 to 3 exhibited identical growth curves; cohort 4 (treated with glucose, MIBG, CNCn, and cisplatin) showed an 8-day growth delay, $p = .02$. Previous studies have demonstrated that the intracellular pH (pHi) in the tumor is decreased from $\approx$7.0 to 6.2 to 6.3 by the metabolic acidification procedure; the pHi of liver, muscle, and brain was unaffected. **Conclusions:** The experiment demonstrates the potential feasibility of systemic chemotherapy of melanoma with cisplatin following selective metabolic acidification of the tumor. Further studies are in progress to optimize the protocol, evaluate toxicity, and implement these principles in the clinic.

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PREDICTING TUMOR METASTATIC POTENTIAL ON THE BASIS OF DYNAMIC CONTRAST-ENHANCED AND T1RHO MAGNETIC RESONANCE IMAGING AND OPTICAL REDOX RATIO. Lin Z. Li,[1] Rong Zhou,[1] Lily Moon,[1] He N. Xu,[1] Tuoxiu Zhong,[2] Hui Qiao,[1] Mary J. Hendrix,[3] Dennis B. Leeper,[4] Britton Chance,[2] Jerry D. Glickson.[1] [1]Molecular Imaging Laboratory, Department of Radiology, University of Pennsylvania, Philadelphia, PA, USA; [2]Johnson Research Foundation, Department of Biophysics and Biochemistry, University of Pennsylvania, Philadelphia, PA, USA; [3]Children's Memorial Research Center, Northwestern University, Evanston, IL, USA; [4]Department of Radiation Oncology, Thomas Jefferson University, Philadelphia, PA, USA; contact e-mail: linli@mail.mmrrcc.upenn.edu.

Introduction: Development of imaging methods for prediction of tumor metastatic potential would greatly facilitate the clinical management of melanoma. Human melanoma xenografts with well-defined metastatic and invasive potential were grown in nude mice and compared for significant differences by MRI and low-temperature surface fluorescence imaging. **Subjects and Methods:** MRI experiments were performed on a 4.7 T/50 cm Varian spectrometer. Dynamic contrast-enhanced (DCE) MRI was implemented with simultaneous determination of the arterial-input function by cardiac gated imaging of the left ventricle and analysis by the BOLERO method. T1rho-weighted imaging utilized a fast spin-echo sequence. Cryoimaging of tumors surgically excised after rapid freezing was performed by the method of Quistorff and Chance (1985). **Results:** The DCE experiments indicated significant differences in K-trans between the centers of the most aggressive and most indolent melanomas and between the centers and the rims of the most aggressive melanoma. 1/T1rho was significantly higher in the aggressive tumors ($n = 6$) than in the indolent tumors ($n = 6$), $p < .01$. Aggressive melanomas exhibited significant increases in the mitochondrial redox ratios of the center versus the rim of the same tumor, whereas the indolent tumors exhibited a reduced redox ratio. The relative areas under the oxidized redox regions of tumor histograms increased linearly with the invasive potential. **Discussion/Conclusions:** These studies demonstrate three potential methods for clinically distinguishing aggressive and indolent melanomas: DCE MRI, T1rho MRI, and cryofluorescence imaging of biopsy specimens. Tumor invasiveness is clearly linked to tumor mitochondrial metabolism.

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OPTICAL IMAGING OF ANGIOGENESIS MARKERS IN AN IN VITRO MODEL FOR SENESCENT AND PROLIFERATING ENDOTHELIAL CELLS. Tibor Vag, Werner A Kaiser, Ingrid Hilger. Institute of Diagnostic and Interventional Radiology, University Hospital Jena, Jena, Germany; contact e-mail: Tibor.Vag@med.uni-jena.de.

Objective: The design of specific contrast agents for molecular imaging of angiogenesis requires the availability of adequate in vitro models. In this context, we propose an in vitro cell model for senescent and proliferating endothelial cells mimicking both physiological and angiogenic vasculature. **Materials and Methods:** Human umbilical vein endothelial cells were cultured using different conditions to establish a proliferating and a senescent cell subset. Proliferation rate and morphology were assessed by determination of cellular dehydrogenases and light microscopy. Presence of the pan-endothelial marker von Willebrand factor (vWF) and selected angiogenic markers CD105, VEGFR2, TEM7 on the cell surface were evaluated using flow cytometry and gene expression using reverse-transcription PCR. Visualization of surface ligands was performed after specific labeling with fluorophores using a near-infrared fluorescence (NIRF) imager. **Results:** Proliferation assay confirmed distinct cell proliferation and no proliferation respectively in the two culture subsets. Light microscopy revealed a tubular alignment of the senescent cells, which was absent in proliferating cells. Flow cytometry demonstrated the presence of vWF on both subsets and an upregulation of CD105 but not of VEGFR2 and TEM7 on proliferating endothelial cells. While CD105 and VEGFR2 gene expression was detectable both in proliferating and in senescent cells, TEM7 was not expressed in any of the subsets. NIRF imaging revealed the highest fluorescence signal for CD105 in proliferating endothelial cells. No relevant signal was observed in VEGFR2 and TEM7. **Conclusion:** The proposed cell model was verified on morphological, genetic, and protein levels and might, in principle, be useful in future angiogenesis applications, like evaluating new fluorophores and other contrast media.

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INFRARED-EXCITED MULTIPHOTON MICROSCOPY: SYSTEM DESIGN AND APPLICATION IN PRECLINICAL CANCER RESEARCH. Volker Andresen,[1] Wolfgang-Moritz Heupel,[2] Gudrun Kohl,[3] Ingo Rimke,[4] Gregory Harms,[2] Robert M. Hoffman,[5] Edward Geissler,[3] Peter Friedl.[2] [1]LaVision BioTec GmbH, Bielefeld, Germany; [2]University of Würzburg, Rudolf-Virchow Center for Experimental Biomedicine and Department of Dermatology, Würzburg, Germany; [3]Institute for Experimental Surgery, University of Regensburg, Regensburg, Germany; [4]APE GmbH, Berlin, Germany; [5]Department of Surgery, University of California-San Diego, San Diego, CA, USA; contact e-mail: peter.fr@mail.uni-wuerzburg.de.

Multiphoton microscopy has defined standards for 3D fluorescence and higher harmonic generation analysis of cells and tissue structures in vitro and in vivo. We here extend conventional two-photon excited biomedical imaging by using a tuneable optical parametric oscillator emitting laser light in the range of 1,060–1,500 nm for live cancer imaging. Infrared-excited two-photon microscopy above 1 micron allowed multifold enhanced excitation efficiency of red fluorophores (eg, RFP) and second harmonic generation at submicron optical resolution, supported 80 to 100% deeper tissue penetration, and reduced phototoxicity and photobleaching by 80 to 95%, compared to excitation below 1 micron. Due to enhanced tissue penetration deep tumor microenvironments became accessible by intravital microscopy, revealing subregions that support high-frequency cancer cell exit via blood vessels. Due to minimized phototoxicity, 4 h intravital time-lapse microscopy and cell tracking revealed the collective invasion of cell masses together with high-frequency proliferation, thus directly visualizing invasive growth at cellular and subcellular resolution. In conclusion, infrared-shifted two-photon excitation

allows high-penetration live histopathology for the reconstruction of cancer progression and will enhance monitoring of anticancer therapy.

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FLUOROMETHYLCHOLINE-(18F) POSITRON EMISSION TOMOGRAPHY/COMPUTED TOMOGRAPHY MOLECULAR IMAGING FOR LOCALIZATION OF INTRAPROSTATIC CANCER LESIONS; IMPROVEMENT BY MEANS OF FACTOR ANALYSIS. Sona Balogova, Nacer Kerrouche, Khaldoun Kerrou, Virgine Huchet, Jean-Noel Talbot. Hopital Tenon; contact e-mail: sona.balogova@kapor.sk.

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Objectives: In many patients with high PSA serum levels, multiple prostate biopsies are unable to localize the probable cancer. In this context, fluoromethylcholine-(18F) (FCH) positron emission tomography/computed tomography (PET/CT) has been proposed to guide further biopsies. However, inflammation and benign hyperplasia result in a nonspecific FCH uptake that jeopardizes an accurate detection of the most active cancer lesions. We made the hypothesis that processing FCH PET/CT images with FA may improve the localization of the carcinoma tissue inside the prostate. In a prospective study, the blinded readings of images obtained by means of the manufacturer's reconstruction software and images processed through FA were compared to histology, the ''standard of truth.'' **Methods:** The data of prostate histology were available in eight patients enrolled in a prospective study of FCH PET/CT in the pretreatment work-up of their prostate cancer. Just after injection of 4 MBq/kg of body weight of FCH (Iasocholine, Iason Laboratory), a dynamic acquisition was started over the pelvic area on a PET/CT machine (Gemini, Philips) during 8 min at a one frame per minute rate; a whole-body scan was subsequently acquired. FA was performed on the eight images of the dynamic acquisition. Two independent factors were extracted to explain the common variance in the data set. It was assumed that cancer tissue has constant accumulation kinetics throughout the acquisition period, as it takes up FCH very early after injection; the urinary tract kinetics were supposed nought for the first 3 to 4 minutes and then very high for the rest of the acquisition time. The series of eight dynamic images reconstructed according to the manufacturer's procedure and the parametric images obtained using FA were presented in a random order and blind-read by the same nuclear medicine physician, during one single session. The localized or diffuse character of FCH prostate uptake and the topography of the most active lesions were noted, both for the ''raw'' images and the FA images. **Results:** In all cases, the first factor actually matched the anatomical landmarks of the urinary tract and thus the second factor was expected to delineate the cancer tissue; they respectively explained around 80% and 20% of the total variance. Confrontation with histology revealed that FA permitted in three cases (38%) to localize focal lesions of active cancer that could not be identified on ''raw'' images. **Conclusion:** FA was able to improve the localization of intraprostatic cancer lesions by visual interpretation in a significant number of patients with a known prostate cancer, in this preliminary study. FCH PET/CT with FA could therefore be useful to guide biopsies in patients suspicious for prostate cancer.

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INTRAOPERATIVE FLUORESCENCE REFLECTANCE IMAGER. Nicolas Laustriat, Michel Berger, Philippe Peltié, Philippe Rizo. CEA; contact e-mail: nicolas.laustriat@cea.fr.

In this presentation we describe a new handheld intraoperative reflectance fluorescence imager. This device is aimed at being used during a surgical operation in order to ease the detection and exerese of cancerous nodules. The patient must have been previously intravenously injected with a fluorescent probe such as CEA (carcinoembryonic antigen) coupled to a specific fluorochrome (derived from indocyanine green) in case of colon cancer. This imager excites and detects the exogenous fluorescence, which is emitted by the fluorescent molecules attached to cancerous cells. The intraoperative setup is composed of two 690 nm fibred lasers, which are scattered in order to uniformly illuminate the field of examination at a distance of 20 cm from the exit of the device. A filtered CCD camera records the fluorescence image, which is displayed on a dedicated screen. The display screen shows an overlay of a white light image and of the fluorescence image. Preclinical tests obtained with this setup on mice and rat will be presented in order to assess for the capabilities of this system in terms of sensitivity and functionalities.

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A NOVEL SERIES OF NITROMIDAZOLE PROBES FOR PO2 MEASUREMENTS BY 1H MAGNETIC RESONANCE SPECTROSCOPY. Jesús Pacheco-Torres,[1] Pilar López-Larrubia,[1] Paloma Ballesteros,[2] Sebastián Cerdán.[1] [1]Instituto de Investigaciones Biomédicas ''Alberto Sols,'' CSIC, Madrid, Spain; [2]Instituto Universitario de Investigación - UNED, Madrid, Spain; contact e-mail: jpacheco@iib.uam.es.

Hypoxia is known to be an important physiological parameter determining tumor progression and malignancy. Consequently, a variety of methods have been developed to measure tumor oxygenation. In this study we report the use of a novel series of nitroimidazoles for the measurement of oxygen tension in preparations of C6 astrocytoma cells and the kinetics of transformation of these probes under normoxic or hypoxic conditions. Several nitroimidazolyl derivatives were synthesized by Michael addition of the corresponding nitromidazol to the appropriate acceptor. Of these, we report here the evaluation of dimethyl 2-(2-nitroimidazol-1-yl)succinate (JP1) only. The NADPH:cytochrome P450 reductase and xanthine/xanthine oxidase (XOD) enzymatic systems were used to measure in vitro reduction of hypoxia marker under anoxic and normoxic conditions. Only the P450 system was found to reduce these compounds at a rate comparable to other nitroimidazolyl probes in the absence of oxygen. Incubation of JP1 with C6 cells under different oxygen concentrations depicted clearly visible changes in the 1H-NMR spectrum at the probe, which depended on the degree of hypoxia. We analyzed the kinetics of JP1 transformation by C6 cells under normoxic or hypoxic incubation conditions with two different mathematical models. Model 1 involved a sequential transformation of the parental probe JP1 into an intermediate (JP11) and a final NMR invisible adduct (JP12). Model 2 involved the simultaneous transformation of JP1 into two final products, JP11 and JP12, only one of them being NMR invisible. Our results indicate that JP1 is reduced to JP12 through a mechanism compatible with model 1.

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DUAL-PHASE FLUORODEOXYGLUCOSE–POSITRON EMISSION TOMOGRAPHY: DELAYED ACQUISITION IMPROVE THE HEPATIC DETECTABILITY OF PATHOLOGICAL UPTAKE. Anastasios Douroukas,[1] Andrea Skanjeti,[1] Vincenzo Arena,[1] Roberta Casoni,[2] Ettore Pelosi.[1] [1]Centro PET IRMET, Torino, Italy; [2]SCDU Medicina Nucleare, ASO S. Giovanni Battista, Torino, Italy; contact e-mail: douroukas@tiscali.it.

Introduction: It is recognized that FDG-PET sensitivity at the liver level is low in cases of lesion diameter that are less than 1.5 cm, due to the high background values and the respiratory motion. The aim of this study was to evaluate if the acquisition of delayed images could improve the detectability of liver pathological uptakes. **Methods:** Ninety-five consecutive patients with suspected liver metastases underwent a dual-phase PET scan. All patients underwent a whole-body PET/CT scan (PET-1), which was acquired 1 hour post–FDG injection, and a liver PET/CT scan (that is one or two fields of view of the upper abdomen; PET-2), which was acquired 2 hours post-injection. In all cases, the image reconstruction was performed as 3D reconstruction algorithm FORE-Iterative, FOV 50 cm, image matrix size 128 × 128. Both studies were evaluated qualitatively and semiquantitatively (background SUVmean of the liver, lesion SUVmax and mean, and ratio lesion/background). **Results:** Thirty-seven of the 95 included patients (38.9%) presented liver lesions at both PET-1 and PET-2 exams, whereas 2 (2.2%) only at PET-2. Eighty-one liver lesions were identified at both PET studies, whereas nine (11.1%) only at PET-2. Furthermore, at PET-2, we had a statistically significant reduction of the SUVmean background values ($p < .001$, Wilcoxon test) and a concomitant increase in the SUVmean lesion values ($p < .001$, Wilcoxon test), the ratio lesion to background ($p < .001$, Wilcoxon test). **Conclusions:** The acquisition of delayed images of the liver parenchyma improves the hepatic detection of pathological uptakes.

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DEVELOPMENT OF AN IN VITRO PROTOCOL FOR THE TREATMENT OF CANCER CELLS BASED ON THEIR DIFFERENT P53 STATUS. Sebastian Schindler, Susanne Gruener, Gunter Wolf, Sergey V. Tokalov, Nasreddin D. Abolmaali. OncoRay - Center for Radiation Research in Oncology, Medical Faculty Carl Gustav Carus, TU Dresden, Dresden, Germany; contact e-mail: sebastian.schindler@oncoray.de.

The loss of p53 function is responsible for increased aggressiveness of cancers; at the same time it could be exploited therapeutically to selectively kill p53-deficient ($p53^{-/-}$) cancer cells and to protect p53 wild-type cells (p53wt) cells. The aim of this study was to develop a preliminary in vitro protocol of treatment that protects p53wt cells from radiation whereas making $p53^{-/-}$ cells more vulnerable by arresting them in radiosensitive metaphase. Two NSCLC lines A549 (p53wt) and H1299 ($p53^{-/-}$) and a SCC (FaDu, $p53^{-/-}$) were used, which were treated with chemical compounds (Taxol, cisplatin, doxorubicin, roscovitine) and irradiated with x-rays. Also the in vivo growth of these tumor cell lines was proofed using a xenograft nude rat model by magnetic resonance imaging (MRI). Two treatment protocols were developed: first the administration of the chemical compounds and irradiation with 2 Gy together and second the irradiation 24 h after the administration of cisplatin or doxorubicin/roscovitine and Taxol. After treatment the cells were analyzed by flow cytometry using propidium iodide (PI) for cell cycle analysis and carboxyfluorescein diacetate succinimidyl ester (CFSE) to separate protected and blocked cells. In both protocols, the applied chemical compounds protected p53wt but not $p53^{-/-}$ cells from radiation. When irradiation was administered 24 h after chemical treatment, a significantly higher proportion of $p53^{-/-}$ cells were in radiosensitive metaphase in comparison to the other protocol. In conclusion, the lack of p53wt in cancer cells could be exploited for therapeutic advantage by selectively killing $p53^{-/-}$ cells using defined succession of radiation and two or more drugs.

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FLUORODEOXYGLUCOSE POSITRON EMISSION TOMOGRAPHY/COMPUTED TOMOGRAPHY: EARLY EVALUATION OF CHEMOTHERAPY IN HODGKIN'S AND NON-HODGKIN'S LYMPHOMAS. Manuela Racca,[1] Valeria Pirro,[1] Paola Scapoli,[1] Teresio Varetto,[1] Marilena Bellò,[1] Umberto Vitolo,[2] Ettore Pelosi,[3] Gianni Bisi.[1] [1]SCDU Medicina Nucleare, ASO San Giovanni Battista, Torino, Italy; [2]SCDO Ematologia, ASO San Giovanni Battista, Torino, Italy; [3]Centro PET Irmet, Torino, Italy; contact e-mail: manuelaracca@gmail.com.

Aim: The use of positron emission tomography with 18F-fluorodeoxyglucose (FDG PET/CT) for evaluation of Hodgkin's lymphoma (HL) and aggressive non-Hodgkin's lymphoma (NHL) has increased during the last few years. However, the use and the timing of FDG PET/CT in the assessment of chemotherapy response are still under debate. Is early scan during treatment predictive of the final response to therapy? **Materials and Methods:** Ninety-one consecutive patients were staged by FDG PET/CT (PET1) for HD ($n = 57$) and aggressive NHL ($n = 34$). All of them underwent interim PET/CT (PET2) after two to four cycles of chemotherapy and final restaging (PET3) at the end of the treatment. **Results:** In the HL group 43/57 patients resulted disease-free at PET2: 41 still had complete response, while 2 had a positive scan for disease relapse at PET3. The remaining 14 patients had partial response at PET2: 8 still had partial response and 6 had a complete response at PET3. In the NHL group 23/34 resulted disease-free at PET2: all of them still had complete response at PET3. The remaining 11 patients had partial response to treatment at PET2: 7 of them still had partial response and 4 had complete response at PET3. The negative predictive value of PET2 resulted 100% in NHL and 95% in HL patients. **Conclusions:** Our preliminary data suggest that FDG PET/CT scan seems to be a powerful imaging modality in early identifying nonresponder patients and in optimizing therapeutic management.

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CLINICAL AND PATHOLOGICAL PARAMETERS CORRELATING WITH SUVMAX IN NON–SMALL CELL LUNG CANCER. Andrea Skanjeti,[1] Anastasios Douroukas,[1] Andrea Billè,[2] Vincenzo Arena,[1] Francesco Ardissone,[2] Piero Borasio,[2] Ettore Pelosi.[1] [1]Centro PET IRMET, Torino, Italy; [2]Università di Torino, Dipartimento di Scienze Cliniche e Biologiche ASO S. Luigi, Orbassano, Torino, Italy; contact e-mail: askanjeti@yahoo.it.

Aim: The SUVmax measurement is considered an important prognostic index in patients with non–small cell lung cancer (NSCLC). The aim of this study was to investigate the correlation between the maximum standardized uptake value (SUVmax) of the primary lesion and different clinical and pathological parameters. **Methods:** One hundred seventeen consecutive patients with NSCLC (adenocarcinoma $n = 85$, squamous cell carcinoma $n = 32$) underwent FDG-PET/CT followed by curative surgery. SUVmax was calculated in all the primary tumor lesions and was categorized by 10 (SUV10). Clinical parameters include age and sex; histopathological parameters include histology, grading, tumor necrosis, tumor vascular invasion, tumor size, and disease stage. Univariate and multivariate statistical analyses were performed. **Results:** Mean SUVmax was 9.2 (SD = 5.1); 73 patients had an SUVmax value ≤ 10; 44 had an SUVmax value > 10. At univariate analysis, SUV10 resulted statistically correlated with age ($p = .006$), histology ($p = .0003$), tumor necrosis ($p = .03$), tumor vascular invasion ($p = .001$), and tumor dimension ($p < .0001$). At the multivariate analysis SUV10 was significantly correlated with tumor dimension ($p < .0001$), histology (squamous cell carcinoma > adenocarcinoma; $p = .004$), and vascular invasion (presence > absence; $p = .043$). **Conclusion:** SUVmax measurement has to be considered the ''weighted expression'' of several different pathological parameters (in our experience: tumor dimension, histology, and vascular invasion), thus explaining its crucial role as a prognostic index.

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SCINTIGRAPHY IMAGING OF IN VIVO DRUG BIODISTRIBUTION PREDICTS REGIONAL THERAPEUTIC EFFECTIVENESS OF A PACLITAXEL–HYALURONIC ACID BIOCONJUGATE. Maria Rondina,[1] Alessandra Banzato,[1] Elena Zangoni,[2] Laura Meléndez-Alafort,[2] Davide Renier,[3] Ulderico Mazzi,[2] Antonio Rosato.[4] [1]Department of Oncology and Surgical Sciences, University of Padova, Padova, Italy; [2]Department of Pharmaceutical Sciences, University of Padova, Padova, Italy; [3]Fidia Pharmaceuticals; [4]Department of Oncology and Surgical Sciences, University of Padova and Istituto Oncologico Veneto, IOV, Padova, Italy; contact e-mail: maria.rondina@unipd.it.

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Position-sensitive gamma-ray detectors capable of imaging gamma emitters distributed in biological organisms represent sensitive and noninvasive instruments to perform in vivo studies of transport processes or metabolic trapping of radiopharmaceuticals as well as of molecular therapeutic agents. We employed a small gamma camera, equipped with a highly segmented yttrium-aluminate perovskite (YAP) scintillator coupled to a position-sensitive photomultiplier, which provides high-resolution images (about 1 mm) on a field of about 4 × 4 cm, to assess in vivo biodistribution of a new prototype derivative bioconjugate composed of paclitaxel linked to hyaluronic acid (ONCOFID-P). Biodistribution of the compound was studied following intravenous, intraperitoneal, intravesical, and oral administration. After anesthetization, mice were inoculated with 99mTc-labeled ONCOFID-P and subsequently underwent imaging by the YAP camera for a 2 h period. Intravenous inoculation of the compound was followed by a strong liver uptake that reached 80% of the radioactive signal after 10 minutes and remained constant thereafter, thus indicating that this route of administration could be well suited to target primitive or metastatic liver neoplasias. Imaging of the bladder and abdomen after regional administration disclosed that the radiolabeled bioconjugate remained confined to the cavities, suggesting a potential regional application for vesical, ovarian, and gastric cancers. Therefore, preventive studies based on imaging analysis of new drugs may potentially dictate their therapeutic application in vivo.

Cardiovascular Imaging

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LOCALIZATION AND VIABILITY ASSESSMENT OF TRANSPLANTED ENDOTHELIAL PROGENITOR CELLS USING GENETIC LABELING WITH THE HUMAN SODIUM/IODIDE SYMPORTER GENE AND MAGNETIC LABELING WITH IRON OXIDES IN THE RAT HEART. Takahiro Higuchi,[1] Martina Anton,[2] Stefan Seidl,[3] Stephan G. Nekolla,[1] Marc C. Huisman,[1] Sybille Reder,[1] Frank M. Bengel,[1] Rene Botnar,[1] Markus Schwaiger.[1] [1]Nuklearmedizinische Klinik und Poliklinik der Technischen Universität München, Klinikum Rechts der Isar, Munich, Germany; [2]Institut für Experimentelle Onkologie und Therapieforschung der Technischen Universität München, Klinikum Rechts der Isar, Munich, Germany; [3]Institut für Allgemeine Pathologie und Pathologische Anatomie der Technischen Universität München, Klinikum Rechts der Isar, Munich, Germany; contact e-mail: higuchi@po2.nsknet.or.jp.

Combination of PET and MRI may provide answers to unresolved questions concerning cell engraftment and viability in cardiac cell transplantation therapy. **Methods:** Human endothelial progenitor cells (EPCs) were transduced with the human sodium/iodide symporter (NIS) gene using a retroviral vector. For MRI studies, EPCs were labeled with superparamagnetic iron oxides. Rats received an intramyocardial injection of 4 million EPCs. MRI (clinical 1.5 T) and I-124 PET (MicroPET) were performed at day 1 ($n = 10$), 3 (4), and 7 (7) after cell transplantation. **Results:** The labeled EPC injection site was readily visualized by T2*-weighted MRI, and cell viability was seen as a focal I-124 accumulation by PET at day 1. The contrast to noise ratio (CNR) with MRI (0.8 [138] 0.2 vs -0.3 ± 0.1, $p < .001$) and percentage of tracer-injected dose (%ID/cc) by PET (4.1 ± 1.0 vs -0.2 ± 0.4, $p < .001$) were significantly higher in iron and NIS-labeled than in nonlabeled EPCs. Postmortem analysis confirmed the presence of iron particles and EPCs at day 1. The CNR remained high at day 3 (3.9 ± 0.6) and day 7 (4.4 ± 1.3). In contrast, I-124 uptake disappeared at day 3 (0.42 ± 0.05%ID/cc) and day 7 (0.33 ± 0.06). Histological analysis confirmed the absence of transplanted EPCs at the site of myocardial injection at day 7. **Conclusions:** Multimodality imaging using magnetic and genetic cell labeling allows for the simultaneous assessment of cell localization and viability early after transplantation. The MRI signal loses specificity for viable transplanted cells as early as 7 days after injection most likely due to cell death and/or phagocytosis. Monitoring of cell viability by tracer techniques appears important for cell therapy control.

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NONINVASIVE IMAGING OF MYOBLAST-MEDIATED HYPOXIA-INDUCIBLE FACTOR 1 ALPHA GENE EXPRESSION USING TWO DIFFERENT OPTICAL BIOLUMINESCENCE REPORTER GENES. Olivier Gheysens,[1] Ian Y. Chen,[2] Martin Rodriguez-Porcel,[2] Paulmurugan Ramasamy,[2] Carmel C. Chan,[2] Juergen K. Willmann,[2] Joseph C. Wu,[2] Sanjiv S. Gambhir.[2] [1]Molecular Imaging Program at Stanford (MIPS), Departments of Radiology and Bioengineering, Bio-X Program, Stanford University, Stanford, CA, USA, and Department of Nuclear Medicine, Catholic University Leuven, Leuven, Belgium; [2]Molecular Imaging Program at Stanford (MIPS), Departments of Radiology and Bioengineering, Bio-X Program, Stanford University, Stanford, CA, USA; contact e-mail: olivier.gheysens@uz.kuleuven.ac.be.

Background: Combined cell-gene therapy using stem cells that carry and overexpress angiogenic genes is a promising strategy for treating ischemic heart diseases. To assess its efficacy, we have developed a novel imaging strategy in which both the survival of transplanted myoblasts and their transgene expression can be separately imaged using firefly luciferase (fluc) and renilla luciferase (hrl) optical bioluminescence reporter genes, respectively. **Methods and Results:** A plasmid vector (pUbi-hrl-pUbi-HIF1α 1-390/VP16x2) was built to express both hrl and HIF1α/VP16x2, with each gene driven by a constitutive ubiquitin (Ubi) promoter. C2c12 myoblasts and c2c12 stably expressing a multimodality trifusion reporter gene (pUbi-mrfp-fluc-HSV1-sr39tk, c2c12-3F) were transiently transfected with the plasmid and assayed 24 hours later for hrl, HIF1α/VP16x2, and downstream VEGF gene expression using in vitro luciferase assay, Western blot, and ELISA, respectively. A high linear correlation was observed between RL activity and the amount of plasmid transfected ($r^2 > .95$) or HIF1α/VP16x2 protein level ($r^2 > .82$) . Downstream angiogenic VEGF level is also highly correlated with RL activity ($r^2 > .82$).Balb-c mice injected with either c2c12-3F or c2c12-3F expressing the plasmid construct (c2c12-3FRH) into the hindlimb were imaged for fluc and hrl expressions 24 hours later. **Conclusion:** Noninvasive optical bioluminescence imaging of myoblast-mediated gene transfer can be performed by using two different optical reporter genes, with one reporter gene used for imaging cell survival and the other one for indirectly monitoring the therapeutic gene expression. Further optimization of this dual reporter gene imaging strategy should help to facilitate the evaluation of combined cell-gene therapy in living subjects.

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RESEARCH OF PHAGE DISPLAY–SELECTED PEPTIDES WITH SPECIFIC AFFINITY FOR VASCULAR CELL ADHESION MOLECULE 1 OVEREXPRESSED IN ATHEROSCLEROTIC PLAQUES. Carmen Burtea,[1] Claire Corot,[2] Sophie Laurent,[1] Marc Port,[2] Eric Lancelot,[2] Sébastien Ballet,[2] Luce Vander Elst,[1] Robert N. Muller.[1] [1]University of Mons-Hainaut, Department of General, Organic and Biomedical Chemistry, NMR and Molecular Imaging Laboratory, Mons, Belgium; [2]Guerbet, Aulnay-sous-Bois, France; contact e-mail: Carmen.Burtea@umh.ac.be.

Acute atherothrombotic syndromes (ie, myocardial infarction, brain stroke, etc.) represent the leading cause of morbidity and mortality in the developed countries. Despite major advances in the treatment of coronary heart disease, a large number of the disease's victims presenting an apparently healthy constitution die suddenly without prior symptoms. Vascular cell adhesion molecule 1 (VCAM-1), overexpressed in inflammatory conditions, is exposed on the endothelial cell surface of the diseased artery itself and of the microvascular network of the vasa vasorum in atherosclerotic plaques. Neovascularization and expression of adhesion molecules by microvessels at sites of vulnerable lipid-rich plaques could contribute to plaque destabilization. The aim of the present work was to screen by phage display for VCAM-1 peptide binders with the final purpose to diagnose vulnerable atherosclerotic plaques by MRI after peptide conjugation to a paramagnetic or superparamagnetic contrastophore (magnetic reporter). The screening was performed in vitro (recombinant mouse VCAM-1 immobilized on magnetic beads) with a disulfide-constrained heptapeptide library. The 42 phage clones isolated after four rounds of biopanning present an important affinity both for mouse and human VCAM-1. The sequences presenting the amino acids T, R, and L were enriched after four rounds of panning. Peptide alignment with adhesion molecules (integrin, protocadherin) or with immunoglobulin receptors shows that their selection was not accidental. Based on K*d and IC*50 values, peptide expressed by phage clone 40 was selected for subsequent in vitro and in vivo evaluation. The in vitro evaluation of this peptide confirms a specific interaction with the targeted biomolecule. Its conjugation to magnetic reporters will provide a helpful tool for the diagnosis of atherosclerotic disease, both during its precocious stages and later, when the plaque is prone to rupture and thrombosis.

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STEREOTACTIC REALIGNMENT OF POSITRON EMISSION TOMOGRAPHY AND HIGH-RESOLUTION COMPUTED TOMOGRAPHY FOR EX VIVO ASSESSMENT OF RADIOTRACER UPTAKE IN AORTIC AND SUPRA-AORTIC PLAQUES IN MICE. Lars Stegger,[1] Petra Keul,[2] Sven Hermann,[1] Uta Schnöckel,[1] Norbert Lang,[1] Klaus P. Schäfers,[1] Bodo Levkau,[2] Michael A. Schäfers.[1] [1]Department of Nuclear Medicine, University of Münster, Münster, Germany; [2]Institute of Cardiovascular Pathophysiology, University of Duisburg-Essen, Essen, Germany; stegger@uni-muenster.de.

Ex vivo imaging of tracer uptake in arterial walls of mice spatially correlated to CT-derived morphology can facilitate assessment of novel PET tracers for visualization of atherosclerotic plaques. In comparison to in vivo imaging this approach allows for imaging with higher spatial resolution and devoid of motion artifacts. **Methods:** We devised a method to image radiotracer uptake of the isolated aorta and supra-aortic arteries inside a small-animal PET scanner and to coregister images with those from high-resolution CT. Prior to imaging the vessel lumens were filled with a contrast agent (microfill) and embedded in paraffin together with three fiducial markers for retrospective image fusion. To evaluate the feasibility of this method we imaged vessels from 14 ApoE-deficient mice, 8 injected with 18F-FDG and 6 with 18F-NaF, and 6 wild-type mice (3 FDG; 3 NaF). **Results:** Stereotactic realignment of PET and CT images was successful in all cases. Radiotracer uptake for both FDG and NaF was markedly elevated in the area of the aortic arch and the proximal parts of the supra-aortic arteries compared to the abdominal aorta. Wild-type mice showed nearly no visible uptake of NaF in the aortic arch; uptake of FDG was visible but less than for ApoE-deficient mice. **Conclusion:** Spatially coregistered molecular-morphological imaging of the aorta and the supra-aortic arteries ex vivo is possible by using fixation/embedding techniques and retrospective image registration using fudicial markers. First applications with FDG and NaF as radiotracers point to the usefulness of this technique for the evaluation of novel tracers in the future.

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AN INVESTIGATION INTO THE EFFECT OF THE RADIOISOTOPE 64-COPPER EMISSION ON NANOBACTERIA OF HEART DISEASE. Paola Panichelli,[1] Lorenzo Castignani,[1] Meri Colonna,[1] Domenico Martini,[1] Gianluca Valentini,[1] Cinzia Nasuti,[2] Luana Quassinti,[2] Massimo Bramucci,[2] Luigi Ninona.[3] [1]ACOM (Advanced Center Oncology Camerino), Camerino, Italy; [2]Department of Biology M.C.A and Chemistry, University of Camerino, Camerino, Italy; [3]Heart Hospital, Civitanova Marche, Italy; contact e-mail: p.panichelli@acompet.it.

Background: In the '90 year in Italy at Viterbo, Lazio, were first discovered by some geologist nanoparticles associated with precipitation of calcium carbonate. Similar structures have been recently isolated from human blood, urine, ovarian cancer, and kidney stones, suggesting that these structures may contribute to calcifying disease in human. These nanoparticles appear self-replicating in culture and therefore have been called nanobacteria. The study of these particles shows that they are encapsulated with hydroxylapatite, the same calcium mineral found in atherosclerotic tissue. Vascular calcification is a multifactorial process, but nanobacteria could be an additional biological factor. Copper mineral is the third metal most present naturally in the human body and is an essential cofactor for a number of biological process and so copper transport at the cell surface is already in normal physiology. Indeed, 64Cu is the most versatile of all copper radionuclides owing to its unique decay scheme, which combines electron capture (41%), beta minus (40%), and beta plus (19%) decays and also results for Auger electron emission with therapeutic potential. Its half life of 12.7 hours is compatible with in vivo kinetics of a variety of large and small molecular carriers. **Methods:** Calcified human arteries, aortic aneurysms, and cardiac valves were collected as surgical waste from patients undergoing vascular or valvular repair at the heart hospital of Civitanova. Tissue segments were embedded in paraffin and sectioned in 5 μm, and immunostaining was performed. Filtered homogenates were prepared from these tissues and then were inoculated into special culture flasks from the Department of Biology of the University of Camerino. Analyses of cultured tissues were executed and also the specificity of 8D1o antibody was studied. From the flasks of nanoparticles were selected the most good growing and were incubated with different concentration of activity of 64-CuCl in ACOM in Montecosaro. **Results:** Radioisotope emission of 64-copper clearly affected vitality and replication of nanobacteria. **Conclusions:** The results indicate that suitable administration of radioactive copper could be instrumental in stopping the growth of calcified structures. The finding could stimulate the design of cooperative therapy concepts that could reduce death caused by myocardial infarcts.

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APPLICATION OF COPPER DEPLETION THERAPY AGAINST FORMATION OF ATHEROSCLEROSIS VASCULAR DISEASE IN THE HEART. Paola Panichelli,[1] Lorenzo Castignani,[1] Meri Colonna,[1] Domenico Martini,[1] Gianluca Valentini,[1] Cinzia Nasuti,[2] Luana Quassinti,[2] Massimo Bramucci,[2] Luigi Ninona.[3] [1]ACOM (Advanced Center Oncology Camerino), Camerino, Italy; [2]Department of Biology M.C.A and Chemistry, University of Camerino, Camerino, Italy; [3]Heart Hospital, Civitanova Marche, Italy; contact e-mail: p.panichelli@acompet.it.

Background: Cholesterol is important for cell function, neurotransmission, and synaptic plasticity but is also an important risk factor for atherosclerosis and formation of plaque, so copper is an essential nutrient but also is implicated as an important factor for Alzheimer disease (AD), so that chelation of copper has been suggested as a potential therapy for AD. In many studies it has been noticed that people who died of heart disease also had beta-amyloid plaques in their brains. **Methods:** A total of 20 male rats of 8 to 10 weeks of age and weighing about 250 g were housed, with free access to food and water, maintained on a 12 h light/12 h dark cycle. The rats were divided in two groups of 10 elements and each group had its respective diet of nutrition. One received distilled water; the other received water supplemented with 0.18 mg of copper sulfate. The hearts and arteries of rats were examined in vivo using the 8D10 antibody labeled with 124 iodine and with the execution of micropet studies (YAPPET Micropet, FWHM = 1.8 mm and sensitivity = 17.3 cps/Kbq). After the sacrifice studies of biodistribution, autoradiography, immunohistochemistry, and histological analysis were executed. All rats were maintained on their respective food and water regimens for a total of 10 weeks before studies in vivo and final euthanasia. **Results:** Rats fed with copper added to their distilled waters revealed a nearly 50% increase of uptake of 124I-8D10 compared with animals fed with unaltered distilled water. **Conclusions:** Similarly to cholesterol, elevated quantity of copper can also be viewed as one of the major risk factors for both atherosclerosis and heart disease. It could be beneficial for all people to limit their overall copper consumption that can be taken in from daily foods and drinking water. For people affected by the pathology of atherosclerotic plaque in the cardiocirculatory system a lifestyle modification could be applied that includes other than an exercise program, stress reduction, alcohol limitations, and a diet poor in cholesterol and copper and indeed to begin a systematic therapy of copper depletion with the assumption of chelators in the way to maintain the level of copper under normal values.

Chemistry

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TRIAZA MACROCYCLIC CHELATES OF GROUP 13 METAL IONS WITH RELEVANCE TO NUCLEAR MEDICINE: IN VITRO CHARACTERIZATION AND IN VIVO RAT IMAGING STUDIES OF THE 67GA RADIOLABELED CHELATES. João Paulo André,[1] Vanda Sofia Lopes,[2] Maria Isabel Prata,[3] Ana Cristina Santos,[3] Carlos Campos Geraldes.[2] [1]Centro de Química, Campus de Gualtar, Universidade do Minho, Braga, Portugal; [2]Departamento de Bioquímica, Centro de RMN e Centro de Neurociências e Biologia Celular, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, Coimbra, Portugal; [3]Instituto de Biofísica e Biomatemática, Faculdade de Medicina, Universidade de Coimbra, Coimbra, Portugal; contact e-mail: jandre@quimica.uminho.pt.

A multinuclear NMR study of Al3+, Ga3+, and In3+ chelates of five triazamacrocyclic ligands (NOTA = 1,4,7-triazacyclononane-N,N′,N′′-triacetate; DETA = 1,4,7-triazacyclodecane-N,N′,N′′-triacetate; NOTP = 1,4,7-triazacyclononane-N, N′, N′′-trimethylenephosphonate; NO2AP = 1,4,7-triazacyclononane-N-methylenephospho nate-N′,N′′-diacetate; NOA2P = 1,4,7-triazacyclononane-N,N′-bis(-methylenephosphonate)-N′′acetate) provided information on the stability, structure, and dynamics of the chelates formed in aqueous solution. In particular, the analysis of 27Al, 71Ga, and 115In NMR spectra gave information on the symmetry and stability toward hydrolysis of the species existing in solution. The 31P NMR spectra reflected the protonation of the noncoordinated oxygen atoms from the pendant phosphonate groups and the number of species in solution. The 1H NMR spectra afforded the analysis of the number of species and their structure and dynamics in solution. Additionally, the 1H NMR titration of ligands NOA2P5- and NO2AP4- contributed toward the knowledge of their protonation schemes. These results were compared with other related triaza chelates.[1] Biodistribution and gamma imaging studies were performed on Wistar rats using the radiolabeled 67Ga(NO2AP)- and 67Ga(NOA2P)2- chelates. These studies demonstrated that both chelates have fast renal uptake and excretion, as expected for highly charged species, and in good agreement with our previous results on the structurally related 67Ga(NOTA), 67Ga(NOTP)3-, and 67 (NOTPME) chelates.[2] Taken together, they show that as the number of the ligand phosphonate groups increases, increasing the negative charge of the chelates, their uptake from the bloodstream is slower and their retention time in the kidneys increases. The absence of liver or bone uptake reflects the high in vivo stability of the two 67Ga3+ chelates studied. The absence of activity in the brain is indicative of these chelates being unable to cross the blood-brain barrier, as expected from charged hydrophilic compounds.

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MONODISPERSE WATER-SOLUBLE SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES REVEALED AS A HIGH-PERFORMANCE T2 CONTRAST AGENT FOR MAGNETIC RESONANCE IMAGING. Jian Qin,[1] Sophie Laurent,[2] Alain Roch,[2] Robert N. Muller,[2] Mamoun Mohammed.[1] [1]Royal Institute of Technology, Stockholm, Sweden; [2]University of Mons-Hainaut, Mons, Belgium; contact e-mail: jian@mse.kth.se.

Recently, high-temperature decomposition strategies have been developed to produce monodisperse and highly crystalline superparamagnetic iron oxide nanoparticles (SPIONs).[1–3] To use SPIONs for biomedical applications, it is essential to disperse them in aqueous phase. In this work, we have developed a reliable and rapid method to transfer the hydrophobic SPIONs to water solution by employing the triblock copolymer Pluronic F127 (PF127), leading to the formation of a hierarchical surface structure, which results in a novel MRI T2 contrast agent. SPIONs were synthesized by the decomposition of iron oleate complex. The PF127-coated water-soluble SPIONs (POA@SPION) were prepared through a ''mix-evaporation-redispersion'' method. Noticeably, the r2/r1 ratios of POA@SPION are 6- and 17-fold higher than those of Resovist at 0.47 T and 1.41 T respectively. We also investigated the relaxivities of SPIONs transferred to aqueous phase by coating with small molecules, eg, tetramethylammonium hydroxide (TMAOH) and dimercaptosuccinic acid (DMSA). The relaxivities and r2/r1 ratios of TMAOH@SPION and DMSA@SPION are similar to those of Resovist. Hence it is believed that the enhancement of the r2/r1 ratio results from the surface characteristics of POA@SPION. It is therefore hypothesized that the PF127 hierarchical structure plays a dual role to simultaneously maintain the T2 relaxation rate and to decrease the T1 relaxation rate.

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SYNTHESIS AND PHYSICOCHEMICAL CHARACTERIZATION OF A NEW POTENTIAL MAGNETIC RESONANCE IMAGING REPORTER: INVESTIGATION OF ITS NONCOVALENT INTERACTION WITH HUMAN SERUM ALBUMIN. Céline Henoumont, Virginie Henrotte, Sophie Laurent, Luce Vander Elst, Robert N. Muller. University of Mons Hainaut, Mons, Belgium; contact e-mail: celine.henoumont@umh.ac.be.

The synthesis and the physicochemical characterization of a new gadolinium MRI contrast agent (Gd-C4-sulfaphenazol-DTPA) showing a strong affinity for human serum albumin (HSA) are reported. The measurements reveal a relaxivity in water of 7.8 s-1 mM-1 at 0.47 T and 310 K, a fast water exchange, and a good stability versus zinc transmetallation. The investigation of the interaction with HSA was performed with three different techniques: proton relaxivity, mass spectrometry, and NMR diffusometry. The relaxometry technique shows an apparent relaxivity of ca 24 s-1 mM-1 for a 1 mM solution of the complex in the presence of HSA 4%. The calculated association constant is ca 9000 M-1 with three binding sites. Mass spectra of the paramagnetic complex confirm its high affinity for the protein and show that at least three binding sites exist. Competition experiments with ibuprofen (Suddlow site II of HSA) and salicylic acid (Suddlow site I of HSA) were carried out by NMR diffusometry. In these experiments, the diffusion coefficient of ibuprofen (10 mM) or salicylic acid (10 mM) was measured in the presence of the europium complex (2 mM) and HSA 4% (buffered solutions). The results show that the presence of the Eu-complex increases markedly the diffusion coefficient of ibuprofen but has little effect on salicylic acid, indicating that the paramagnetic molecule interacts with the Suddlow site II of HSA. This new reporter is thus promising and further in vivo experiments will be undertaken in the near future.

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COMPARATIVE STUDIES OF [64CU]ETHHYLENEDICYSTEINE-NITROIMIDAZOLE AND [18F]FLUOROMISONIDAZOLE. Domenico Martini, Gianluca Valentini, Paola Panichelli, Meri Colonna, Claudio Malizia, Lorenzo Castignani. A.C.O.M. spa Advanced Center Oncology Macerata; contact e-mail: d.martini@unicam.it.

The [64Cu]ethhylenedicysteine-nitroimidazole ([64Cu]NNEC-NIM) can be synthesized at room temperature starting from NNEC-NIM and 64Cu(CH3COO)2. Decay-corrected radiochemical yields based on 64[Cu] copper were 80% ± 5% (n = 10). Copper 64 [64Cu]NNEC-NIM was determinated at radio-thin-layer chromatography to have a radiochemical purity of 90%. The amount of agent injected for high-performance liquid chromatography was 10 μCi. The specific radioactivity was calculated to be 1 mCi/μg. To determine biodistribution [64Cu]NNEC-NIM was injected through the tail vein in three groups of five Swiss albino mice each (male) 30 to 35 g. The animal were sacrificed by cardiectomy under slight ether anesthesia at predetermined time intervals (1, 4, and 16 h). The organs of interest were excised and weighed and the radioactivity counted in gamma counter. Several tumoral cell lines were injected into 18 nude mice, and the animals were then injected with [64Cu]NNEC-NIM or [18F]fluoromisonidazole (FMISO) (0.037–0.074 MBq per mice). The microPET studies were executed using a Yappet System (ISE) 1 hour, 4 hours, and 16 hours after injection. Through microPET analysis we have compared microPET images of the animal group injected with [64Cu]NNEC-NIM, indicated as group 1, and the animal group injected with [18F]fluoromisonidazole (FMISO), indicated as group 2. MicroPET imaging studies in nude mice have evidenced similarities between [64Cu] ECDG and [18F] FMISO uptake in tumors, and study findings supported the potential use of [64Cu] ECDG as a functional imaging agent.

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SELF-ASSEMBLED PEPTIDE AMPHIPHILIC MOLECULE AS SELECTIVE AND HIGH RELAXIVITY CONTRAST AGENTS IN MAGNETIC RESONANCE IMAGING. Antonella Accardo,[1] Diego Tesauro,[1] Luigi Del Pozzo,[1] Luigi Paduano,[2] Eliana Gianolio,[3] Silvio Aime,[3] Giancarlo Morelli.[1] [1]Department of Biological Sciences and CIRPeB University of Naples ''Federico II,'' Naples, Italy; [2]Department of Chemistry, University of Naples ''Federico II,'' Naples, Italy; [3]Department of Chemistry, IFM, University of Turin, Turin, Italy; contact e-mail: antonellaacc@interfree.it.

Magnetic resonance imaging (MRI) is a very powerful diagnostic imaging technique,[1] giving very resolved images; unfortunately, its sensitivity is very poor. To improve this parameter MRI needs high concentration (10-4 M) of a contrast agent such as paramagnetic Gd(III) complexes.[2] To reach the required local concentration many carriers have been developed such as supramolecular aggregates. In the present communication, we describe the synthesis and the characterization of a new peptide amphiphile monomer, containing both CCK8 peptide and a Gd complex, that self-assemble into micelles at the physiological pH. The CCK8 is able to recognize receptors overexpressed in a wide variety of tumor cells. The critical micellar concentration value was determined by a fluorescence-based method using 8-anilinonaphthalene-1-sulfonate (ANS) as the fluorescent probe. The structural data obtained by physicochemical techniques indicate that micelles have ellipsoidal shape with a hydrodynamic radius of ≈50 Å. Relaxivity measurements of the self-assembled aggregates show high relaxivity parameters (R1p = 15.0 mM-1s-1) with a large enhancement with respect to the isolated DTPAGlu(Gd) complex (R1p = 6.2 mM-1s-1). In order to verify the specific binding and the contrast agent accumulation biological assays on tumor cells are in progress.

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A NEW ROUTE TO CELLULAR LABELING. Eliana Gianolio,[1] Anna Ciampa,[1] Stefania Lanzardo,[2] Silvio Aime,[1] Giovanni Battista Giovenzana.[3] [1]Dipartimento di Chimica IFM, Università di Torino, Torino, Italy; [2]Dipartimento di Scienze Chimiche e Biologiche, Università di Torino, Torino, Italy; [3]Dipartimento di Scienze Chimiche Alimentari Farmaceutiche e Farmacologiche, Università del Piemonte Orientale ''A. Avogadro''; contact e-mail: eliana.gianolio@unito.it.

MRI is the technique of choice for tracking cells in vivo. Several methods have been applied to label cells by using either Gd(III) complexes and iron oxide particles. This contribution deals with an alternative route to label cells based on anchoring a micelle containing almost 103 Gd(III) atoms to a cell through a properly designed linker. To visualize cells it is necessary to use assemblies consisting of a high number of Gd(III) centers. The herein reported method deals with the use of a tightly assembled micelle that displays on its surface a high number of negative charges that represent the recognition sites for a positively charged linker that is responsible for the anchoring to the cell's surface. A tightly assembled micelle (cmc < 10-5 M) has been obtained with a lipophilic derivative of Gd-AAZTA containing two saturated alphatic chains that endow each Gd center with a relaxivity of ca. 25 mM-1s-1 (20MHz, 298K). From light scattering measurements a micelle diameter of ca 80 nm and an

aggregation number of ca 850 have been determined. The system has been relaxometrically characterized by recording NMRD and 17O-NMR profiles. The outer surface of the cell membrane is known to present an excess of negative charges and therefore there is a limited interaction with the Gd-containing micelle. To attain a strong binding interaction between the cell and the micelle, a basic polypeptide (Poly-Arg, $n = 173$) has been used. It has been found that each cell can load up to ca. 1.2×10^7 micelles. This corresponds to ca. 1×10^{10} Gd/cell, ie, well above the threshold for MRI visualization.

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NOVEL UTP DERIVATIVE WITH DIENE-CONTAINING SPACER ARM FOR DIELS-ALDER REACTION: POTENTIAL TOOL FOR RADIOACTIVE OR FLUORESCENT DETECTION UPON ITS INCORPORATION INTO RIBONUCLEIC ACID. Zdenek Tocik,[1] Libor Krasny,[2] Sona Kovackova,[1] Dominik Rejman,[1] Ivan Rosenberg.[1] [1]IOCB, Academy of Sciences, Prague, Czech Republic; [2]Institute of Microbiology, Academy of Sciences, Prague, Czech Republic; contact e-mail: tocik@uochb.cas.cz.

A synthetic route was elaborated to the preparation of a UTP derivative containing in the 5-position of the uracil moiety the spacer arm terminated with furfuryl substituent, a diene moiety utilizable for the Diels-Alder reaction. Such a construct was needed for incorporation into RNA and its post labeling with a dienophile carrying fluorescent or radiolabeled groups to serve as reporters in visualization of interactions at the molecular level in diagnosis of cancer- or virus-associated diseases. We present the first stage of the study using fluorescent dye to verify overall plausibility of the method. We prepared the spacer arm first: reaction of furfurylamine with monomethyl adipoyl chloride provided the intermediate, which, upon hydrolysis, yielded a furfuryl-substituted carboxy derivative, which was converted into N-hydroxysuccinimidyl ester. This compound was coupled with aminoallyl-UTP to afford the desired derivative (Fur-UTP). The ability of Fur-UTP to react with dienophile was tested in reaction with N-(4-fluorobenzyl)maleimide. The expected product was identified on MS-ES. We proved that (a) the prepared Fur-UTP can be successfully incorporated into RNA chain using T7 RNA polymerase with pGEM DNA template, and (b) the resulting transcript can be covalently labeled with Alexa Fluor 488 maleimide conjugate. This indicates that Diels-Alder reaction can be a useful method for post labeling of RNA and potentially for introduction of 18F label. Further study to optimize the substrate properties of Fur-UTP and to obtain additional knowledge of Diels-Alder reaction is underway.

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ISOPOLAR PHOSPHONATE 2′,5′ OLIGOADENYLATES WITH P-C BRIDGING INTERNUCLEOTIDE LINKAGE: SEARCH FOR POTENT EFFECTORS OF RIBONUCLEASE L ACTIVITY. Ondřej Páv, Eva Protivínská, Michaela Collinsová, Jiří Jiráček, Jan Snášel, Miloš Buděšínský, Ivan Rosenberg. IOCB, Academy of Sciences, Prague, Czech Republic; contact e-mail: pav@uochb.cas.cz.

Interferon-treated cells exhibit enhanced activity of the ribonuclease L (RNase L). Interferon induces expression of 2′,5′ oligoadenylate synthetases (OAS) utilizing ATP to generate 2′,5′ oligoadenylates (2-5A), which bind to, and activate, the latent RNase L to cleave the ssRNA. The cellular 2-5As are cleaved by a specific phoshodiesterase, which regulates their level and, thus, the RNase L activity. The overexpression of OAS in virus-infected cells led to the inhibition of picornavirus replication; however, the expression of an inactive mutant RNase L caused an increased susceptibility to viral infection and loss of the interferon-mediated inhibition of cell growth. From this point of view, the RNase L activity represents an important mechanism of interferon-induced cellular antiviral defence. In addition, a hyperactive RNase L was found in lymphocytes of people suffering from CFS (chronic fatigue syndrome). In this respect, the RNase L is considered an important marker of CFS and target for chemotherapy. Within the search for potent agonists and antagonists of RNase L we have synthesized distinct libraries of two types of phosphonate oligoadenylates containing isopolar, nonisosteric 3′C-O-C-P-O-5′C[1] and 3′C-O-P-C-O-5′C internucleotide linkages, respectively, to find how the nonisosteric linkage influences binding to, and activation of, the RNase L.

Support from the European Molecular Imaging Laboratory (EMIL) under project Z40550506 of the IOCB is acknowledged.

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A NOVEL GENERATION OF IMPROVED LIPOCEST MAGNETIC RESONANCE IMAGING AGENTS WITH HIGHLY SHIFTED INTRALIPOSOMAL WATER PROTONS. Daniela Delli Castelli,[1] Claudia Cabella,[2] Carla Carrera,[2] Roberta Mazzon,[2] Simona Rollet,[1] Joseph Stancanello,[2] Enzo Terreno,[1] Elisabetta Violante,[1] Massimo Visigalli,[2] Silvio Aime.[1] [1]Department of Chemistry IFM and Molecular Imaging Center, University of Torino, Torino, Italy; [2]CRM Bracco Imaging, Colleretto Giacosa (TO), Italy; contact e-mail: daniela.dellicastelli@unito.it.

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The advent of the molecular imaging era prompts the search for innovative imaging probes in order to set up novel procedures for pursuing early diagnosis and efficient follow-up of therapeutic treatments. Among MRI agents, those named CEST (chemical exchange saturation transfer) have the unique property of yielding a ''frequency-encoded'' contrast that may allow the visualization of different agents in the same region.[1,2] Within the class of CEST agents, LIPOCESTs display the highest sensitivity (subnanomolar scale) owing to the extremely high number of mobile intraliposomal water protons, properly shifted by the presence of an encapsulated paramagnetic Ln(III)-based shift reagent (SR), which can be selectively saturated.[3] In addition to the sensitivity, a very important characteristic of CEST agents is the range of the resonance frequency values of their mobile protons. For the first generation of LIPOCESTs, this interval is rather small (from −4 to 4 ppm with respect to the resonance of bulk water depending on the magnetic anisotropy of the SR), being limited by the concentration of the encapsulated SR that is mainly controlled by osmotic effects. To enhance the chemical shift of the intraliposomal water protons, several routes have been pursued, including the encapsulation of neutral multimeric SRs for increasing the maximum amount of encapsulated SR and the exploitation of the chemical shift contribution arising from bulk magnetic susceptibility effect by inducing an osmotic shrinkage of the liposomes. This effect is proportional to the paramagnetism of the encapsulated Ln(III) ion and it depends on the shape and orientation of the liposomes with respect to the external magnetic field. The incorporation of amphiphilic SRs in the liposome membrane in order to (i) increase (at least for the SR units pointing inwards) the concentration of the SR in the intraliposomal cavity and (ii) to influence the orientation of the shrinked liposomes with respect to the field. It will be shown that the combination of these strategies may enhance the window of the accessible saturation frequencies of LIPOCESTs of almost one order of magnitude, thus making possible, for the first time, the MRI visualization of different LIPOCEST probes in the same region of interest.

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A NOVEL GENERATION OF CONCENTRATION-INDEPENDENT GADOLINIUM(III)-BASED MAGNETIC RESONANCE IMAGING–RESPONSIVE AGENTS. Enzo Terreno,[1] Alberto Bert,[1] Walter Dastrù,[1] Franco Fedeli,[2] Alberto Sanino,[1] Silvio Aime.[1] [1]Department of Chemistry IFM and Molecular Imaging Center, University of Torino, Torino, Italy; [2]LIMA, Biondustry Park, Colleretto Giacosa (TO), Italy; contact e-mail: enzo.terreno@unito.it.

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A responsive (elsewhere referred to as smart or intelligent) MRI agent is a chemical whose contrasting properties are sensitive to a given physicochemical variable that characterizes the microenvironment in which the probe distributes. Typical parameters of primary diagnostic relevance include pH, temperature, enzymatic activity, redox potential, concentration of specific ions, and low-weight metabolites. So far, several Gd(III)-based agents, whose relaxivity is dependent on the above-mentioned parameters, have been investigated.[1] In spite of the good responsiveness displayed by several of such systems, their clinical use is limited by the fact that the detected image contrast cannot be unambiguously ascribed to a change in the parameter of interest if the local concentration of the responsive agent is unknown. So far, this problem has been tackled by an indirect determination of the local concentration of the agent by using a reference compound whose relaxivity is not dependent on the parameter of interest.[2] In this contribution, a novel approach based on a ratiometric method will be presented and discussed. Measuring the ratio between transverse and longitudinal paramagnetic contributions to the water proton relaxation rate, R2p/R1p, one attains the removal of the concentration dependence. In order to act as a ratiometric responsive probe, the R2p/R1p ratio of a Gd(III) agent must be dependent on the parameter of interest. Two systems have been investigated and validated in vitro: a macromolecular pH responsive system consisting of a polyornithine adduct in which a portion of the free amino groups has been covalently linked to a macrocyclic Gd(III) complex. At magnetic fields higher than 1 T, the R2p/R1p ratio of this compound is dependent on the molecular tumbling of the metal complex covalently attached to the polymer (nanoseconds scale). Since it has been reported that the tumbling rate of cationic polyamino acids, like polyornithine, is inversely dependent on the protonation degree of their basic sites; the R2p/R1p ratio of this macromolecular adduct will increase toward the basic side. In addition, Figure 1 (left) demonstrates the concentration independence of the R2p/R1p ratio. A Gd(III)-loaded liposomes as potential temperature MRI reporters. In this case, the temperature dependence of the R2p/R1p ratio has been attained by exploiting the R2-specific magnetic susceptibility contribution generated by the encapsulation of the paramagnetic agent in the lipidic vesiscle. Interestingly, R2p is dominated by the magnetic susceptibility contribution, which decreases upon increasing temperature. Conversely, the R1p values of the same system are determined by the water permeability of the liposome membrane and consequently R1p increases with the temperature. As a consequence, R2p/R1p is temperature dependent and, thanks to the ratiometric approach, concentration independent. In conclusion, the ratiometric approach may be considered a promising route for designing a novel generation of concentration-independent MRI-responsive agents.

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PARAMAGNETIC DY(III)-LOADED LIPOSOMES AS T2 SUSCEPTIBILITY MAGNETIC RESONANCE IMAGING AGENTS. Daniela Delli Castelli,[1] Claudia Cabella,[2] Carla Carrera,[2] Linda Chaabane,[2] Roberta Mazzon,[2] Joseph Stancanello,[2] Enzo Terreno,[1] Massimo Visigalli,[2] Silvio A.[1] [1]Department of Chemistry IFM and Molecular Imaging Center, University of Torino, Torino, Italy; [2]CRM Bracco Imaging, Colleretto Giacosa (TO), Italy; contact e-mail: daniela.dellicastelli@unito.it.

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Paramagnetic low molecular weight Dy(III)-based complexes have been investigated in the late 1980s as T2-susceptibility MRI agents in virtue of their heterogeneous tissue distribution.[1] In spite of the promising results obtained (also in humans) in several diagnostic applications, the interest for these agents has slowly diminished, mainly for the advent of the much more sensitive iron oxide particles, which affect the images on the basis of analogous relaxation processes.[2] Recently, the challenge brought about by molecular imaging applications prompted the design of highly sensitive nano-sized systems aimed at lowering the contrast agent concentration threshold for MRI detection. Among the available nano-sized platforms, liposomes have received much attention, primarily for their high chemical versatility, high biocompatibility, and peculiar pharmacokinetic properties.[3] As T2-susceptibility effects arise from the compartmentalization of a paramagnetic compound, it is expected that the encapsulation of a hydrophilic Dy(III) complex in the intraliposomal cavity leads to a significant T2 shortening. In this contribution, the relaxometric properties of a series of Dy(III)-loaded liposomes are discussed and compared with those of the reference iron oxide particles. In addition, the potential of these agents has been tested in MRI targeting experiments in cellulo and in vivo on mice models. The large T2-sensitivity enhancement determined by the encapsulation of a Dy(III) complex (Dy-HPDO3A) in a liposome is shown in Figure 1, where the T2-contrast of a liposome filled with the paramagnetic complex is compared with an aqueous solution of Dy-HPDO3A at the same concentration (7 T, 25°C). In these experimental conditions, a 10-fold increase in R2p was observed when the relaxation data are normalized to the metal content, but this difference increases enormously (ca. 107) if the transverse relaxivity is normalized to the concentration of the paramagnetic nanoparticle. The efficiency of the Dy(III)-loaded liposomes is affected by the concentration of the entrapped compound, the liposome size, and, of course, the magnetic field strength. In addition, a further enhancement in the T2 effects can be gained by increasing the amount of encapsulated complex (eg, by using neutral multimers) or by incorporating amphiphilic Dy(III) complexes in the liposome membrane. The latter strategy has two advantages: (i) to enhance the magnetic susceptibility of the liposome by increasing the concentration of the paramagnetic ion in the intraliposomal cavity (contribution of the amphiphilic Dy(III) units pointing inwards) and (ii) to exploit the Curie relaxation mechanism, which is expected to be relevant at high fields for such slowly rotating systems. The transverse relaxivities of the different Dy(III)-loaded liposomes investigated in this work are comparable and often even higher than the values reported for iron oxide particles of similar size and measured at the same experimental conditions. The promising T2 properties found for the aqueous suspensions of Dy(III)-loaded liposomes have been confirmed in some MRI targeting experiments in vitro (targeting fibrin on human clots), in cellulo (targeting glutamine transporters on Neuro 2A cell line), and in vivo on mice models (targeting xenografted neuroblastoma). In conclusion, Dy(III)-loaded liposomes may be considered as an interesting alternative to the use of iron oxide particles as T2-susceptibility MRI agents. In addition to their high sensitivity, these systems can also be potentially used as dual, T2 and CEST, agents.

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DIVALENT BOMBESIN ANALOGUES FOR TUMOR TARGETING: FACILE SYNTHESIS OF NOVEL DOTA-DERIVED PROCHELATOR FOR DIVALENT PEPTIDE CONJUGATION. Keelara Abiraj, Sibylle Tschumi, Martin Kretzschmar, Helmut R. Maecke. Division of Radiological Chemistry, Department of Radiology, University Hospital of Basel, Basel, Switzerland; contact e-mail: keelaraa@uhbs.ch.

Application of multimeric targeting vectors may improve tumor targeting as multivalency enhances receptor binding affinity, tumor specificity, and tumor retention time. Development of multimeric targeting vectors grafted on DOTA-derived prochelators is highly advantageous as DOTA accommodates many metals that are useful in different fields of molecular imaging like Gd(III) for MRI, In-111, Ga-67 for SPECT, Ga-68, Cu-64 for PET, and Eu(III), Tb(III) for optical imaging. We have synthesized a novel DOTA-based prochelator functionalized with glutamic acid for the divalent conjugation with peptidyl ligands. The synthetic strategy included the bromination of L-glutamic acid 5-benzyl ester followed by protection of carboxylic group by tert-butyl group to yield the racemic alpha-bromoglutamic acid 1-tert-butyl ester 5-benzyl ester (02). Subsequent N-alkylation of DO2A (tert-butyl ester) with 02 followed by catalytic hydrogenation produced the new prochelator (04). The prochelator 04 has two free carboxylic groups for divalent conjugation with peptidyl ligands and exists in different isomeric forms (RR, SS, RS, and SR). Coupling of 04 with different bombesin analogues gave the corresponding DOTA-conjugated divalent bombesin analogues, which showed excellent chelating properties with Gd(III) (for MRI applications) and also with radionuclides such as In-111, Lu-177, and Ga-68 (for radiopharmaceutical applications). Both In-111- and Lu-177-labeled divalent radiopeptides showed rapid internalization and slower externalization rate as studied with the PC-3 cell line. In conclusion, we have developed a novel prochelator to synthesize DOTA-conjugated divalent targeting vectors, which could be employed for the multimodal imaging such as PET/MRI or SPECT/MRI with improved tumor targeting capabilities.

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NEW CATECHOL DERIVATIVES COATED IRON OXIDE COLLOIDS FOR MAGNETIC RESONANCE IMAGING. Soraya Benderbous,[1] Sékouba Traore,[1] Souad Ammar,[2] François Chau,[3] Pierre-Antoine Eliat,[4] Françoise Heymans,[5] Fernand Fievet.[2] [1]LEPG, FRE-CNRS 2969, University of Tours, Tours, France; [2]ITODYS, UMR, CNRS 7086; [3]Unité de Pharmacochimie Moléculaire et Systèmes Membranaires EA 2381, Université de Paris VII, Paris, France; [4]PRISM, ImagiVeC -UPRES-EA 3890, Université de Rennes 1, Rennes, France; [5]Unité de Pharmacochimie Moléculaire et Systèmes Membranaires EA 2381, Université de Paris VII, Paris, France; contact e-mail: benderbous@univ-tours.fr.

Superparamagnetic iron oxide nanoparticles have been developed in order to increase MRI sensitivity at the cell level. Usually, these nanoparticles are produced by aqueous co-precipitation at room temperature, which does not allow a simultaneous control of their size and crystallinity.[1] They are then coated by dextran or polyethylene glycol to be biocompatible.[2] Such polymeric coated magnetic particles present a high hydrodynamic radius, which is a limiting factor to cross the endovascular epithelium[3] and exhibit a very weak magnetization. Recently, we have developed an original synthetic route to prepare monodisperse and well-crystallized nanoparticles, using a forced hydrolysis of metallic salts in polyol.[4] We succeeded in producing 7 nm sized Fe2O3 and 18 nm sized Fe3O4 nanoparticles and coat them with catechol derivatives since spectroscopic study[5] suggested that bidentate enediol could tightly bind to iron oxide, leading to a stable chelate. Dopamine and catechaldehyde are an interesting anchor because they can be functionalized with other molecules of interest, thanks to amino and formyl group. Contrary to 3,5-bis(dimethyl-/diethyl-sulfonamide) catechol reported as iron chelates,[6] the new monomeric coated iron oxide nanoparticles present a superparamagnetic behavior and exhibit an enhanced saturation magnetization (about 35 and 59 emu.g-1). By dynamic light scattering measurement, the nanoparticle size does not exceed 50 nm with narrow distribution. The FT-IR and zeta potential experimental results proved that monomeric molecules bound to the the iron oxide nanoparticles via the coordination between the two oxygen atoms of the enediol function of the catechol derivative and the Fe3+ ions of the surface. The measured r2 relaxivities in water at 200 MHz and 25°C lie between 25 and 162 mM-1.s-1. The present study suggests that the potential application of these nanoparticles for in vivo imaging depends now upon molecular chemistry to functionalize the complexes to target specific proteins, tumors, and cancer cells.

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TARGETING TUMOR CELLS WITH GADOLINIUM(III) CHELATES BY MEANS OF THE GLUTAMINE TRANSPORTING SYSTEM. Silvia Fuzerova,[1] Rachele Stefania,[1] Alessandro Barge,[2] Lorenzo Tei,[3] Simonetta Geninatti Crich,[1] Franco Fedeli,[1] Lorena Bertrami,[1] Silvio Aime.[1] [1]Department of Chemistry I.F.M.,University of Torino, Torino, Italy; [2]Department of Drug Science and Technology, University of Torino, Torino, Italy; [3]Department of Science, Ambience and Life; Universita del Piemonte Orientale, Alessandria, Italy; contact e-mail: silvi_f@seznam.cz.

Contrast agents for magnetic resonance imaging have become familiar in hospitals all around the world. Still with respect to other molecular imaging modalities such as PET or SPECT, the low sensitivity is the main limitation of the magnetic resonance molecular imaging (MRMI) approach. To overcome this limitation the imaging probe for MRMI applications must identify the target with high specificity and should provide sufficiently intense signal enhancement within the imaged volume to be easily distinguishable from unenhanced tissue. The next generation of contrast agents will include systems able to recognize specific molecules on the cellular surface that act as early reporters of a given pathology. We have recently exploited the glutamine transporting system as a route to deliver a large number of Gd(III) contrast agents to the tumor cells. It is known that proliferating cells consume more glucose and amino acids (and their derivatives) than their benign counterparts. Transport of glucose and amino acids into cells is mediated by specific membrane proteins called transporters, which are responsible for the translocation of the substrate from one side of the membrane to the other. The increased expression or upregulation of these transporters correlates with the greater transport of glucose and amino acids and is strictly related to the cell growth. Novel systems for efficient conjugation have been synthetized: (a) Gd-DOTA monoamide (Gd-DOTAMA) derivatives in which the glutamine residue is conjugated through different functionalities; (b) Gd-DOTAMA derivatives endowed with a different spacer between the chelate and the glutamine moieties; (c) multivalent systems containing more glutamine residues per Gd complex; (d) a Gd-loaded liposome functionalized with glutamine vectors on its outer surface. All Gd complexes were tested in vitro on HTC, C6, and hepatocyte cell lines and the best compounds also in vivo on A/J mice grafted with the murine neuroblastoma cell line Neuro-2a and in Her-2/neu transgenic mice developing multiple mammary carcinomas.

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THERMODYNAMIC AND KINETIC EVALUATION OF MONOPHOSPHORUS ACID DERIVATIVES OF DOTA. Jan Kotek,[1] Michaela Försterová,[1] Petr Hermann,[1] Přemysl Lubal,[2] Alice Lázničková,[3] Milan Lázníček.[4] [1]Department of Inorganic Chemistry, Universita Karlova, Prague, Czech Republic; [2]Department of Analytical Chemistry, Masaryk University, Czech Republic; [3]Department of Biophysics and Physical Chemistry, Universita Karlova, Hradec Králové, Czech Republic; [4]Department of Pharmacology and Toxicology, Universita Karlova, Hradec Králové, Czech Republic; contact e-mail: modrej@natur.cuni.cz.

Dissociation constants of phosphonic acid and two bifunctional phosphinic acid derivatives of DOTA and stability constants of their complexes with some transition metal and lanthanide ions were determined. Phosphinate ligands exhibit basicity similar to those of DOTA and phosphonate derivative is more basic. Thermodynamic stabilities of the complexes correspond to the overall basicity of the ligands and, therefore, they are similar to stability constants of DOTA complexes. Formation and decomplexation kinetics of copper(II) and yttrium(III) complexes depend on the nature of the phosphorus atom substituent. Hydrophobic aminobenzyl side chain decelerates complex formation as well as complex dissociation. The hydrophilic –OH group and propionate side chain form complexes faster. The behavior can be explained by an ability of the side chain to assist proton transfer from/to nitrogen atom(s) of the ring inside/outside the macrocyclic cavity. Kinetic inertness of yttrium(III) complexes of the phosphorus ligands is higher than that of the yttrium(III)-DOTA complex. Copper(II) complexes of the investigated ligands are less kinetically inert if compared with the Cu(II)-DOTA complex. Biodistribution studies of 90Y-complexes with the bifunctional ligands in rats showed that the complexes had a similar pharmacokinetic profile as the analogous 90Y-DOTA complex; they are hydrophilic with preferential extraction through kidney. The ligands are suitable for possible medicinal utilizations.

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FLUORINE-18 LABELING OF SMALL INTERFERING RIBONUCLEIC ACIDS FOR POSITRON EMISSION TOMOGRAPHY IMAGING. Thomas Viel,[1] Bertrand Kuhnast,[2] Raphael Boisgard,[1] Françoise Hinnen,[2] Bertrand Tavitian,[1] Frédéric Dollé.[2] [1]CEA, DSV, I2BM, SHFJ, LIEG, INSERM U803, Institut d'Imagerie Biomédicale, Service Hospitalier Frédéric Joliot, Laboratoire d'Imagerie de l'Expression des Gènes, Orsay, France; [2]CEA, DSV, I2BM, SHFJ, LIME, Institut d'Imagerie Biomédicale, Service Hospitalier Frédéric Joliot, Laboratoire d'Imagerie Moléculaire Expérimentale, Orsay, France; contact e-mail: thomas.viel@cea.fr.

Small interfering ribonucleic acids (siRNAs), a class of macromolecules constituted by the association of two single-stranded ribonucleic acids of short sequences, are naturally responsible for the cleavage of mRNA at defined sites. This process (called RNAi process), which is related to a natural defense against viruses and the mobilization of transposable genetic elements, can also be a powerful tool in the hands of the biologist to efficiently and specifically block the expression of a gene at the RNA level. Three selected siRNAs, showing high plasmatic stability toward nucleases and high in vitro cellular capacity to interfere with the target mRNA coding for luciferase, were labeled with the positron emitter fluorine-18 (half-life 109.8 minutes), permitting in vivo dynamic and quantitative molecular imaging with positron emission tomography (PET). The strategy used involves (1) prosthetic conjugation of a single-stranded oligonucleotide with the phosphorothioatemonoester-selective [18F]FPyBrA (N-[3-(2-[18F]fluoropyridin-3-yloxy)-propyl]-2-bromoacetamide) reagent,[1] followed by (2) formation of the target duplex (annealing) using the complementary sequence. About 0.55–1.11 GBq of fluorine-18-labeled siRNAs (specific activity: 74-148 GBq/μmol at EOB) could be obtained within 165 minutes starting from 37.0 GBq of starting [18F]fluoride (1.5% to 3.0%, non-decay-corrected isolated yields). Radiochemical purity of the labeled duplexes was verified by HPLC and nondenaturing polyacrylamide gel electrophoresis and was found to be greater than 90%.

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ONE-STEP FLUORINE-18 LABELING OF LBT-999, A SELECTIVE RADIOLIGAND FOR THE VISUALIZATION OF THE DOPAMINE TRANSPORTER WITH POSITRON EMISSION TOMOGRAPHY. Frederic Dolle,[1] Julie Helfenbein,[2] Patrick Emond,[3] Françoise Hinnen,[1] Sylvie Mavel,[3] Zoia Mincheva,[3] Wadad Saba,[1] Marie-Anne Schöllhorn-Peyronneau,[1] Heric Valette,[1] Lucette Garreau,[3] Sylvie Chalon,[3] Christer Halldin,[4] Jean-Claude Madelmont,[5] Jean-Bernard Deloye,[6] Michel Bottlaender,[1] Joel Le Gailliard,[2] Denis Guilloteau.[3] [1]CEA, Service Hospitalier Frédéric Joliot, DSV, Orsay, France; [2]Orphachem, ZATE INSERM U484, Clermont-Ferrand, France; [3]INSERM, U619, Université François-Rabelais de Tours, Tours, France; [4]Karolinska Institute, Department of Clinical Neuroscience, Karolinska Hospital, Stockholm, Sweden; [5]INSERM U484, Laboratoire Etude Métabolique des Molécules Marquées, Clermont-Ferrand, France; [6]Laboratoires Cyclopharma, Biopôle Clermont Limagne, Saint Beauzire, France; contact e-mail: frederic.dolle@cea.fr.

LBT-999 (8-((E)-4-fluoro-but-2-enyl)-3-beta-p-tolyl-8-aza-bicyclo[3.2.1]octane-2-beta-carboxylic acid methyl ester) is a recently developed cocaine derivative belonging to a new generation of highly selective DAT ligands.[1–3] Initial fluorine-18-labeling[4] was based on the robust and reliable two-step radiochemical pathway often reported for such tropane derivatives, involving first the preparation of (E)-1-[18F]fluoro-4-tosyloxybut-2-ene followed by its coupling to the appropriate nor-tropane moiety. In order to facilitate both the automation and the purification process, a simple one-step fluorine-18-labeling of LBT-999 has been developed, based on a chlorine-for-fluorine aliphatic substitution. The process involves reaction of K[18F]F-Kryptofix222 at 165°C for 10 min in DMSO (0.6 mL) containing the chloro analogue (3.5–4.5 mg), followed by C-18 Sep-Pak cartridge prepurification and finally semipreparative HPLC purification (Symmetry C-18). Typically, 2.59–3.07 GBq of [18F]LBT-999 (> 95% chemically and radiochemically pure) could be obtained with specific radioactivities ranging from 37 to 111 GBq/micromol within 80–85 min (HPLC purification and Sep-pak-based formulation incl.), starting from a 37.0 GBq [18F]fluoride batch (overall RCY: 7.0–10.0%, non-decay-corrected).

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RADIOSYNTHESIS OF [11C]DPA-713, [11C]DPA-715, [11C]CLINME, AND [18F]DPA-714, SELECTED NOVEL POTENTIAL RADIOLIGANDS FOR IMAGING THE PERIPHERAL BENZODIAZEPINE RECEPTORS WITH POSITRON EMISSION TOMOGRAPHY. Frederic Dolle,[1] Cyrille Thominiaux,[1] Michelle James,[2] Anna Creelman,[2] Bertrand Kuhnast,[1] Francoise Hinnen,[1] Johnny Vercouillie,[3] Ivan Greguric,[4] Filomena Mattner,[4] Christian Loch,[4] Roger Fulton,[2] Stephane Demphel,[1] Stephane Le Helleix,[1] Fabien Chauveau,[1] Herve Boutin,[1] Anne-Sophie Herard,[1] Silvia Selleri,[5] Denis Guilloteau,[3] Philippe Hantraye,[1] Bertrand Tavitian,[1] Andrew Katsifis,[4] Michael Kassiou.[2] [1]Service Hospitalier Frédéric Joliot, CEA/DSV, Orsay, France; [2]Brain and Mind Research Institute, University of Sydney, Camperdown, NSW, Australia; [3]INSERM, U619, Université François-Rabelais de Tours, Tours, France; [4]Radiopharmaceuticals Research Institute, Australian Nuclear Science Technology and Organisation, Lucas Heights, NSW, Australia; [5]Dipartimento di Scienze Farmaceutiche, Università degli Studi di Firenze, Firenze, Italy; contact e-mail: frederic.dolle@cea.fr.
[11C]PK11195 is not only the oldest but also the most widely used PET radiotracer for in vivo imaging of the peripheral benzodiazepine receptors (PBR or translocator protein [18 kDa, TSPO]). Being already in use for two decades in humans, it suffers from low brain uptake, extensive binding to plasma proteins, and relatively high nonspecific binding. With the aim of developing a new PET imaging probe for the in vivo study of the PBR, three pyrazolo[1,5-a]pyrimidineacetamides (DPA-713, DPA-714, and DPA-715) and one imidazo[1,2-a]pyridineacetamide (CLINME) were radiolabeled with the positron emitters carbon-11 (half-life 20.38 min) and fluorine-18 (half-life 109.8 min). Briefly, CLINME (2-[6-chloro-2-(4-iodophenyl)-imidazo[1,2-a]pyridin-3-yl]-N-ethyl-N-methyl-acetamide) was labeled at its methylacetamide moiety chain from the corresponding nor-analogue using [11C]methyl iodide (in DMSO/DMF (100/200 μL) containing powdered KOH (3–5 mg) at 110°C for 3 min). DPA-713 (N,N-diethyl-2-[2-(4-methoxyphenyl)-5,7-dimethylpyrazolo[1,5-a]pyrimidin-3-yl]acetamide) and DPA-715 (N,N-diethyl-2-[2-(4-methoxyphenyl)-5,7-bis-trifluoromethylpyrazolo[1,5-a]pyrimidin-3-yl]acetamide) were labeled at their aromatic methoxy groups from the corresponding nor-derivatives using [11C]methyl triflate (in acetone (300 μL) containing aq. 3M NaOH (4 μL) at 110°C for 1 min). DPA-714 (N,N-diethyl-2-[2-[4-(2-fluoroethoxy)phenyl]-5,7-dimethyl-pyrazolo[1,5-a]pyrimidin-3-yl]acetamide) was labeled at its aromatic fluoroethoxy group from the corresponding tosyl-derivative using the K[18F]F-Kryptofix®222 (in CH3CN (3 mL) at 85°C for 5 min or DMSO (600 μL) at 130°C for 5 min). All radioligands were purified using semipreparative reverse-phase HPLC (Zorbax, XTerra or Symmetry C-18 column), were adequately formulated for IV injection, and were found to be > 95% chemically and radiochemically pure. Total synthesis time: less than 30 min (including HPLC purification and formulation) for [11C]CLINME, [11C]DPA-713, and [11C]DPA-715; 90 min for [18F]DPA-714.

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SYNTHESIS OF HIGHLY AFFINE CLOSO-BORANE CONJUGATED TYR3-OCTREOTATE DERIVATES FOR BORON NEUTRON CAPTURE THERAPY. Tobias Hess,[1] Thomas Betzel,[1] Frank Rösch,[1] Jean Claude Reubi.[2] [1]Institute of Nuclear Chemistry, University of Mainz, Mainz, Germany; [2]Institute of Phathology, University of Bern, Bern, Switzerland; contact e-mail: hesst@uni-mainz.de.
Aim: Despite the improvements in cancer therapy during the last years, many other types of cancer are still extremely resistant to all current forms of therapy. Boron neutron capture therapy (BNCT) provides a way in theory to destroy cancer cells without damaging healthy tissue. However, BNCT in practice is still limited because of the lack of the selective delivery of boron to cancer cells. Since many neuroendocrine tumors show an overexpression of the somatostatin receptor, it was the aim to synthesize compounds, which are highly affine toward this receptor. **Methods:** The synthetic peptide octreotate was picked as the high affine tumor-targeting vector. It was the intention to insert a spacer consisting of different lengths of sarcosine between the peptide and the closo-borane-containing molecule (linker) to increase the affinity. The different linker molecules were synthesized starting from different benzoic acid derivates and decaborane. Using common coupling reagents the various systems were synthesized and purified by HPLC. **Results:** Starting from methyl-4-hydroxy-benzoate and two different benzoic acids-derivates various linker molecules containing 10 or 20 boron atoms were synthesized in three steps and yields of up to 53–66%. Further on the desired spacers with chain lengths of 2, 4, 6 were coupled with octreotate and the linker. Overall seven different closo-borane-octreotate derivates with yields of up to 22% were obtained. The in vitro affinities showed excellent values. **Conclusions:** Via the developed synthesis strategy linker molecules containing 60 boron atoms could be achieved. Using the successful building block chemistry various products can be synthesized quickly and easily with good yields. The first target compounds showed high affinities of up to 1.2 nM toward the somatostin receptor and its potential for the use in BNCT.

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A NOVEL GADOLINIUM-BASED MAGNETIC RESONANCE IMAGING CONTRAST AGENT RESPONSIVE TO THE FACTOR XIII TRANSGLUTAMINASE ACTIVITY. Lorenzo Tei,[1] Alessandro Barge,[2] Dario Longo,[3] Linda Chaabane,[4] Luigi Miragoli,[4] Vito Lorusso,[4] Silvio Aime.[3] [1]DISAV, University of Eastern Piedmont "A. Avogadro," Alessandria, Italy; [2]Department of Drug Science and Technology, University of Torino, Torino, Italy; [3]Department of Chemistry I.F.M., University of Torino, Torino, Italy; [4]Bracco Imaging S.p.A., Milano, Italy; contact e-mail: lorenzo.tei@mfn.unipmn.it.
Fibrin-stabilizing factor XIII is involved in many pathologies, such as thrombotic disorders, myocardial infarction, and cerebrovascular disease. The MRI "in vivo" visualization and quantitation of the factor XIII activity may be a very useful tool for the diagnosis of these pathologies and the monitoring of therapeutic follow-up. Activated factor XIII (factor XIIIa) is a transglutaminase that stabilizes the clot crosslinking proteins in the fibrin thrombus by reaction between the carbonyl group of a glutamine in one protein and the amino group of a lysine residue in a nearby protein.[1] Using molecular mechanics and docking computational methodologies we investigated the quaternary structure of factor XIII and its catalytic center and we designed a peptide (DCCP16) able to bind the protein and crosslink with fibrinogen. We have tested "in vitro" our Gd-DCCP16 against GdHPDO3A as negative control and also against an imaging probe for MR imaging of factor XIII activity already published.[2] A marked signal enhancement was observed when Gd-DCCP16 is present during the clot formation whereas a much reduced effect is detected when the contrast agent is added to an already formed clot. The signal enhancement in the NMR image is therefore only due to the formation of covalent bonds between peptide and fibrin. Our probe also gave a higher signal enhancement (about 70% increase at 0.4 mM concentration) with respect to the published imaging probe.
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GRAFTING OF A GADOLINIUM COMPLEX ON POLYMERIC NANOPARTICLES: A MACROMOLECULAR T1 CONTRAST AGENT FOR MAGNETIC RESONANCE IMAGING. Caroline Cannizzo,[1] Emmanuel Allard,[2] Loïck Moriggi,[1] Angélique Sour,[3] André E. Merbach,[1] Chantal Larpent,[2] Lothar Helm.[1] [1]EPFL-ISIC, Lausanne, Switzerland; [2]Institut Lavoisier, Versailles, France; [3]Université de Strasbourg 1, Strasbourg, France; contact e-mail: caroline.cannizzo@epfl.ch.
During the last two decades, the use of paramagnetic gadolinium chelates for medical magnetic resonance imaging has increased considerably. In order to slow down the correlation time of the complex, and therefore enhance proton relaxivity, it is possible to graft it on macromolecules. Moreover, when using macromolecules as a support it is possible to obtain a great number of ligands in a confined space, with a relative rigidity of the system. The polymerization of organic monomers in oil-in-water microemulsion affords means to produce stable and translucent suspensions of monodisperse nanoparticles (diameter smaller than 50 nm). The proper choice of the monomers allows one to synthesize reactive polymeric particles with high degrees of chemical functionalization. The grafting of a new layer at the surface of the particles can be readily achieved by post-functionalization of the polymeric material, directly in the aqueous media. Here we report the synthesis of EPTPA-functionalized nanoparticles: the ligand EPTPA-bz-NCS is bound at the surface of aminated nanoparticles via a thiourea linkage. The ligand content was evaluated by elemental analysis, colorimetric titration, and magnetic susceptibility of the Gd-loaded particles. These nano-objects could later be used for the design of multiple-imaging devices, for example, with a fluorescent dye encapsulated in the core of the particles, and a MRI ligand grafted at the surface.

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FIRST EXPERIENCE WITH RADIOPAQUE MICROSPHERES IN COMPUTED TOMOGRAPHY IMAGING. Soenke Heinrich Bartling,[1] Chagit Biton,[2] Anna Galperin,[2] Jochen Huppert,[1] Wolfhard Semmler,[3] Fabian Kiessling,[1] Shlomo Margel.[2] [1]Junior Group Molecular Imaging, German Cancer Research Center, Heidelberg, Germany; [2]Department of Chemistry, Bar-Ilan University, Ramat-Gan, Israel; [3]Department of Medical Physics in Radiology, German Cancer Research Center, Heidelberg, Germany; contact e-mail: s.bartling@dkfz.de.
Purpose: To test whether radiopaque microspheres can be used as CT contrast media for blood-pool, liver, and lymph node imaging. These microspheres can be produced in various sizes, which might provide different distribution kinetics and hence possibly new means for pathology characterization. **Material and Methods:** Radiopaque nanoparticles of 50 ± 10% nm diameter were formed by emulsion polymerization of the monomer 2-methacryloyloxyethyl(2,3,5-triiodobenzoate) and enriched so that a solution with 100 mg/mL iodine resulted. To increase tolerability 5% dextrose was added. The aqueous dispersion was injected into the tail veins of rats (2 ml) and mice (200 μL). Furthermore, it was injected into the subcutaneous tissue and intraperitoneal cavity of rats. Over a period of 5 days flat panel–based CT scans were performed. **Results:** After intravenous injection a blood-pool imaging effect can be observed. However, this is shortly followed (< 5 min) by a complete uptake into the liver, spleen, and enteric wall, where it causes an enhancement longer than 5 days. A minutes-lasting slight enhancement of the left basal lungs suggests at least a partial trapping of the microspheres in the basal lung areas. After intraperitoneal and subcutaneous injection a clear enhancement of draining lymph nodes was visible that enriched over time and lasted at least 5 days. **Conclusions:** Iodinated microspheres can be used as a CT contrast media. It might be useful for blood-pool, liver, and lymph node imaging. These results open the opportunity to further investigate differently sized microspheres.

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CHARACTERIZATION OF NEW NEAR-INFRARED FLUORESCENT DYES FOR MOLECULAR IMAGING. Tibor Vag,[1] Jutta Pauli,[2] Romy Haag,[1] Ute Resch-Genger,[2] Werner A. Kaiser,[1] Ingrid Hilger.[1] [1]Institute of Diagnostic and Interventional Radiology, University Hospital Jena, Jena, Germany; [2]Federal Institute for Materials Research and Testing, Berlin, Germany; contact e-mail: Tibor.Vag@med.uni-jena.de.
Purpose: To determine cytotoxicity, stability, and fluorescence properties of recently introduced near-infrared fluorescent (NIRF) dyes in comparison to indocyanine green (ICG), the only clinically approved fluorescence dye until now. **Methods and Materials:** Stability of the dyes DY-676, DY-681, DY-731, DY-751, and DY-776 (Dyomics, Germany) was determined by measuring their absorption spectra dissolved in a protein-supplemented solution (PSS) for up to 72 hours at 37°C. Dye cytotoxicity was assessed by incubating different dye concentrations with mouse SVEC-endothelial and J774-macrophage cell lines for up to 72 hours and measuring the activity of cellular dehydrogenases in treated and nontreated cells. Fluorescence quantum yields (QYs) were calculated from integrated, blank-, and spectrally corrected emission spectra employing in IR 125 (Lambda Physik) in DMSO and oxazine-1 (Lambda Physik) in ethanol as fluorescence standards. **Results:** ICG revealed an absorption intensity (Iabs) of 92% after 72 compared to 0 hours (fresh solution). Dyes of the DY series generally showed higher stabilities with Iabs values between 91 and 100%. Highest stability was observed for DY-681 and DY-776 (Iabs of 99% and 100% respectively) after 72 hours of incubation. ICG revealed higher cytotoxicity on macrophages than on endothelial cells (93% decrease of cell vitality versus 40%). Dyes of the DY series generally exhibited lower cytotoxic effects on both cell types with decrease of cell vitality only between 0 and 30%. Highest QYs were obtained for DY-676, DY-681, and DY-731 (QY of 0.3). **Conclusion:** Highest stability and QY in conjunction with low cytotoxicity were revealed by DY-681, making it an interesting candidate for NIRF in vivo applications.

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POTENTIAL POSITRON EMISSION TOMOGRAPHY RADIOLIGAND FOR BRAIN DOPAMINE D3 RECEPTOR VISUALIZATION: DESIGN, SYNTHESIS, BINDING PROFILE, AND INTERACTION WITH THE EFFLUX PUMP P-GLYCOPROTEIN. Marcello Leopoldo, Enza Lacivita, Paola De Giorgio, Marialessandra Contino, Nicola Antonio Colabufo, Francesco Berardi, Roberto Perrone. Dipartimento Farmaco-Chimico Università degli Studi di Bari, Bari, Italy; contact e-mail: leopoldo@farmchim.uniba.it.
The D3 dopamine receptor belongs to the D2-like family of dopamine receptors. From the beginning, attention has been attracted to the restricted distribution of the D3 receptor in the brain (islands of Calleja, ventral striatum/nucleus accumbens, dentate gyrus, and striate cortex), seemingly related to functions of dopamine associated with the limbic brain. Various pharmacological studies have investigated it as an interesting therapeutic target for the treatment of schizophrenia, Parkinson's disease, drug-induced dyskinesia, and sustance abuse (cocaine, alcohol, tobacco). To date, a few attempts have been made to develop PET radioligand to visualize brain dopamine D3 receptors. They failed because of the high lipophilicity of the radioligands that resulted in high nonspecific accumulation in the brain. With the scope to obtain more effective PET radioligands, we have designed and prepared a series of N-[4-(4-arylpiperazin-1-yl)butyl]arylcarboxamides, characterized by

lipophilicity within a range considered optimal for blood-brain barrier penetration and low nonspecific binding. The affinities of the new compounds for dopamine D3 receptors and selectivity over other monoaminergic receptors have been assessed by radioligand binding assays. Also, interaction with the P-glycoprotein, an efflux pump that can actively transport lipophilic drugs out of the brain, has been evaluated.

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DESIGN AND SYNTHESIS OF ARYLPIPERAZINE-BASED FLUORESCENT LIGANDS FOR SEROTONIN 5-HT1A AND DOPAMINE D3 RECEPTORS. Marcello Leopoldo, Enza Lacivita, Elena Passafiume, Marialessandra Contino, Nicola Antonio Colabufo, Francesco Berardi, Roberto Perrone. Dipartimento Farmaco-Chimico Università degli Studi di Bari, Bari, Italy; contact e-mail: leopoldo@farmchim.uniba.it.

Fluorescent high-affinity ligands represent a class of widely applicable tools. For example, fluorescent ligands have allowed the localization of several GPCR. Fluorescent ligands can also give information on the biophysical characteristics of the ligand binding site because some fluorophores show quantum yield depending on lipophilicity or the pH of the environment. During the last decade, our research group has been involved in the structure affinity-elucidation of compounds with arylpiperazine structure as a versatile framework to obtain high-affinity ligands for some GPCR such as serotonin 5-HT1A and 5-HT7 and dopamine D2 and D3 receptors. Recently, we became interested in the preparation of fluorescent probes targeting such receptors. The most common approach for this task is the labeling of a known ligand with a fluorophore into an area of the structure that would have minimal influence on receptor binding. However, the binding properties of the labeled molecules can be significantly altered with respect to the native ligand. For our purpose, we have prepared new fluorescent ligands by including a fluorescent core into a framework endowed with affinity for the target receptors. In this way, the above-mentioned shortcomings should be overcome and an entire series of fluorescent ligands could be characterized

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PHYSICAL AND RELAXOMETRIC CHARACTERIZATION OF IRON OXIDE NANOPARTICLES AS T1 MAGNETIC RESONANCE CONTRAST AGENTS. Elisenda Rodriguez,[1] Elena Taboada,[2] Anna Roig,[2] Isabelle Mahieu,[3] Sebastien Boutry,[3] Alain Roch,[3] Robert N. Muller.[3] [1]Center for Molecular Imaging Research, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA, USA; [2]Institut de Ciència de Materials de Barcelona, Consejo Superior de Investigaciones Cientificas (ICMAB-CSIC), Esfera de la UAB, Catalunya, Spain; [3]NMR and Molecular Imaging Laboratory, Department of General, Organic and Biomedical Chemistry, University of Mons-Hainaut, Mons, Belgium; contact e-mail: evrodriguez@mgh.harvard.edu.

Iron oxide nanoparticles play an important role as negative MR T2 contrast agents due to their sizes and magnetic properties.[1] In spite of their widespread use, inherent problems of these systems, for example, large particle size distributions, remain unsolved. We have recently described the synthesis of ultrasmall iron oxide nanoparticles with narrow particle size distribution and a high saturation magnetization value. They have been stabilized in water at physiological pH and proved to be useful as a positive T1 MRI contrast agent.[2] After functionalization with specific biological ligands, as citrate anions, they could be used as magnetic reporters for molecular imaging probes. Cytotoxicity of those particles is currently being investigated on the basis of intracellular uptake. Preliminary results show that a high level of cell internalization of our particles is achieved even at low incubation times.

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PREPARATION OF THERAPEUTIC DOSES OF 177LU OR 90Y DOTATATE FOR RECEPTOR-MEDIATED RADIOTHERAPY. Dariusz Pawlak, Renata Mikolajczak. IAE, Radioisotope Centre POLATOM; contact e-mail: d.pawlak@polatom.pl.

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The therapeutic efficacy of receptor-mediated therapy depends on the ability of the carrier molecule to recognize the tumor cell receptors and on the physical properties of the selected radionuclide and is strongly enhanced by the specific activity of the labeled molecule. The aim of our study was to establish border lines for DOTATATE labeling conditions with 177Lu and 90Y. DOTATATE was received from PiChem, Austria, and the radionuclides 90Y (carrier-free) and 177Lu (about 15 Ci/mg Lu) were obtained as chlorides at Radioisotope Centre POLATOM. Chemical impurities (Zn, Cu, Fe, Ni, Co) in 90Y and 177Lu were measured prior to labeling by ICP-OES spectrometry and did not exceed 15 μg/Ci. The labeling was carried out in ascorbic acid solution at pH = 4.5–5.3 followed by 30 min incubation at 90°C. Radiochemical purity was assessed by TLC, HPLC, and Sep-Pak. The complexes of DOTATATE with 90Y and 177Lu were obtained with over 99% radiochemical purity when the minimal molar ratio of peptide:radionuclide was about 20 for 90Y and about 2 for 177Lu. Good agreement of results obtained with HPLC, TLC, and Sep-Pak separation was observed (ie, 24 hours after labeling the radiochemical purity of 177Lu-DOTATATE was 99.52%, 98.78%, and 98.95%, respectively). No significant differences between the stability of peptide labeled with 90Y and 177Lu were observed (the radiochemical purity values were at 4 hours 99.91% and 99.40%, after 24 hours 99.08% and 99.62%, respectively). However, the radioactive concentration of the radiolabeled peptides strongly influenced their stability.

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ESTIMATION OF BASELINE DA D2 RECEPTOR OCCUPANCY IN STRIATUM AND EXTRASTRIATAL REGIONS IN HUMANS USING POSITRON EMISSION TOMOGRAPHY WITH [18F]FALLYPRIDE. Patrizia Riccardi, Ronald Baldwin, Ronald Salomon, Sharlet Anderson, M.S. Ansari, Rui Li, Benoit Dawant, Amy Bauernfeind, Dennis Schmidt, Robert Kessler. Vanderbilt University, Nashville, TN, USA; contact e-mail: riccardip@aol.com.

Background: Previous alphamethylparatyrosine (AMPT) administration has been used with SPECT and PET studies to assess the baseline occupancy of striatal dopamine D2 receptors by endogenous dopamine in vivo in normal and schizophrenic subjects. As dopaminergic neurotransmission in cortical and limbic regions plays an important role in a number of psychiatric disorders, we examined whether PET studies using [18F]fallypride performed prior to and following AMPT depletion could be used to estimate baseline DA D2 occupancy by endogenous DA. **Methods:** Six normal subjects without any history of psychiatric, neurological, or medical illness were recruited. MRI scans were performed. PET studies were performed using a GE Discovery LS PET scanner using a 3-D emission acquisition and a transmission attenuation correction. [18F]Fallypride PET scans (5.0 mCi, specific activity greater than 2,000 Ci/mmol) were performed prior to and following AMPT administration (71.4 mg/kg PO administered in six doses) over 26 hours. Serial scans were obtained for 3.5 hours. Blood samples were collected for determination of HVA plasma levels. **Results:** Paired two-tailed *t*-tests and Wilcoxon rank tests demonstrated significant increases in binding potentials following AMPT treatment ($p < .001$) for the caudate, putamen, ventral striatum, and substantia nigra. A trend-level increase was seen in the medial thalamus. **Conclusions:** The results of this study demonstrate that [18F]fallypride PET studies performed prior to and following AMPT depletion of cerebral DA can be used to estimate the baseline occupancy of DA D2 receptors in striatum and extrastriatal regions. Using this method, future research can help to identify the abnormalities in dopaminergic neurotransmission in neuropsychiatric illnesses.

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LONGITUDINAL MANGANESE-ENHANCED MAGNETIC RESONANCE IMAGING STUDIES OF THE SOMATOSENSORY TRACT IN RATS. Guadalupe Soria, Aurore Bogaert, Mathias Hoehn. In-vivo-NMR Laboratory, Max-Planck-Institute for Neurological Research, Cologne, Germany; contact e-mail: gsoria@nf.mpg.de.

Introduction: Manganese-enhanced magnetic resonance imaging (MEMRI) has been described as a powerful tool to depict architecture of neuronal circuits (Pautler et al, 1998, 2003; Van der Linden, 2004; Bilgen, 2006). The aim of this study was to optimize the experimental conditions of MEMRI to study in a longitudinal way the somatosensory pathway and provide functional information on corticothalamic connectivity. **Methods:** Anesthetized Wistar rats were placed in a stereotactic holder and immobilized by earplugs and a teeth bar. A hole was drilled in the skull 3.0 mm lateral to Bregma. A Teflon guide-screw (Plastics One Co.) was inserted into the hole to allow repetitive injections at the same stereotactic coordinates; 200 nL 0.3 M MnCl2 were injected 1.5 mm below the dura by using a calibrated microcapillary and the Picospritzer (Parker-Hannifin). After the injection, the ipsilateral somatosensory cortex was stimulated by electrical pulses (2.0 mA; 3 Hz; 0.3 ms) on the contralateral forepaw during 1 h. Animals were injected with manganese three times every 15 days. MRI experiments were conducted at 7 T. 3D-FLASH and T1- and T2-weighted images were acquired before each injection, and 0 h, 24 h, 48 h, 72 h and 7 and 10 days after each MnCl2 injection. Animals were then perfusion fixated and brains were cryoprotected for histological analysis. **Results:** T1 maps were calculated to quantify T1 values of the selected ROIs. Twenty-four hours after MnCl2 injection we observed Mn2+ enhancement along the corticothalamic pathway, including somatosensory cortex, globus pallidus, caudate putamen, thalamus, internal capsule, and substantia nigra. Forty-eight hours and 72 h after MnCl2 injection Mn2+ enhancement was also visible in some contralateral structures such as the contralateral globus pallidus and thalamus. Seven days after the injection a very light contrast was still detectable. This was totally absent after 15 days. A total of three injections were performed in each animal; however, no signs of manganism were observed through this period. **Conclusions:** We have determined the optimal experimental conditions to perform MEMRI longitudinal studies in rats. Therefore, we have a valid tool of great interest to study the time-course changes of the corticothalamic connections following stroke in the rat.

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[11C]-MEJDTIC: A SELECTIVE POSITRON EMISSION TOMOGRAPHY RADIOTRACER FOR KAPPA OPIOID RECEPTOR IMAGING. Cécile Perrio, Géraldine Poisnel, Farhana Oueslati, Martine Dhilly, Jérôme Delamare, Ahmed Abbas, Danièle Debruyne, Louisa Barré. Groupe de Développements Méthodologiques en Tomographie par Emission de Positons, UMR CEA 2E, CEA/DSV, Université de Caen-Basse Normandie, Centre Cyceron, Caen, France; contact e-mail: perrio@cyceron.fr.

PET imaging of kappa receptor could provide important information on the in vivo assessment of the opioidergic system in healthy volunteers and patients with clinical brain disorders (Machulla et al. J Nucl Med 2005;46:386). Recently, MeJDTic has been described as a potent and selective antagonist (Ki = 0.053 nM; Ki (mu)/Ki (kappa) = 700; Ki (delta)/Ki (kappa) > 10,000) (Thomas et al. J Med Chem 2003;46:3127). Here we report the radiosynthesis and the evaluation, in mouse, of [11C]-MeJDTic. The radiosynthesis of [11C]-MeJDTic was performed by N-methylation reaction using [11C]-methyl triflate. In production mode, batches of 6–30 mCi (222–1110 MBq) were obtained in 55 min. Ex vivo biodistribution studies in mouse brain demonstrated that [11C]-MeJDTic crossed the blood-brain barrier readily and localized, at 10 min post-injection, in brain regions known to contain kappa receptors such as the hypothalamus, thalamus, cerebellum, hippocampus, olfactory tubercules, and cortex. Radioactive metabolite analysis carried out at this time showed that [11C]-MeJDTic was 90 and 60% of the total radioactivity in brain and plasma, respectively. Blocking studies were consistent with selective binding to kappa receptor, eg, U50,488 (a kappa referring agonist) induced an important blockade of specific binding while morphine (a mu agonist) and naltrindole (a delta antagonist) had no or little effect. These results demonstrated the potentiality of [11C]-MeJDTic as radiotracer.

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NOVEL REFERENCE REGION MODELING REVEALS INCREASED MICROGLIAL AND REDUCED VASCULATURE BINDING OF [11C]-(R)-PK11195 IN ALZHEIMER'S DISEASE PATIENTS. Federico E. Turkheimer,[1] Giampaolo Tomasi,[2] Paul Edison,[3] Alessandra Bertoldo,[4] Federico Roncaroli,[5] Poonam Singh,[5] Claudio Cobelli,[2] Alexander Gerhard,[6] David J. Brooks.[1,5] [1]Department of Clinical Neuroscience, Division of Neuroscience and Mental Health, Imperial College London, London, UK; [2]Department of Information Engineering, University of Padova, Padova, Italy; [3]MRC-Clinical Sciences Centre, Hammersmith Hospital, London, UK; [4]Department of Information Engineering, University of Padova, Padova, Italy; [5]Department of Neuropathology, Division of Neuroscience and Mental Health, Imperial College London, UK; [6]Department of Psychiatry, University of Mainz, Mainz, Germany; contact e-mail: federico.turkheimer@imperial.ac.uk.

Aims: [11C]-(R)-PK11195 is a PET radiotracer for the quantification of microglial activation in brain. We employed a modified reference tissue model that accounts for cerebral blood volume both in reference and target tissues (SRTMV) to investigate the effects of vascular binding in the calculation of binding potential (BP) in Alzheimer's disease (AD). **Methods:** Subjects consisted of 10 AD patients and 10 age-matched normal subjects (NC). After normalization to the MNI stereotaxic space, the time-activity curves (TACs) were extracted using the Hammersmith Maximum Probability Atlas. BPs were estimated using standard simplified reference tissue model (SRTM) with the reference TAC computed with a supervised selection algorithm and SRMTV. The whole blood tracer activity (Cb(t)) was extracted directly from the images. PBBS expression in the vasculature was also assessed by immunocytochemistry on a separate cohort of young and elderly controls and three AD postmortem brains. **Results:** The use of SRTMV increased BPs by about 11.9% in NC and 16.8% in AD patients while Vb values were 4.22% for NC but only 2.87% in patients. Average Cb(t) was also lower for AD patients, indicating reduction of PBBS expression in the vascular components of AD. Immunocytochemistry confirmed reduced PBBS expression in the vasculature in AD due to marked fibrosis. **Discussion:** SRTMV amplifies the BP due to microglial binding in AD more than in controls

because of a decrease in PK binding to the vasculature in AD. The same fibrosis may cause a decrease in the size of lumens and therefore be the reason behind the observed decrease of blood volume.

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IN VITRO CHARACTERIZATION OF AB42-SPECIFIC HEPTAPEPTIDES SELECTED BY PHAGE DISPLAY AND PRELIMINARY STUDIES ON ALZHEIMER'S DISEASE DIAGNOSIS BY MAGNETIC RESONANCE IMAGING WITH PEPTIDE-FUNCTIONALIZED CONTRAST AGENTS. Lionel Larbanoix,[1] Carmen Burtea,[1] Sophie Laurent,[1] Oltea Murariu,[1] David Vansthertem,[2] Gérard Toubeau,[2] Fred Van Leuven,[3] Luce Vander Elst,[1] Robert N. Muller.[1] [1]Department of General, Organic and Biomedical Chemistry, NMR and Molecular Imaging Laboratory, University of Mons-Hainaut, Mons, Belgium; [2]Laboratory of Histology, University of Mons-Hainaut, Mons, Belgium; [3]Experimental Genetics Group, KULeuven, Leuven, Belgium; contact e-mail: lionel.larbanoix@umh.ac.be.

As the predominant cause of senile dementia, Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by amyloid beta (Ab) amyloidosis and tauopathy. The deposition of Ab in the brain parenchyma and vasculature represents one of the key steps in the pathogenesis of AD. Although the senile plaques by themselves may not be the direct cause of AD symptoms, a noninvasive method to detect their presence in the brain will allow diagnosis and preventive treatment before occurrence of neurological symptoms and irreversible neurodegeneration. Aiming at the evaluation of the affinity constants of two phage display selected peptides for their target, Ab, various protocols based on ELISA and on NMR relaxometry were conceived and assessed. The ELISA protocols were carried out with biotinylated peptides detected either in the absence or in the presence of an amplification system based on antibodies. Peptides were conjugated either to USPIO or to biotin and their relaxometric detection was performed with the system streptavidin-biotin conjugated to USPIO. Our results suggest that relaxometry has the potential to detect molecular binding and to evaluate the apparent dissociation constant. The peptides conjugated to USPIO or to Gd-DTPA were subsequently assessed in vivo by MRI to investigate their diagnosis potential. The efficacy of two AD transgenic models and of the mannitol to open the blood-brain barrier was evaluated. Finally, several histological studies were carried out to confirm the MRI results. In conclusion, the results obtained with these peptides are promising and prompt further studies.

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RAT BRAIN CONNECTIVITIES: COMBINED APPLICATION OF MAGNETIC RESONANCE IMAGING OF DIFFUSION TENSOR IMAGING AND MANGANESE-ENHANCED MAGNETIC RESONANCE IMAGING. Aurore Bogaert, Guadaluppe Soria, Mathias Hoehn. In-vivo-NMR Laboratory, Max-Planck-Institute for Neurological Research, Cologne, Germany; contact e-mail: abogaert@nf.mpg.de.

Introduction: Diffusion tensor imaging (DTI) has been described as a powerful technique of MRI to describe the architecture of neuronal circuits in adult or neonatal human brain in vivo (Huppi et al, 2006). By applying this technique on small animals, the systematic monitoring of brain diseases and recovery effects should become accessible. DTI studies on small animal had been conducted ex vivo, due to the necessary long experiment time in order to have results comparable to human studies (Wang et al, 2006). Some encouraging recent studies (Sizonenko et al, 2007; Mayer et al, 2007; Jiang et al, 2006) showed results in vivo but with rather poor resolution at still a long experiment time. The aim of the present study was to find, improve, and optimize experimental parameters of DTI sequence in order to map neuronal tracts in rat brain with high spatial and temporal resolution and to combine such data for a correlation with parallel manganese-enhanced magnetic resonance imaging (MEMRI) results to investigate corticothalamic connectivity. **Methods:** Wistar rats were anesthetized with 1.5% isoflurane in O_2/N_2O (30%/70%). DTI was performed on a 11.7 T BioSpec system equipped with 600 mT/m gradient sets, using 2D multislice diffusion tensor EPI, resulting in quantitative maps of tensor, FA, and ADC trace values under BRUKER Paravision 4.0 software. MEMRI results on the same animals were obtained, 24 hr after stereotactic MnCl2 injection (200 nL; 0.3 M) in the cortex followed by ipsilateral stimulation. MEMRI was recorded with a Flash 3D T1-weighted gradient echo sequence. **Results:** With the optimized DTI sequence used, we have obtained in vivo a map of the rat brain with spatial resolution to characterize the main structures of the brain by FA, ADC values while assigning fiber orientations with quantitative DTI maps. The experiment time to cover the whole brain was 21 minutes. Comparison with MEMRI data permitted us to follow the corticothalamic connectivity and to gain structural information about the somatosensory pathway. **Conclusions and Perspectives:** With the present experimental conditions we are now able to perform longitudinal DTI studies in lesioned rat brain (eg, after stroke) in order to monitor structural changes as correlates for functional disturbances. This will be explored as a potential tool for the prediction of functional recovery.

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UPTAKE AND BINDING OF [18F]ALTANSERIN IN RAT BRAIN, WITH AND WITHOUT THE P-GLYCOPROTEIN INHIBITOR CYCLOSPORIN A. Mikael Palner,[1] Stina Syvänen,[2] Ulrik S. Kristoffersen,[3,4] Nic Gillings,4 Andreas Kjaer,[3,4] Gitte Moos Knudsen.[1] [1]Neurobiology Research Unit and Center of Integrated Molecular Brain Imaging, Rigshospitalet, Denmark; [2]Uppsala Imanet and Uppsala University, Uppsala, Sweden; [3]Cluster for Molecular Imaging, Panum Institute, Copenhagen University, Copenhagen, Denmark; [4]Department of Clinical Physiology, Nuclear Medicine & PET, Rigshospitalet, Denmark; contact e-mail: mikael@nru.dk.

Fluorine-18-labeled altanserin {3-(2-[4-(4-fluorobenzoyl)piperidin-1-yl]-ethyl)-1,2-dihydro-2-thioxo-quinazolione} is a widely used 5HT2A receptor-selective positron emission tomography (PET) tracer (Ki 0.13 nM) in humans. Ex vivo studies in the rat show, however, that altanserin has a limited brain uptake and the altanserin binding potential (BP2) is highly variable with a low reproducibility. We hypothesized that altanserin—in parallel to what has been reported for the radiotracers [11C]carazolol, [11C]verapamil, and [18F]MPPF—is a substrate for P-glycoprotein (P-gp), an efflux transporter in the blood-brain barrier. Small-animal PET was used to estimate brain percent standard uptake value (%SUV) of radioactivity in control and CsA-treated rats. [18F]Altanserin (1–10 MBq) was injected IV and arterial blood samples were obtained from a femoral cathether during the scan. To assess the specific binding before and after CsA treatment, a displacement study with IV injection of the potent 5HT2(A/C) antagonist ketanserin was performed. The area under the arterial input curves (AUC) was computed to compare radioligand levels in plasma and blood. When normalized with the corresponding AUCs, brain images of CsA-treated rats had a 52% higher total brain uptake of [18F]altanserin, and a 6.46-fold increase in frontal cortex BP2 compared to rats injected with [18F]altanserin alone. The distribution of activity in the brain was found to be similar to the distribution found by in vitro 5HT2A receptor autoradiography. It is concluded that CsA pronouncedly affects [18F]altanserin uptake and binding in the rat brain. By contrast, [18F]altanserin shows a high uptake and binding in the human brain.

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NIGROSTRIATAL INVOLVEMENT IN ATAXIA WITH OCULOMOTOR APRAXIA TYPE 1:A FP-CIT-SPECT STUDY. Elena Salvatore,[1] Andrea Varrone,[2] Giuseppe De Michele,[1] Chiara Criscuolo,[1] Pietro Mancini,[1] Valeria Sansone,[2] Caterina Strisciuglio,[2] Valentina Scarano,[1] Marco Salvatore,[2,3] Sabina Pappatà,[2] Alessandro Filla.[1] [1]Department of Neurological Science, University "Federico II," Napoli, Italy; [2]Biostructure and Bioimaging Institute, CNR, Napoli, Italy; [3]Department of Biomorphologic and Functional Science, University "Federico II," Napoli, Italy; contact e-mail: elena.salvatore@unina.it.

Aim: To assess dopaminergic nigrostriatal function in ataxia with oculomotor apraxia type 1 (AOA1) patients by FP-CIT-SPECT. **Background:** AOA1 is an autosomal recessive neurodegenerative disease associated with mutations in the aprataxin gene. The main features are usually early-onset cerebellar ataxia, oculomotor apraxia, and peripheral neuropathy, possibly associated with choreoathetosis, dystonia, or cognitive impairment. A postmortem study in an AOA1 patient did not disclose any neuronal loss in the striatum and substantia nigra. **Materials and Methods:** Four AOA1 patients (3 M/1 F; mean age at examination: 44 ± 17 yrs; mean onset: 23 ± 13 yrs) and eight normal controls underwent SPECT with FP-CIT, a dopamine transporter (DAT) radioligand. All patients showed ataxia, neuropathy, and cerebellar atrophy at MRI. Only one patient had chorea. SPECT scans were normalized in the MNI space using SPM'99. Outcome measures were the specific:nondisplaceable binding ratio, V3" (ROIstriatum-ROIoccipital/ROIoccipital). Significance was assessed by *t*-test at the $p < .05$ level. **Results:** Overall, striatal DAT density was reduced by 22.9% in the four AOA1 patients as compared with controls ($p = .015$). Striatal DAT density was below 2 SD from the control mean in two patients. Average DAT density was reduced by 21.1% in the caudate ($p = .015$) and 22.8% in the putamen in AOA1 patients ($p = .025$). **Conclusions:** AOA1 patients showed a slight reduction in the average striatal DAT density compared with controls. Nigrostriatal abnormalities were bilateral and uniform in caudate and putamen. The nigrostriatal impairment appears to occur even in the absence of extrapyramidal features. Our data suggest neuropathological involvement of basal ganglia in some AOA1 patients.

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MULTIMODAL IMAGING OF NEURAL PROGENITOR CELL FATE IN RODENTS. Yannic Waerzeggers,[1] Markus Klein,[1] Uwe Himmelreich,[2] Hong-Feng Li,[1] Mathias Hoehn,[2] Andreas Winkeler,[1] Andreas H. Jacobs.[1] [1]Laboratory for Gene Therapy and Molecular Imaging at the Max-Planck Institute for Neurological Research with Klaus-Joachim-Zülch-Laboratories of the Max-Planck Society and the Faculty of Medicine of the University of Cologne and Departments of Neurology at the University of Cologne and Klinikum Fulda, Cologne, Germany; [2]Max-Planck Institute for Neurological Research with Klaus-Joachim-Zülch-Laboratories of the Max-Planck Society and the Faculty of Medicine of the University of Cologne, Cologne, Germany; contact e-mail: yannic@nf.mpg.de.

Objective: Sequential multimodal molecular imaging of fate and functional status of implanted neural progenitor cells (NPCs) in rodent brain. **Methods and Materials:** C17.2 NPCs were genetically engineered to stably express firefly luciferase (L), HSV-1 thymidine kinase (T), and green fluorescent protein (G). C17.2-LITG cells were inoculated into the left striatum of nude mice ($n = 5$) whereas Gli36dEGFR glioma cells were implanted on the contralateral site. C17.2-LITG cells were also injected within the right striatum either within pre-established Gli36dEGFR gliomas ($n = 2$) or as a mixture together with Gli36dEGFR glioma cells ($n = 4$). Control animals ($n = 2$) received only C17.2-LITG cells. All animals were examined with bioluminescence imaging (BLI) and 9-[4-[18F]fluoro-3-hydroxymethyl)butyl]guanine positron emission tomography (18F-FHBG PET). To verify tumor growth magnetic resonance imaging (MRI) was performed. **Results:** Using BLI, in all animals with bihemispheric cell inoculation, C17.2-LITG cells could be detected as an almost spherical signal with stable intensity over the experimental period. Beginning with day 8, stem cells started to migrate toward the contralateral tumor site. In no animal a complete shift of the BLI signal to the contralateral hemisphere could be detected. Moreover, 18F-FHBG PET did not visualize inoculated stem cells. Following unilateral co-injection of C17.2-LITG and Gli36dEGFR cells, specific signals could be detected with BLI and 18F-FHBG-PET with increasing signal intensities over time, suggesting uncontrolled C17.2-LITG proliferation. In control animals, uncontrolled NPC migration toward the cerebellum was observed. **Conclusion:** Our data indicate that molecular imaging should be used as a safety switch for early detection of aberrant NPC migration and proliferation.

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PK11195 BINDING TO THE PERIPHERAL BENZODIAZEPINE RECEPTOR FOR MONITORING MICROGLIA ACTIVATION IN GLOBOID CELL LEUKODYSTROPHY. Ilaria Visigalli,[1] Sara Belloli,[2] Maria Rosa Moresco,[2] Elisa Coradeschi,[2] Elia Turolla,[2] Mario Matarrese,[2] Letterio S. Politi,[3] Luigi Naldini,[1] Ferruccio Fazio,[2] Alessandra Biffi.[1] [1]San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET), San Raffaele Scientific Institute, Milan, Italy; [2]IBFM-CNR, Nuclear Medicine Department, San Raffaele Scientific Institute, University of Milan-Bicocca, Milan, Italy; [3]Neuroradiology Department, San Raffaele Scientific Institute, Milan, Italy; contact e-mail: i.visigalli@hsr.it.

Globoid cell leukodystrophy (GLD) is a lysosomal storage disorder (LSD) due to the deficiency of the enzyme galactocerebrosidase (GALC). The defect results in intracellular storage of undegraded metabolites in oligodendrocytes and Schwann cells, leading to severe demyelination. More recently, a pathogenic role of microglia activation has been suggested. Allogeneic hematopoietic stem cell transplantation (HSCT) has been shown to improve disease phenotype in GLD patients. Its efficacy relies on the replacement of activated microglia by donor-derived cells, providing reduction in local inflammation and a source of functional enzyme for correction of surrounding cells. The peripheral benzodiazepine receptor (PBR) is expressed in a negligible amount in normal brain parenchyma and at a high level on activated microglia. We assessed whether [11C]-PK11195 binding to the PBR could be used for measuring microglia activation in GLD mice. Applying a combined approach based on ex vivo radioligand binding techniques and immunohistochemistry on brains retrieved from GLD mice, we found a correlation between [11C]-PK11195 binding and immunoreactivity for activated microglia/macrophage markers. Their results were confirmed also by ex vivo autoradiography on mice brain slices. The distribution of [11C]-PK11195 binding into the brain varied over time, according to disease evolution, with a typical posteroanterior fashion. Interestingly, in GLD mice undergoing HSCT, we observed a marked reduction of [11C]-PK11195 binding when compared to age-matched untreated controls, thus confirming the progressive replacement of activated microglia by nonactivated, donor-derived cells. These results suggest that [11C]-PK11195 may be exploited for monitoring disease evolution and the efficacy of HSCT in GLD and possibly other LSD.

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SEX DIFFERENCES IN THE RELATIONSHIP OF REGIONAL DOPAMINE RELEASE TO AFFECT AND COGNITIVE FUNCTION IN STRIATAL AND EXTRASTRIATAL REGIONS USING POSITRON EMISSION TOMOGRAPHY AND [18F]FALLYPRIDE. Patrizia Riccardi, Sohee Park, M. Sib Ansari, Sharlet Anderson, Rui Li, Mikisha Doop, Benoit Dawant, Dennis Schmidt, Ronald Baldwin, Robert Kessler. Vanderbilt University, Nashville, TN, USA; contact e-mail: pitrizia.riccardi@vanderbilt.edu.

Studies of sex differences have revealed that dopaminergic neurotransmission is modulated by sex steroids. As dopaminergic neurotransmission is involved in the pathophysiology of neuropsychiatric disorders, we examined sex differences in the correlations of d-amphetamine (dAMPH)-induced displacements of [18F]fallypride in relation to affect and cognition. Six females and eight males underwent positron emission tomography (PET) with [18 F]fallypride before and 3 h after an oral dose of d-AMPH. Percent displacements in striatal and extrastriatal regions were calculated using both regions of interest analysis and on a pixel by pixel basis using parametric images of dopamine (DA) D2 receptor binding potential (BP). Neuropsychological testing was performed at baseline and 1 hour after dAMPH administration. Significant sex-related differences were seen in the correlations of regional dAMPH-induced DA release with changes in cognition and affect. Men but not women demonstrated significant positive correlations of DA release in the left thalamus and a trend level in the right substantia nigra with change in spatial working memory and in the left medial thalamus and temporal cortex with change in Stroop interference. Digit symbol search, a test of psychomotor speed, demonstrated a significant cluster in female subjects and a strong negative correlation in the right basal forebrain not seen in male subjects. Sex differences were seen in correlations of changes in positive affect with DA release in ROIs. dAMPH-induced DA release in the left substantia nigra was correlated with change in positive affect in males but not in females. There was a trend-level correlation of right ventral striatal DA release with positive affect in men and a nonsignificant correlation in females.

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ABNORMAL BRAIN INTEGRITY IN BOTH WHITE AND GRAY MATTER WAS OBSERVED WITH QUANTITATIVE DIFFUSION TENSOR IMAGING IN AN ALZHEIMER MOUSE MODEL ACCUMULATING NONFIBRILLAR INTRACELLULAR AMYLOID DEPOSITS (APP_T714I). Greetje Vanhoutte,[1] Bianca Van Broeck,[2] Christine Van Broeckhoven,[2] Samir Kumar-Singh,[2] Annemie Van der Linden.[1] [1]Bio-Imaging Lab, University of Antwerp, Antwerp, Belgium; [2]Neurodegenerative Brain Diseases Group, VIB, University of Antwerp, Antwerp, Belgium; contact e-mail: Greetje.Vanhoutte@ua.ac.be.

The amyloid cascade hypothesis states that Aβ plaques propagate the neurodegenerative cascade in AD. Still, in vivo surrogate markers of disease progression are required for measuring treatment effects of the putative disease-modifying therapies in development. Recently, Kumar-Singh et al described the APPT714I pathology that leads to intraneuronal nonfibrillar diffuse amyloid deposits.[1] This is one of the earliest AD pathologic events that yet results in neurodegeneration[2] and therefore makes this model well suited for studying early disease processes in AD. Brains of APPT714I transgenic mouse models ($n = 4$) at the age of 20 months were compared with age-matched controls ($n = 4$) for changes detectable with in vivo diffusion tensor imaging (DTI). All MR experiments were performed on a 7 T horizontal bore magnet (Bruker) using multislice DTI-EPI (30 directions). The primary finding of this study is that in a mouse model with accumulation of diffuse intracellular amyloid plaques, in vivo DTI demonstrates both white and gray matter anisotropy abnormalities. Anatomy-based ROI analysis observed significantly decreased FA in the corpus callosum, the hippocampus, and the somatosensory cortex due to genotype. As a result, examination of FA within the gray matter is rather a sensitive probe for microscopic changes in brain tissue, reflecting the progression of the disease in association with, in our case, the prevalence of the diffuse plaques in the cortex and hippocampus. This finding strengthens the hypothesis that diffuse nonfibrillar amyloid plaques have an essential role in AD pathology, which was undermined before.

1. Kumar-Singh S, et al. Hum Mol Genet 2000.
2. Van Broeck B, et al. Neurobiol Aging 2006.

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GRAFT OF ADULT BONE MARROW MESENCHYMAL STEM CELLS IN A RAT MODEL OF PARKINSON'S DISEASE. Gaëlle Bouchez,[1] Luc Sensebé,[2] Patrick Vourc'h,[1] Denis Guilloteau,[1] Pierre Charbord,[2] Jean-Claude Besnard,[1] Sylvie Chalon.[1] [1]INSERM U619, Tours, France; [2]INSERM-ESPRI EA3855, EFS, Tours, France; contact e-mail: bouche_g@med.univ-tours.fr.

Cellular therapy is an opportunity for treatment of Parkinson's disease. To validate this approach, we studied the effects of grafted adult bone marrow mesenchymal stem cells (MSCs) in a rat model. Animals were unilaterally lesioned in the striatum with 6-OHDA. Two weeks later, group I was not grafted, group II was sham grafted, and group III was grafted intrastriatally with MSCs cultured in an enriched medium. The number of amphetamine-induced rotations was measured during 6 weeks. One week after grafting, this was stably decreased by 50% in group III (10.8 [138} 1.7 vs 22.0 ± 2.1 turns/min before graft) whereas it remained constant in groups I/II. At 8 weeks post-lesion, several presynaptic dopaminergic markers were increased in the grafted group: TH+ neurons in the substantia nigra, 52.5 ± 8.2% in the lesioned vs intact side in group III compared to 24.2 ± 6.7% in group I/II; DAT in the striatum, 44.6 ± 9.1% vs 17.7 ± 6.3% in group I/II, and in the substantia nigra 50.5 ± 6.8% vs 23.4 ± 6.5% in group I/II; VMAT-2 in the striatum, 35.3 ± 5.1% in group III vs 17.9 ± 3.6% in group I/II, and in the substantia nigra 62.0 ± 7.6% vs 36.4 ± 8.1% in group I/II. We showed using microdialysis that while the pharmacologically stimulated release of dopamine was significantly reduced in the lesioned vs intact striatum of rats not grafted (452 ± 83 vs 1,.163 ± 228% basal release), it was similar in both sides in animals receiving MSCs (955 ± 115 and 752 ± 76%, respectively). These findings demonstrate that the graft of adult MSCs partially restore the dopaminergic markers and function in this model, thus being a potential therapy for Parkinson's disease.

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CHARACTERIZATION OF THE IMMUNE SYSTEM AFTER STROKE IN GFPT-CELL MICE: AN OPTICAL IMAGING STUDY. Abraham Martin,[1] Juan Aguirre,[2] Anikitos Garofalakis,[2] Heiko Meyer,[2] Dimitris Kioussis,[3] Clio Mamalaki,[4] Anna Planas,[1] Jorge Ripoll.[3] [1]Instituto de Investigaciones Biomédicas de Barcelona, CSIC, Barcelona, Spain; [2]Institute of Electronic Structure and Laser, FORTH, Heraklion, Greece; [3]National Institute for Medical Research, Medical Research Council; 4Institute of Molecular Biology and Biotechnology, FORTH, Heraklion, Greece; contact e-mail: ammfat@iibb.csic.es.

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Stroke-induced immunodepression was recently described in mice (Prass et al. J Exp Med 2003;198:725–36), but this response remains poorly understood. Immunodepression rends animals more susceptible to develop infection after stroke. Likewise, stroke patients often suffer infections, which are associated with further complications and mortality. The proposed work is the study of the response of the immune system after stroke in mice that express the green fluorescent protein (GFP) in T cells (de Boer J, et al. Eur J Immunol 2003;33:314–25). Permanent brain ischemia was induced by occlusion of the middle cerebral artery with craniotomy (Tamura et al. J Cereb Blood Flow Metab 1981;1:53–60). Animals were studied in vivo by optical imaging tomography and the tissues were processed postmortem for fluorescence-activated cell sorting (FACS) analysis. We studied the distribution of the GFPT cells in different tissues such as the thymus, cervical lymph nodes, and spleen at 0, 1, 2, 4, and 7 days post-ischemia. In parallel, blood samples were collected to study the percentage of GFPT-cell by flow cytometry at the same days. The number of GFP cells measured by FACS decreased at 7 days in the spleen, thymus, and lymph nodes in stroked animals compared with controls (sham operated). The number of T cells in blood started to decrease from 2 days, and this effect was maintained beyond 4 days. The tomographic fluorescence data detected by optical imaging showed a decrease in the lymph nodes and thymus over time after ischemia. Importantly, we found a linear correlation between the signal intensity obtained by tomography and the number of GFPT cells measured by FACS. So, we can conclude that brain ischemia in mice induces immunodepression as evidenced by a reduction in the number of T cells in blood and in different lymphoid tissues.

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MULTIMODAL IMAGING OF FUNCTIONAL BRAIN ACTIVATION BY FOREPAW STIMULATION IN RATS: PRELIMINARY RESULTS. Heiko Backes,[1] Yannic Waerzeggers,[1] Dirk Wiedermann,[2] Uwe Himmelreich,[2] Rudolf Graf,[2] Mathias Hoehn,[2] Andreas Jacobs.[3] [1]Department of Neurology, University of Cologne, and Max-Planck-Institute for Neurological Research with Klaus-Joachim-Zülch-Laboratories of the Max-Planck Society, Cologne, Germany; [2]Max-Planck-Institute for Neurological Research with Klaus-Joachim-Zülch-Laboratories of the Max-Planck Society, Cologne, Germany; [3]Laboratory for Gene Therapy and Molecular Imaging, Max-Planck Institute for Neurological Research with Klaus-Joachim-Zülch-Laboratories of the Max Planck Society and the Faculty of Medicine of the University of Cologne, and Center for Molecular Medicine (CMMC) and Institute of Genetics, University at Cologne, and Department of Neurology, University of Cologne, and Klinikum Fulda, Cologne, Germany; contact e-mail: backes@nf.mpg.de.

The long-term examination of neuronal disease's progress or response to therapy demands the use of noninvasive in vivo imaging methods. We developed a protocol for imaging brain functionality in terms of measuring the response of the lesioned brain area to an external stimulus using different imaging modalities. The effects of electrical forepaw stimulation of a rat on the glucose consumption in the related somatosensory cortex measured by 18F-FDG positron emission tomography (PET) and 18F-FDG autoradiography (AR) are compared with the change in cerebral blood flow measured by MRI using the blood oxygen level–dependent (BOLD) effect. Although AR is the gold standard for the determination of glucose consumption, for the establishment of a long-term functional study, AR is not a useful candidate. We show that PET is capable of locating the stimulated part in the somatosensory cortex in the same way as AR and can therefore replace AR in its ability of measuring glucose consumption. The activated brain region displayed in the 18F-FDG PET matches the region with increased BOLD effect on functional MRI.

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OPTICAL IMAGING TO STUDY ESTROGEN RECEPTOR ACTION IN MAMMALIAN BRAIN. Azzurra Ravizza, Alessia Stell, Paolo Ciana, Adriana Maggi. Center of Excellence on Neurodegenerative Disease, and Department of Pharmacological Sciences, University of Milan, Milan, Italy; contact e-mail: azzurra.ravizza@libero.it.

The ability to obtain a dynamic view of the expression of selected genes in brain is severely limited by the complexity and heterogeneity of this tissue. By combining the power of molecular genetics and optical imaging, we succeeded in creating a novel methodology to measure the activity of transcription factors in well-defined brain areas. The system we developed was applied to the study of hormone-regulated activity of brain estrogen receptors in the reporter mouse model system ERE-Luc.[1,2] Gonadectomy severely reduced basal luciferase expression in male and female brains. Hormone replacement with increasing concentrations of 17beta-estradiol (SC) showed an estradiol-, dose-dependent induction of photon emission. The study of female mice during the estrous cycle provided the pattern of the changes in ER activity occurring with the cycle. All the above experiments show that the method set-up enables the quantitative assessment of ER activity in selected brain areas. This is of major interest for understanding hormonal or genetic effects causing gender-specific behaviors. Furthermore, this novel technology may be useful for the study of pathologies associated with female aging and for the development of drugs for hormone replacement therapies. The approach here presented can be generally exploited to the investigation of spatiotemporal activity of any transcription factors in brain regions during all mouse life, from embryos to adulthood.

1. Maggi A, Ciana P. Reporter mice and drug discovery and development. Nat Rev Drug Discov 2005;4:249–55.
2. Ciana P, Raviscioni M, Mussi P, et al. In vivo imaging of transcriptionally active estrogen receptors. Nat Med 2003;9:82–6.

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[18F]LBT-999, A NEW RADIOLIGAND TO STUDY THE DOPAMINE TRANSPORTER WITH POSITRON EMISSION TOMOGRAPHY: CHARACTERIZATION IN BABOONS. Wadad Saba,[1] Marie-Anne Schollhorn,[1] Heric Valette,[1] Sylvie Chalon,[2] Frédéric Dolle,[1] Lucette Garreau,[2] Patrick Emond,[2] J.B. Deloye,[3] Denis Guilloteau,[2] Michel Bottlaender.[1] [1]SHFJ/I2BM/DSV/CEA, France; [2]INSERM U619, Tours, France; [3]Cyclopharma, Clermont-Ferrand, France; contact e-mail: wadad.saba@cea.fr.

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The dopamine transporter (DAT) is the main regulator of the synaptic concentration of dopamine in the brain and plays a key role in many neurological and psychiatric diseases. The goal of the study was to characterize the properties of [18F]LBT-999 in baboons. After IV injection of a tracer dose of [18F]LBT-999 the highest accumulation was observed in the striatum with a peak uptake at 50 min (about 5% ID/100 mL). The radioactivity uptake peaked at 8 min in the midbrain (2.3% ID/100 mL) and decreased rapidly. The lowest uptake was observed in the cerebellum (0.4%ID/100 mL). In the plasma, [18F]LBT-999 was rapidly metabolized. Unchanged [18F]LBT-999 accounted for around 21% at 30 and 7% at 120 min. The region to cerebellum ratio reached a maximum of 22 in the striata at 110 min and remained stable until 240 min. This ratio was 4 in the midbrain and less than 2 in the thalamus and cortical structures. Binding potential calculated using a 2.2 in ± 2.9 in the putamen and 13.7 ± Simplified Reference Tissue Model were 17.2 caudate. Blocking studies using unlabeled LBT-999 prevent striatal accumulation (ratio was reduced by 96%), while desipramine pretreatment did not modify the striatal uptake. These results confirmed, in vivo, the specificity of [18F]LBT-999 for DAT versus the norepinephrine transporter. The high brain uptake, the selectivity and the specificity of [18F]LBT-999, as demonstrated in our study, indicated that this radiotracer is an excellent candidate for the in vivo imaging of the DAT in humans.

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IMPACT OF RECONSTRUCTION WITH SPATIAL RESOLUTION MODELING ON A CLINICAL POSITRON EMISSION TOMOGRAPHY STUDY OF THE DOPAMINE TRANSPORTER. Florent C. Sureau,[1] Claude Comtat,[2] Andrew J. Reader,[3] Claire Leroy,[4] Maria J. Santiago Ribeiro,[2] Regine Trebossen.[2] [1]Service Hospitalier Frederic Joliot, I2BM, DSV, CEA/Siemens S.A.S. Medical Solutions, Orsay, France; [2]Service Hospitalier Frederic Joliot, I2BM, DSV, CEA, Orsay, France; [3]School of Chemical Engineering and Analytical Science, University of Manchester, Manchester, United Kingdom; [4]Service Hospitalier Frederic Joliot, URM 0205, INSERM-CEA, Orsay, France; contact e-mail: florent.sureau@cea.fr.

Aim: Brain PET imaging in small structures is challenged by low resolution inducing bias in the quantification. Improved spatial resolution may be obtained using dedicated tomographs such as the ECAT HRRT (Siemens Molecular Imaging). New reconstruction schemes using a more comprehensive model of the acquisition system would fully benefit from their high intrinsic spatial resolution without increasing noise in the images. In this work we assessed the impact of reconstruction with resolution modeling on clinically relevant parametric images. **Methods:** Dynamic time series were acquired for 1 hour on the HRRT after injection of a selective DAT tracer ([11C]-PE2I) to five healthy volunteers. These data sets were reconstructed using an OP-OSEM algorithm that does not include any specific resolution model (OP-OSEM) and an OP-OSEM algorithm including a stationary spatial resolution model of the HRRT (RM-OP-OSEM). Anatomical regions of interest (caudate, putamen, ventral striata, and cerebellum) were drawn on MRI. The PET time series were used to compute the binding potential (BP) of [11C]-PE2I using a simplified reference tissue model with the cerebellum as the nonspecific region. **Results:** The definition of cortical and subcortical structures is visually improved on images reconstructed with RM-OP-OSEM. The BP values measured for all subjects are systematically and significantly increased in all regions of interest when using RM-OP-OSEM (from about 10% in the ventral striatum to 20–25% in the caudate and putamen). These results are in accordance with previous phantom studies.

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[11C] MP4A BAYESIAN QUANTIFICATION OF ACETYLCHOLINE ESTERASE ACTIVITY AT PIXEL AND REGION OF INTEREST LEVEL IN NORMALS AND ALZHEIMER PATIENTS. Ioana Florea,[1] Alessandra Bertoldo,[1] Rosamaria Moresco,[2] Assunta Carpinelli,[2] Andrea Panzacchi,[2] Valentina Garibotto,[2] Daniela Perani,[3] Maria Carla Gilardi,[2] Ferruccio Fazio,[2] Claudio Cobelli.[1] [1]Department of Information Engineering, University of Padova, Padova, Italy; [2]IBFM-CNR, San Raffaele Scientific Institute, University of Milan Bicocca, Milan, Italy; [3]San Raffaele Scientific Institute, University Vita-Salute San Raffaele, Milan, Italy; contact e-mail: florea@dei.unipd.it.

The aim of the study is the development of a method for a rapid and reliable quantification of [11C]MP4A PET images at both pixel and region of interest (ROI) level for the in vivo study of acetylcholine esterase activity (AChE). We implemented a bayesian mathematical approach to measure in normal controls (NC) and Alzheimer disease patients (AD) AChE activity in terms of the rate constant for hydrolysis of [11C]MP4A, k3. Bayesian k3 values were estimated both on a pixel by pixel basis or from TAC obtained from dynamic images. In both cases individual k3 values were obtained from automatically generated ROI. ROIs have been applied on PET images (parametric or dynamic scans) normalized to the MNI stereotactic space and coregistered to the single-subject MRI template using SPM2 software. Bayesian k3 values from areas with different levels of AChE activity were compared. We obtain low variation between pixel and ROI k3 estimates for both NC and AD in a region with low enzyme activity (CV = 5%) but high differences between pixel and ROI k3 estimates in an area with medium-high AChE activity (CV = 20% in NC and CV > 50% in AD). Our method, based on the automatic extraction of anatomical areas and on the application of a bayesian approach to both dynamic PET images or TAC, is an accurate method for the quantification of AChE activity in low-activity regions. The differences between pixel and ROI k3 Bayes estimates observed in area with medium-high AChE activity in AD subjects need further investigation.

Infection, Inflammation, and Other Topics

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INDUCTION OF DIFFERENTIATION OF MESENCHYMAL PROGENITOR CELLS INTO OSTEOBLASTS USING THE HSP70B PROMOTER AND HEAT. Claire Rome,[1,2] Eric Kaijzel,[2] Hetty Sips,[2] Karien de Rooij,[2] Geertje van der Horst,[2] Marcel Karperien,[2] Chrit Moonen,[1] Clemens Löwik.[2] [1]UMR5231 CNRS/Université Victor Segalen, Bordeaux, France; [2]Department of Endocrinology, Leiden University Medical Center, Leiden, the Netherlands; contact e-mail: c.rome@lumc.nl.

Rationale: BMP7 and RunX2 splice variant MASN could be used in bone tissue engineering, but currently used approaches in gene manipulation do not allow tight control of transgene expression in vitro and in vivo. The heat-inducible hsp70B promoter has the potential of robust gene regulation in vitro and in vivo. **Specific Aim of This Study:** To test the feasibility of the hsp70B promoter as an inducible promoter able to drive expression of transgenes that drive differentiation of mesenchymal progenitor cells (MPCs) into the osteoblast lineage, first in vitro and then in vivo. **Methodology:** 4D3 mouse MPCs are derived from KS483 cells and have been engineered to contain a unique single FRT entry site in the genome. Flp-mediated recombination is an efficient and reproducible method for the generation of isogenic-stable cell lines expressing any gene of interest. 4D3 MPCs were transfected with the Flp vector containing a hsp70B-luciferase, hsp70B-BMP7 or hsp70B-MASN cassette. First, the conditions for maximal transgene expression in MPCs should be optimized. To this end, stable cell lines, transfected with the Flp vector containing a hsp70B-luciferase, were utilized and a simple water-bath method for heat induction. The cells were subjected to different thermal treatments for different times and heat efficacy of the gene expression was evaluated by measuring luciferase activity. **Results:** We have successfully generated isogenic clones of 4D3 expressing hsp70B-BMP7, hsp70B-MASN, and hsp70B-Luc. Using the latter it was shown that heating induces luciferase expression, allowing analysis of kinetics of induced transgene expression.

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99MTC-NTP 15-5 AS A TRACER FOR EXPERIMENTAL OSTEOARTHRITIS IMAGING. Elisabeth Miot-Noirault,[1] Aurelien Vidal,[1] Philippe Pastoureau,[2] Jacques Bonafous,[3] Agnès Chomel,[2] Jean-Claude Madelmont,[1] Nicole Moins.[1] [1]UMR 484 INSERM, Clermont Ferrand Cedex, France; [2]Institut de Recherches Servier, Suresnes, France; [3]Centre Jean Perrin, Clermont Ferrand Cédex, France; contact e-mail: noirault@inserm484.u-clermont1.fr.

Background and Objective: Our lab develops the ''cartilage targeting imaging strategy'' with a tracer (99mTc-NTP 15-5) that selectively binds to the proteoglycans. This study performed in the meniscectomized guinea pig model aimed to demonstrate that 99mTc-NTP 15-5 would have pathophysiologic validity for in vivo osteoarthritis imaging. **Methods:** 99mTc-NTP 15-5 imaging was performed at 20, 50, 80, 115, 130, 150, and 180 days after medial meniscectomy ($n = 10$ MNX) or sham operation ($n = 5$) and scintigraphic ratios (operated/contralateral) calculated for femur (F) and tibia (T) areas. F and T ratios were compared with those of 99mTc-MDP bone scintigraphy. At study ending, autoradiographic analysis of joint 99mTc-NTP 15-5 distribution and macroscopic scoring of cartilage integrity were performed. **Results:** The high and specific accumulation of 99mTc-NTP 15-5 in normal cartilage that allowed a highly contrasted joint imaging was affected by osteoarthritis. In MNX animals, 99mTc-NTP 15-5 accumulation in cartilage within the operated joint versus contralateral was observed to change in the same animals as pathology progressed. During osteoarthritis progression, the time course of femoral and tibial scintigraphic ratios evidenced (1) an initial ''increased scintigraphic ratio phase'' associated with the hypertrophic response of cartilage and (2) a ''decreased scintigraphic ratio phase'' correlated with a decrease in proteoglycan content. No change in 99mTc-MDP uptake was observed over 6 months. Macroscopic analysis confirmed osteoarthritis features only in MNX knees. **Conclusion:** These results underlined the potential of 99mTc-NTP 15-5 for both the early diagnosis and longitudinal monitoring of osteoarthritis pathology at the molecular level.

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QUANTITATIVE IMAGING OF MOUSE THYROIDS WITH 99MTC AND 123, 131I USING NANOSPECT/CT. Domokos Mathe,[1] Ivan Foldes,[2] Lajos Balogh,[1] Gyozo A. Janoki.[1] [1]Nat. Res. Inst. Radiobiol. Radhyg. Budapest, Hungary; [2]BM Central Hospital, Budapest, Hungary; contact e-mail: dmathe@freestart.hu.

Aims: Production of quantitative in vivo data about isotope uptake of healthy mouse thyroids using Tc-99m and iodine isotopes. **Material and Methods:** Calibrated mouse thyroid phantoms were prepared and filled with Tc-99m pertechnetate or I-131 and I-123 solutions. SPECT measurements were performed on the phantoms (0.1–0.3 MBq of activity per thyroid model). Voxel of interest (VOI) analysis was performed on the data sets using a dedicated software InterView XP. After the phantom measurements, 3-3 mice were injected IV with respective isotope solutions, with activities ranging from 5 to 30 MBq. SPECT-CT imaging with VOI analysis was performed. Then mice were dissected and thyroid and salivary glands were removed and counted in a well-type counter. **Results:** Tc-99m and I-123 phantom measurements equalled original phantom activities with two-digit exactness if the total counts were over 3,0000,000. Imaging time was not longer than 40 minutes. With both isotopes, mouse thyroid VOI activities were identical to the measured ones with a 6% mean difference. I-131 measurements allowed a very good quality imaging even below 0.1 MBq, although imaging time was essentially longer, over 1 h. **Conclusions:** NanoSPECT/CT imager allows the exact quantification of iodine and Tc-99m isotope uptake of mouse thyroids, well discernable from the salivary glands. Submillimeter resolution imaging combined with quantification possibilities offers a new tool for therapeutic model experiments. High-energy gamma radiation of I-131 does not present a problem in imaging either.

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IN VIVO STUDY OF NEAR-INFRARED FLUORESCENT NANOPARTICLE BIODISTRIBUTION. Laura Calderan[1] Federico Boschi, Pasquina Marzola, Andrea Sbarbati. University of Verona, Verona, Italy; contact e-mail: laura.calderan@univr.it.

Nanotechnology represents a new frontier for science progress and there are great expectations in relation to potential diagnostic and therapeutic applications. Our current knowledge of nanoparticle toxicology is poor but suggests that nanoparticles may be able to have adverse effects at their portal of entry in organisms or may also escape the normal defenses and have diverse effects in other target organs (lungs, liver, kidneys, and other districts to study). Nanoparticles have no intrinsic toxicity but a supposed ''size toxicity.'' Tracers used are nanoparticles with optical properties: fluorescent semiconductors, about 15–20 nm in size. They are known as quantum dots (QDs). These nanoparticles fluoresce in a completely different way than do traditional fluorophores. In this work we have investigated the biodistribution of nanoparticles in an in vivo animal model: kinetic, T1/2, biodistribution, and tissue accumulation. We would extrapolate from optic parameters physiological ones, in specific districts so as liver and lungs that are the most probable targets of toxicity. For all experiments we have used a VivoVision Systems, IVIS 200 Series (Xenogen Corporation, Alameda, USA). We have used two groups of nu/nu athymic mice, pharmacologically anesthetized, about two tracer dosages (40 pmol/0.1 mL; 20 pmol/0.1 mL, IV). The acquisition protocol was about a first preacquisition and post tracer injection, to study kinetic and tissue accumulations of tracers in different time points (continuously for 3 h and after 24 h, 72 h, and 1 week tracer administration). Tracers used are nontargeted QDs, (Q-traker800, InvitrogenTM, Milan, Italy). Images are acquired and analyzed with Living Image 2.5 software (Xenogen Corporation).

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QUENCHING OF T1 RELAXIVITY IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS TARGETED WITH PARAMAGNETIC RGD LIPOSOMES. Maarten Kok,[1] Sjoerd Hak,[1] Willem Mulder,[2] Gustav Strijkers,[1] Klaas Nicolay.[1] [1]Biomedical NMR, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, the Netherlands; [2]Department of Radiology, Mount Sinai School of Medicine, New York, NY, USA; contact e-mail: m.b.kok@tue.nl.

Molecular MR imaging is a fast-growing field of research. Quantification of the MRI contrast changes in terms of marker activity remains very difficult due to limited knowledge about the dependence of local T1 and T2 changes on location and concentration at the cellular level. In vitro experiments give a better understanding of the behavior of contrast agents and the processes that are involved in contrast enhancement in vivo. To that aim, the uptake of both nontargeted (NT) and $\alpha v\beta 3$-targeted RGD-liposomes was investigated in human umbilical vein derived endothelial cells (HUVECs). Analysis with CLSM showed clear differences in uptake between RGD- and NT-liposomes. NT-liposomes showed a diffuse intracellular distribution, whereas RGD-liposomes were primarily localized in 1–5 μm structures in the perinuclear region. Surprisingly, in both experiments T1 decrease in cell pellets was similar for RGD- and NT-liposomes. Interestingly, ICP-MS data showed a threefold higher gadolinium concentration in cells targeted with RGD-liposomes compared to cells incubated with NT-liposomes. Cells incubated with NT-liposomes showed a linear relationship between gadolinium concentration and relaxation rate, resulting in a relaxivity (r1) of 3.7 mM-1s-1, which is slightly lower than the relaxivity of liposomes in bulk solution (r1 = 5–6 mM-1s-1). Cells incubated with RGD-liposomes showed a nonlinear relationship, ie, a decreasing relaxivity for higher concentrations of gadolinium, ranging from 2.8 to 0.3 mM-1s-1. It is proposed that the accumulations of liposomes, as observed with CLSM, cause quenching of T1 relaxation, due to a limitation of the water exchange between the aggregates and the cytosol.

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DISCREPANCY IN DATA OBTAINED FROM SMALL-ANIMAL POSITRON EMISSION TOMOGRAPHY AND DIGITAL AUTORADIOGRAPHY: IMPLICATIONS FOR TRACER VALIDATION. Ella Hirani, Rabia Ahmad, Erik Arstad. Hammersmith Imanet Ltd, GE Healthcare, Hammersmith Hospital, London, UK; contact e-mail: ella.hirani@ge.com.

Small-animal PET allows for dynamic studies of tracer candidates, but the method suffers from relatively poor resolution. In contrast, digital autoradiography allows for high-resolution imaging of tracer binding (in vitro and ex vivo) but lacks the dynamic component. Here, we compare data from small-animal PET and digital autoradiography using three PET ligands for the serotonergic system, [11C]MDL100907, a 5-HT2A receptor antagonist; [11C]WAY100635, a 5-HT1A receptor antagonist; and [11C]DASB, a 5-HT transporter blocker, and asses the implications for tracer development. With in vivo scanning, quantification of [11C]MDL100907 and [11C]WAY100635 binding is susceptible to spillover and partial volume effects because of the relatively diffuse distribution of the postsynaptic receptors. Unlike scan data, in vitro autoradiographs of [11C]MDL100907 showed that specific binding was confined and concentrated to discrete layers of the frontal cortex. In lesion studies where 5-HT1A autoreceptors in the raphe were eliminated, in vitro [11C]WAY100635 binding allowed rapid confirmation of a successful lesion, an effect not detected with scanning due to partial volume effects. Finally, in vitro displacement of [11C]DASB binding with increasing serotonin concentration suggested that the ligand is unlikely to be useful to measure fluctuations in endogenous agonist concentration with PET. In conclusion, small-animal PET is a useful tool for obtaining dynamic data, but the relatively low resolution limits make assessment of binding data difficult. On the other hand, digital autoradiography provides detailed data on tracer binding and distribution and should therefore be used in conjunction with PET for tracer validation.

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TUMOR VERSUS INFLAMMATION IMAGING. Anu Autio,[1] Tiina Pöyhönen,[1] Pauliina Virsu,[1] Satu Salomäki,[1] Sirpa Jalkanen,[2] Anne Roivainen.[1] [1]Turku PET Centre, Turku University Hospital, Turku, Finland; [2]MediCity Research Laboratory, University of Turku, Turku, Finland; contact e-mail: akauti@utu.fi.

Differentiation between cancerous growth and inflammatory reaction may sometimes be difficult. The glucose analogue 2-18F-fluoro-2-deoxy-D-glucose (18F-FDG) accumulates in the areas of high glucose metabolism, such as rapidly growing tumor or active inflammatory foci. However, FDG is not specific either for cancerous growth or inflammation, thus causing false-positive findings. Vascular adhesion protein 1 (VAP-1) is a human endothelial protein whose cell surface expression is induced under inflammatory conditions, thus making it a highly promising target molecule for studying inflammatory processes in vivo. We hypothesized that positron emission tomography (PET) with gallium-68-labeled 1,4,7,10-tetraazacyclododecane-N′,N′′,N′′′,N′′′′-tetraacetic acid conjugated synthetic peptide targeted to VAP-1 (68Ga-DOTA-peptide) could be feasible for differentiating tumors and inflammatory foci. We compared the biodistribution of 18F-FDG and 68Ga-DOTA-peptide using an experimental rat model. Athymic rats were SC implanted with human BxPC-3 pancreatic cells and sterile skin/muscle inflammation was caused by turpentine oil. Dynamic 2 h PET imaging and ex vivo organ distribution measures were performed 24 h after induction of inflammation. According to ex vivo biodistribution studies 18F-FDG showed a higher tumor/muscle ratio (7.88 $\pm$ 3.19 vs 3.41 $\pm$ 0.51), inflammation/muscle ratio (5.82 $\pm$ 2.97 vs 4.07 $\pm$ 0.97), and tumor selectivity index (1.82 $\pm$ 1.24 vs 0.85 $\pm$ 0.08) than 68Ga-DOTA-peptide. However, 68Ga-DOTA-peptide showed a better inflammation selectivity index (1.18 $\pm$ 0.12 vs 0.79 $\pm$ 0.51) than 18F-FDG. In addition, PET imaging with 68Ga-DOTA-peptide was able to visualize inflammation better than tumor. Our results indicate that 68Ga-DOTA-peptide targeting VAP-1 was capable of differentiating tumor from inflammatory foci in our animal model. However, it was less sensitive than 18F-FDG.

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CELL-SPECIFIC LABELING BY IMMUNOPORATION FOR MAGNETIC RESONANCE IMAGING MONITORING. Po-Wah So,[1] Amy H. Herlihy,[1] Jimmy D. Bell.[2] [1]Imperial College London, London, UK; [2]MRC Clinical Sciences Centre; contact e-mail: po-wah.so@imperial.ac.uk.

Imaging the dynamics of specific cell populations in vivo is essential for the development of cell-based therapies. Established methods of labeling cells for in vivo tracking by MRI often involve incubation of cells with superparamagnetic iron oxide nanoparticles (SPIOs), either in the presence or absence of transfection agents. This method is essentially nonspecific and would lead to labeling of all incubated cells. Although useful for monocultured cells, its use is limited for mixed cell populations. In this study, we investigate the use of immunoporation for SPIO labeling of cells. Immunoporation is achieved by binding of antibody-coated magnetic beads to specific cell surface antigens and then the shearing off of the beads by mixing, creating transient pores in the plasma membrane, allowing SPIO entry into the cells. Immunoporation performed on IGROV1 cells in the presence of SPIOs (≈50 nm) exhibited negative enhancement in the MRI image (TR/TE = 2000/200 ms) compared to cells only exposed to SPIOs, the former cells also having a significantly higher T2 value ($p < .05$). Viability as assessed by trypan blue exclusion was, however, generally lower in the cells exposed to both immunoporation and SPIOs compared to those exposed to SPIOs only. Thus, these preliminary data suggest that cell-specific labeling for MRI can be achieved with high viability by immunoporation. This method has the advantage of labeling only specific cells in a mixed cell population due to the employment of the antigen-antibody reaction in the immunoporation process, as compared to other established cell labeling methods.

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TRACKING AUTOIMMUNE DISEASE BY IMAGING OF NUCLEAR FACTOR KAPPA B ACTIVATION IN LIVE MICE. Harald Carlsen,[1] Ludvig Munthe,[2] Michael Mehrdad Zangani,[2] Audun Os,[2] Harald Hauglin,[1] Anders Kielland,[1] Rune Blomhoff,[1] Bjarne Bogen.[2] [1]Department of Nutrition, University of Oslo, Oslo, Norway; [2]Institute of Immunology, University of Oslo, Oslo, Norway; contact e-mail: harald.carlsen@medisin.uio.no.

It would be desirable to have early and sensitive detection of autoimmune disease in intact animals. NF-κB is a transcription factor that has been associated with inflammatory responses and immune disorders. We have here investigated if NF-κB activation, detected by bioluminescence in intact mice, could be an early and sensitive indicator for development of autoimmune disease. B cells present endogenous Ig V region peptides (idiotypic [Id] peptides) on their MHC class II molecules to $CD4^+$ T cells. Chronic Id-driven T-B collaboration in mice doubly transgenic for paired Ig and TCR transgenes has been described to result in systemic autoimmune disease with SLE-like features. Here an NF-κB-responsive luciferase reporter transgene was introduced into autoimmune doubly transgenic mice. Triply transgenic mice developed bioluminescent signals from diseased organs prior to onset of clinical symptoms and autoantibody production. Signals were obtained from secondary lymphoid organs, inflamed intestines, skin lesions, and arthritic joints. Signal intensity correlated with disease progression and presence of autoantibodies. Detection of luciferase by immunohistochemistry revealed NF-κB activation in collaborating B and T cells as well as in macrophages. The results show that in vivo imaging of NF-κB activation can be used for early and sensitive detection of autoimmune disease in experimental autoimmune models and should offer new possibilities for evaluation of anti-inflammatory drugs.

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ORGANIZATION OF NADPH OXIDASE ON HEMATOPOIETIC STEM CELL MEMBRANE INVESTIGATED BY SCANNING NEAR-FIELD OPTICAL MICROSCOPY. Maria Cristina Frassanito,[1] Claudia Piccoli,[1] Vito Capozzi,[1] Domenico Boffoli,[1] Antonio Tabilio,[2] Nazzareno Capitanio.[1] [1]Dipartimento di Scienze Biomediche, Università degli Studi di Foggia; [2]Dipartimento di Medicina Interna e Sanità Pubblica, Università degli Studi de L'Aquila; contact e-mail: cristinafrassanito@libero.it.

Scanning near-field optical microscopy (SNOM) is a high-resolution imaging technique that is gaining, in the last few years, a prominent role in cell biology research.[1] SNOM provides simultaneous topographic and fluorescence imaging of the sample with nanometric resolution (≈50–100 nm), thus overcoming the diffraction limit of light. In this study we exploited the SNOM approach to characterize the local distribution and organization of the plasma membrane NADPH-oxidase (NOX) in human hematopoietic stem cells (HSCs). The presence of NOX in HSCs is thought to have a functional role as O_2 sensor and/or as low-level ROS producer to be used as redox messenger for controlling cell growth and differentiation.[2] Given the harmful potential of ROS a fine-tuning of NOX activity is required. HSCs were seeded onto polylysine-coated glass bottom dishes and treated according to standard immunofluorescence protocol for NOX staining. The fluorescence image of HSC membrane displays a highly resolved spotted feature of the NOX antigen on the cell surface. Notably, some regions present a closer packing, suggesting the presence of specialized clusters. The high-resolution imaging obtained in this study suggests in HSCs the occurrence of integrated platforms where the assembly/activation of the membrane-bound NOX catalytic subunit to the regulatory subunits located near the cell membrane along with signal transducing factors[3] is possibly space-controlled.

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TARGETS AND STRATEGIES FOR IMAGING DYNAMICS OF MOLECULAR-CELL INTERACTIONS IN AUTOIMMUNE DISORDERS. Tomasz Soltysinski. Institute of Precision and Biomedical Engineering, Warsaw University of Technology, Warsaw, Poland; contact e-mail: solek@mchtr.pw.edu.pl.

Autoimmune disorders are the reason for serious complication or damage in functionality of human bodies. They affect multiple parts of the central nervous system (CNS), particularly tissue like thyroid gland, blood cells, or the hematopoietic system, leading often to unrecoverable damage of the target or its functionality. The study of the dynamics of particular cells and the molecular components of the immune system is still hard to perform in living organisms. The techniques of molecular imaging (MI) seem to be the most promising tool to monitor the immune system at work. A review of currently used techniques is presented and their possible applications in particular autoimmune disorders, especially those related to blood disorders like immunocythopenia purpura (ITP), are discussed. These imaging techniques are suitable to investigate in precision already known immunosuppressive therapies leading to understanding the underlying processes responsible for their effectiveness or its lack. A number of successful treatment methods for ITP, including intravenous gammaglobulin, corticosteroids, cytostatics, and monoclonal antibodies, have been proven to be clinically useful. However, these therapies usually fail on long-term scale. The molecular-cell interaction responsible for development of autoimmune disorders of this kind is poorly understood in vivo in humans and is hard or expensive to diagnose. In the scope of this study some possible targets (like mAb CD20) for MI in ITP and related disorders are discussed. Indication of novel targets and dynamic multimodal imaging strategies may lead to breaking through current limitations, opening the path toward human in vivo autoimmune system imaging.

Technology

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IN VIVO TOMOGRAPHIC AND ENDOSCOPIC IMAGING OF EXPERIMENTAL ORTHOTOPIC COLORECTAL CANCER. Sylvie Kossodo,[1] Jeff Peterson,[1] Steve Louie,[1] Akihiro Horii,[2] Tianyu Xie,[2] Wael Yared.[1] [1]VisEn Medical, Inc.; [2]Olympus Corporation; contact e-mail: skossodo@visenmedical.com.

The aim of this study was to detect colon cancer in an orthotopic tumor model using a fluorescent protease-activated near-infrared probe and multiple imaging modalities. CT-26 cells were implanted orthotopically into the colons of nude mice. Mice were injected with a cathepsin B–activated probe, imaged 24 hours later with a custom colonoscope and with fluorescence molecular tomography (FMT), a novel quantitative in vivo 3D imager. Results were corroborated by excision of the colons for ex vivo planar imaging and histology. We obtained in situ images and tumor fluorescence data with both endoscopy and FMT with a high tumor to background ratio and clear differentiation from the colons of control animals. Ex vivo imaging and histology confirmed the presence, localization, and size of tumors. Colorectal cancer can be imaged in vivo noninvasively with protease-activatable agents via endoscopy and 3D fluorescence tomography, validating the benefits of these new imaging modalities in cancer research.

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MONTE CARLO SIMULATION–BASED SCATTER ANALYSIS FOR CLEARPET NEURO. Anna Fulterer,[1] Stephan Schneider,[2] Thorsten Buzug,[3] Brigitte Gundlich,[4] Simone Weber.[4] [1]Institute of Material Physics, Technical University of Graz, Graz, Austria; [2]Department of Mathematics and Technology, RheinAhrCampus, Remagen, Germany; [3]Institute of Medical Engineering, University of Luebeck, Luebeck, Germany; [4]Central Institute for Electronics, Forschungszentrum Juelich GmbH, Juelich, Germany; contact e-mail: fulterer@sbox.tugraz.at.

Scatter reduces the image quality in positron emission tomography (PET). In small-animal PET, like ClearPET Neuro, organs with high tracer uptake, eg, heart or bladder, might be located close to the edge of the field of view (FOV), which causes scatter of activity into the FOV. In this paper we focus on the analysis of the different scatter components (eg, object scatter, scatter originating from gantry or animal bed) with activity in and outside the FOV as well as methods to estimate the scatter fraction of

the scanner. We use Monte Carlo simulations to examine both the origin of the scatter from outside the FOV and the relative importance of the gantry, animal bed, etc. as scatter medium and to test the different scatter fraction estimation methods. The results show that most scattered events with OFOV activity arise from scatter in the object and the animal bed. The amount of object scatter depends on the phantom geometry whereas the amount of gantry scatter is similar for the different phantom sizes and changes with source position. The investigation on the origin of gantry scatter revealed that the detected photons were mainly scattered in material in the FOV, ie, the PMMA animal bed. The results of these investigations are used for a new classification of coincidences that expands the current subdivision into ''trues,'' ''scatter,'' and ''random'' in order also to regard the source position (in and out of FOV).

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A COMBINED TWO-D/THREE-DIMENSIONAL APPROACH FOR SEMIAUTOMATIC VOLUME-OF-INTEREST SEGMENTATION. Gudrun Wagenknecht, Markus Losacker, Markus Surudo. Central Institute for Electronics, Research Center Juelich, Juelich, Germany; contact e-mail: g.wagenknecht@fz-juelich.de.

Aim: Volume-of-interest (eg, anatomical structure, tumor) segmentation is an important prerequisite for analyzing structure and function in molecular imaging research. The new 2D/3D approach should allow flexible volume-of-interest (VOI) segmentation with minimum user interaction. **Methods:** 2D sample slices of the VOI are segmented with a live-wire approach (LW).[1] The segmented 2D regions are interpolated to form a 3D volume, the interface between the 2D LW and 3D active-surface approach (ASM).[2] From this volume, a start mesh is generated and deformed until a balance between internal and external ''forces'' is found. The most important influence parameters are the GGVF field to attract the ASM to the edges and the Taubin filter approach to steer the smoothness of the surface. The result can be saved as surface mesh, binary, and gray value volume. MITK provides the interface to the user. **Results:** To show the advantage of this new approach, software phantoms of different size and image quality (noise level) were used to evaluate the segmentation performance quantitatively compared to a simple polygon initialization. The results show that for each phantom the LW-based initialization yields better or similar results with less than half the expense in user interaction. The application to patient and animal studies shows the performance for different kinds of VOIs. **Conclusions:** The hybrid 2D/3D approach is a good choice for VOI segmentation regarding user interaction and segmentation quality.

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AVIDIN-INDUCED CLEARANCE OF BIOTINYLATED PARAMAGNETIC LIPOSOMES FOR IMPROVED MAGNETIC RESONANCE MOLECULAR IMAGING. Geralda van Tilborg,[1] Willem Mulder,[2] Nico Sommerdijk,[3] Klaas Nicolay,[1] Gustav Strijkers.[1] [1]Biomedical NMR, Eindhoven University of Technology, Eindhoven, the Netherlands; [2]Mount Sinai School of Medicine, New York, NY, USA; [3]Biomedical Engineering, Eindhoven University of Technology, Eindhoven, the Netherlands; contact e-mail: g.a.f.v.tilborg@tue.nl.

Magnetic resonance molecular imaging requires strong accumulation of potent contrast agents at the targeted site and low background. Therefore, in the ideal case contrast agents should be rapidly removed from the circulation as soon as sufficient targeting has been obtained. In this study we describe the use of a so-called avidin chase for the fast clearance of biotinylated paramagnetic liposomes from the circulation. Bimodal liposomes were prepared to allow the visualization of these particles both with MRI and fluorescence microscopy. A total of nine mice were used. Groups 1 and 2 received a bolus of liposomes intravenously. After 30 minutes avidin (1) or saline (2) was infused through the same catheter. Group 3 received a bolus of nonbiotinylated liposomes, followed by the infusion of avidin. 3D-FLASH images of the abdomen were acquired at 6.3 T during 60 minutes. MRI showed a significant signal enhancement in the abdominal aorta after infusion of contrast agent, which persisted after infusion of saline. Comparable results were found for nonbiotinylated paramagnetic liposomes that were coinjected with avidin. The observed signal enhancement following injection of biotinylated liposomes rapidly decreased back to its initial value after the onset of avidin infusion. Avidin-chased contrast agents were cleared from the circulation by the spleen and liver. This strategy can be used to increase both the sensitivity and specificity of molecular MRI by enhancing the target to background ratio. This opens novel possibilities for the detection of weakly expressed molecular markers with MRI and the optimization of nanoparticulate contrast agent formulations.

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COMPOSITE MAGNETIC RESONANCE CONTRAST FOR CELL IMAGING USING SUPERPARAMAGNETIC IRON OXIDE PARTICLES AND GADOLINIUM CHELATES. Seung-Schik Yoo,[1] Matthew Marzelli,[2] Krisztina Fischer,[1] Jong-Hwan Lee,[1] Robert V. Mulkern.[3] [1]Department of Radiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; [2]Department of Biomedical Engineering, Boston University, Boston, MA, USA; [3]Department of Radiology, Children's Hospital in Boston, Harvard Medical School, Boston, MA, USA; contact e-mail: yoo@bwh.harvard.edu.

Contrast agents are utilized in magnetic resonance imaging (MRI) to visually exploit and enhance differences in physical structures and/or physiological processes. Gadolinium chelates (Gd-DTPA) and superparamagnetic particles of iron oxide (SPIO) are two commonly used MR contrast agents that exhibit inherently different relaxation properties. The creation of composite relaxation characteristics predicted from these contrast agents may be applied to contrast-enhanced dynamic MRI studies related to tumor oncology. In addition, it can be applied to recent cellular MRI techniques in which magnetically labeled cells can be traced and detected. The use of a single contrast agent in the context of cellular imaging may provide limited information (for example, hypointense signal from SPIO-labeled cells may be misinterpreted to be a site of hemorrhaging, or vice versa). Assuming minimal mutual interaction between these two agents, we were motivated to investigate the creation of composite relaxation properties by mixing the two in aqueous solutions. Concentration-dependent relaxivity coefficients were first obtained from each contrast agent, independently, in saline solution at 3 Tesla. These coefficients were then used to predict relaxation rates of a composite contrast agent using a linear model combining the effects of both contrast media. We found that the combination of SPIO and Gd-DTPA in an aqueous solution exhibits unique and predictable relaxivity properties that are unattainable via the individual use of either agent. The method may be applied to create 'user-tunable' contrast conditions for the visualization of magnetically labeled cells in the context of cell replacement therapy.

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INNER VOLUME APPROACH IN CELLULAR MAGNETIC RESONANCE IMAGING: PRELIMINARY EXPERIENCE. Seung-Schik Yoo,[1] Dimitrios Mitsouras,[1] Krisztina Fischer,[1] Jong-Hwan Lee,[1] Robert V. Mulkern,[2] Frank J. Rybicki.[1] [1]Department of Radiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; [2]Department of Radiology, Children's Hospital in Boston, Harvard Medical School, Boston, MA, USA; contact e-mail: yoo@bwh.harvard.edu.

Cell-replacement therapy refers to a set of therapeutic strategies to replace damaged or defective cells with cells of normal function or functional potency. One of the crucial elements is to monitor the survival, growth, and migration of implanted cells as well as to monitor the potential for tumor formation. Magnetic resonance imaging (MRI), with its interventional capability, has become an excellent candidate in visualizing cells labeled with MR-sensitive agents such as paramagnetic or superparamagnetic contrast agents or fluoride compounds (F-15). Nonetheless, high spatial resolution, on the order of submillimeter microscopic resolution, and a signal-to-noise ratio (SNR) appropriate for the detection of labeled cell are needed. Additionally, both must be achieved in a clinically acceptable imaging time and magnetic field strength (< 4 T). Because of these limitations, current cellular MRI has been conducted mostly on small objects using small-diameter coils to boost the SNR or by using ultra-high-field (> 7 T) scanners. The objective of the study is to develop and implement an MRI method capable of providing such flexibility using an Inner Volume 3-Dimensional Fast Spin Echo (IV-3DFSE) sequence, thus achieving near-microscopic spatial resolution (less than $400 \times 400 \times 500$ cubic μm) of a small localized volume located within a larger in vitro phantom. The SPIO-based dextran-coated contrast agent (Feridex, Berlex Inc.) was used to magnetically label the fibroblasts, and the location of the cells in the agar gel was visualized in near-microscopic spatial resolution ≈20 min using a conventional head coil.

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QUANTITATIVE REAL-TIME BIOLUMINESCENCE IMAGING IN FREELY MOVING RODENTS. Emilie Roncali,[1] Kelly Rogers,[2] Raphael Boisgard,[1] Philippe Brulet,[2] Serge Maitrejean,[3] Bertrand Tavitian.[1] [1]CEA, Institut d'Imagerie Biomédicale, Service hospitalier Frédéric Joliot, Laboratoire d'Imagerie Moléculaire Expérimentale, Orsay, France; INSERM, U803, Orsay, France; [2]Plate-forme d'imagerie dynamique, Institut Pasteur, Paris, France; 3Biospace Lab, Paris, France.

The increasing number of optical probes has promoted in vivo optical imaging to a versatile tool with applications in numerous fields such as oncology or real-time in vivo cell tracking. However, until now in vivo molecular imaging has been restricted to large time-scale processes (about 1 second), banning investigations of transient phenomena such as the activation of signaling pathways. New developments for whole-animal bioluminescence imaging have opened the way for real-time imaging in nonanesthetized and freely moving animals. The photon-counting technology provided by an intensifier tube coupled to a CCD camera has an exquisite sensitivity and enables us to perform fast imaging in the subsecond scale. Biospace Lab applied this technique to design an in vivo dedicated to BLI and FLI.®optical imaging instrument called the Photon Imager. Here we present an upgrade of our system that allows simultaneous recording of bioluminescent signals along with the tracking video of the animal, with a high time resolution.[1] Bioluminescence is recorded by an intensified CCD camera at 25 Hz, using the same instrumentation as the Photon Imager. The video tracking of the animal is provided by infrared lighting and a CCD camera. We present a description of the system and signal-to-noise and time resolution measurements. Validations of its ability to provide real-time imaging with a high time resolution and a good sensitivity were investigated in several murine models. Potential applications of this new methodology will be presented.

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COMPUTED TOMOGRAPHY–BASED ATTENUATION CORRECTION FOR SMALL-ANIMAL POSITRON EMISSION TOMOGRAPHIC IMAGES. Daniela D'Ambrosio,[1] Antonello Enrico Spinelli,[2] Carmelo Quarta,[2] Romano Zannoli,[3] Stefano Boschi,[2] Roberto Franchi,[2] Mario Marengo.[4] [1]Scuola di Specializzazione Fisica Sanitaria, Università degli studi di Bologna, Bologna, Italy; [2]Servizio di Medicina Nucleare Policlinico S. Orsola-Malpighi, Bologna, Italy; [3]Istituto di Cardiologia Policlinico S. Orsola-Malpighi, Bologna, Italy; [4]Servizio di Fisica Sanitaria Policlinico S. Orsola-Malpighi, Bologna, Italy; contact e-mail: d_ambrosiodaniela@hotmail.com.

Objectives: Photon attenuation is an important effect in PET imaging because it leads to inaccurate estimates of radiotracer concentration. The objective of this work was to compare two attenuation correction methods for small-animal PET images based on CT or segmented emission images. **Methods:** PET and CT images of a cylindrical rat-sized phantom (diameter equal to 5 cm) with a hot sphere were acquired using a small-animal PET (GE eXplore Vista) and a CT scanner (GE eXplore Locus). The phantom and sphere were filled in order to give a sphere to background ratio equal to 5. Images were then coregistered using rigid body transformation and an attenuation map at 511 keV was calculated from a CT image using a calibration function. The second method is based on an attenuation map obtained from a two-level segmentation process on a PET image: air and water attenuation coefficients at 511 keV were used. In order to compare the methods, ROI analysis of uncorrected and corrected images was performed and mean values were then compared. **Results:** Profiles showed counts and shape recovery in both corrected images. The increment of counts in the hot sphere with respect to the original image is 42% and 35% for CT- and emission-based methodz, respectively. **Conclusions:** Preliminary results showed that a segmentation-based attenuation correction method provides a good quantitative count recovery and requires less time with respect to the standard method based on CT images.

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QUANTITATIVE DYNAMIC IMAGING OF SMALL-ANIMAL POSITRON EMISSION TOMOGRAPHIC IMAGES USING CLUSTER ANALYSIS. Antonello Enrico Spinelli,[1] Sara Domenichelli,[2] Giovanni Testoni,[2] Stefano Boschi,[3] Roberto Franchi,[3] Mario Marengo.[2] [1]Servizio di Fisica Sanitaria Policlinico S. Orsola-Malpighi, Bologna, Italy; [2]Dipartimento di Fisica, Università degli Studi di Bologna, Bologna, Italy; [3]Servizio di Medicina Nucleare, Policlinico S. Orsola-Malpighi, Bologna, Italy; contact e-mail: spinellia@aosp.bo.it.

Introduction: In order to perform quantitative PET imaging it is necessary to acquire a dynamic scan to measure the arterial input function (IF) and the tissue time-activity curves (TACs). By combining IF and TAC with adequate mathematical models it is possible to obtain useful physiological information. The objective of this work was to implement a method for automatic curve delineation of small-animal dynamic PET studies using cluster analysis (CA). **Material and Methods:** CA allows us to group pixels having the same kinetic; in this work the K-means algorithm was applied. The user must supply the set of pixels that need to be grouped (images acquired at different time) and the number of clusters. The choice of the correct number of clusters was performed by using the Akaike information criteria (AIC). In order to test the proposed method a set of noisy rat cardiac images were simulated considering typical IF and myocardial FDG uptake. A figure of merit (FM) was calculated by taking the ratio of area

of difference between the true and clustered curve to the area under the true curve. **Results:** The values of FM were calculated for several noise realizations and the mean values were respectively equal to 8% and 3% for blood IF and myocardial uptake. The correct number of clusters (equal to 2) was obtained from the minim of the AIC. **Conclusions:** Preliminary results show that using CA it is possible to obtain accurate IF and TAC without the need for manual ROI delineation.

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OPTICAL IMAGING OF MULTIPLEXED MOLECULAR MARKERS IN VIVO AND EX VIVO. 34 James R. Mansfield, David L. Lynch, Peter J. Dwyer, Richard M. Levenson. Cri; contact e-mail: mansfield@cri-inc.com.

Insights gained in characterizing intracellular pathways and other cellular phenotypes have led to increased demands on all kinds of imaging systems, which now are being asked to report on the status of multiple targets simultaneously. One factor that has interfered with the ability to image fluorescently labeled markers in vivo has been unwanted autofluorescent signals. Multispectral imaging (MSI) methodologies can spectrally characterize and computationally eliminate autofluorescence, revealing otherwise invisible molecular targets. Application of MSI can increase sensitivity by orders of magnitude, allowing much less abundant (or dimly labeled) targets to be detected and measured. MSI also is a perfect complement to multiplexed analyses, with as many as five exogenous probes being imaged in vivo simultaneously. Microscopy-based multianalyte immunohistochemistry, in bright field or fluorescence, has many potential applications in the field of drug-target evaluation. However, accurate imaging of two or more colocalized antigens, especially chromogenically labeled ones, has been hindered by difficulty in discriminating and quantifying overlaying signals. MSI can resolve overlapping labels and generate quantitative images of individual analytes. As in the in vivo case, MSI is well suited to detecting and removing autofluorescence in fluorescence microscopy, allowing more sensitive and quantitative studies. Assessment of simultaneous (per-cell) expression of ER, PR, and Her-2 expression in breast cancer using chromogenic labels and the imaging highly multiplexed quantum dot–labeled immunofluorescence signals in tissue will be shown. Thus, MSI can contribute to a gamut of preclinical (small animal) and clinical applications. MSI is a clinically viable technique that holds great potential for further characterizing and subtyping cancers.

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NOVEL BAYESIAN CONSTRAINED SPECTRAL METHOD FOR CELL MORPHOMETRY IN MULTIMODAL MOLECULAR IMAGING. Tomasz Soltysinski. Institute of Precision and Biomedical Engineering, Warsaw University of Technology, Warsaw, Poland; contact e-mail: solek@mchtr.pw.edu.pl.

Molecular imaging is a rapidly growing field of powerful insight into cell and molecule interaction and dynamics. It takes advantage of numerous techniques, from multiwavelength lightning to methods of nuclear physics and histopathology. Its multimodal character requires methods of data analysis that take into account the mutual information gathered during multiple observations realized by different techniques. If the imaging process is described in terms of probabilities then the bayesian inference may be applied to maximize the probability that the multisource maximum knowledge about the object is gathered. This methodology allows for incorporating the knowledge taken in dynamic series or multimodal observations as well as the a priori knowledge. In this study a novel bayesian-spectral method is proposed to quantify cell morphometry from histopathological samples in angiogenesis research. The method is based on image content analysis done first by unique wavelet-based image processing techniques and followed by application of bayesian inference to delineate the cell's initial edge map. This map is further processed by a spectral method adapted to solve partial differential equation that reveals the most probable contour. As the contour is described as a function it may be easily analyzed and the cell dimensions may be quantified, providing an accurate base image for further mapping done on the basis of multimodal molecular imaging. The above method may be modified by manipulation of probabilities and the knowledge included into bayesian inference. This allows us to combine the histopathological, PET, SPECT, bioluminescence, and cellular MRI data to obtain the required information on cell-molecular dynamics.

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PHOTOACOUSTIC IMAGING FOR THE DETECTION OF TARGETED CONTRAST AGENTS. Marc Fournelle, Robert M. Lemor. Fraunhofer Institute for Biomedical Engineering, St. Ingbert, Germany; contact e-mail: marc.fournelle@ibmt.fraunhofer.de.

Background: In optoacoustic imaging the contrast formation for imaging depends on the optical parameters of the investigated tissue. The signal generation is based on the absorption of light and the respective heat generation in the tissue. When irradiated with ultrashort (ns) laser pulses pressure transients are generated in the tissue according to its optical absorption properties. These transients can be detected with ultrasound transducers. For imaging, the received signals are converted into a spatial representation of the absorbed energy. Nanoscaled particles, preferably gold, which strongly absorbs laser radiation and therefore alters the optical absorption properties of the target tissue, were suggested as targeted contrast agents for this imaging modality. **Aim:** In this study we investigated the possibilities of using optoacoustics for the detection of nanoscaled gold particles for the purpose of targeted contrast-enhanced imaging. **Methods:** A numerical simulation of the signal generation in tissue was designed using a Monte Carlo approach for tissue illumination and a linear wave propagation for acoustic detection. The influence of optical absorption and the geometry of the targeted region as well as the transducer transfer function on the achievable imaging resolution and sensitivity were investigated. Numerical results were compared to experimental measurements on tissue phantoms with different kinds of incorporated nanoparticles. **Results:** Phantom measurements are in good agreement with the theoretical expectations. Dependencies of excitation, absorption, and system parameters on the overall performance and the achievable spatial resolution and sensitivity are described. **Conclusion:** The theoretical and experimental evaluation demonstrates that optoacoustic imaging is a promising modality for molecular imaging especially for high-resolution imaging with a penetration range of centimeters and for small-animal imaging in preclinical research.

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HEMATOPORPHYRIN-MEDIATED FLUORESCENCE REFLECTANCE IMAGING MEASUREMENTS ON MICE FOR IN VIVO EARLY DIAGNOSIS OF A HIGHLY MALIGNANT THYROID TUMOR. Maddalena Autiero,[1] Rosanna Cozzolino,[2] Paolo Laccetti,[2] Marcello Marotta,[3] Maria Quarto,[1] Patrizia Riccio,[4] Giuseppe Roberti.[1] [1]Dipartimento di Scienze Fisiche Università degli Studi di Napoli ''Federico II, Naples, Italy; [2]Dipartimento di Biologia Strutturale e Funzionale Università degli Studi di Napoli ''Federico II,'' Naples, Italy; [3]Dipartimento di Medicina Clinica e Sperimentale Università degli studi di Napoli ''Federico II,'' Naples, Italy; [4]Dipartimento di Biologia e Patologia cellulare e Molecolare Università degli studi di Napoli ''Federico II,'' Naples, Italy; contact e-mail: autiero@na.infn.it.

We investigated the capability of fluorescence reflectance imaging (FRI) for the early detection of surface tumors in mice using a hematoporphyrin (HP) compound as an exogenous fluorescent optical contrast agent. The system for image generation and collection consists of a pulsed Nd:YAG laser (λ = 532 nm, energy/pulse = 30 mJ) and a low-noise, high-sensitivity, digital CCD camera. The camera lens provided a field of view of 10.2 × 7.8 cm^2 in order to image the whole mouse body. A cut-on long-wavelength pass filter (cut-on wavelength = 600 nm) allows HP fluorescence radiation to be recorded and backscattered radiation at 532 nm to be rejected. Highly malignant anaplastic human thyroid carcinoma was used as a tumor model. Tumor cells were implanted subcutaneously in the back of a mouse. The fluorescent marker (200 μL of aqueous solution of HP dichlorohydrate, at a concentration of 5 mg HP/mL distilled water), was injected intramuscularly in a posterior leg of the mouse. Fluorescence measurements were performed daily for 5 days starting from the third day after the cell injection and after 6 h from HP injection. The selective HP uptake by the tumor tissues was successfully observed. In fact, we observed the fluorescence of the tumor only 3 days after cancer cell injection, ie, when the tumor mass was neither visible to the naked eye nor palpable. Our results show that FRI is a suitable technique to perform the early tumor detection using HP compound at nontoxic concentrations and without photosensitization effects.

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A NOVEL HORIZONTAL NONCONTACT FLUORESCENCE MOLECULAR TOMOGRAPHY SYSTEM AND APPLICATIONS IN MULTICOLOR IN VIVO FLUORESCENCE IN MICE. Giannis Zacharakis,[1] Anikitos Garofalakis,[1] Rosy Favicchio,[2] Stylianos Psycharakis,[1] Clio Mamalaki,[2] Jorge Ripoll.[1] [1]FORTH-IESL, Heraklion, Crete, Greece; [2]FORTH-IMBB, Heraklion, Crete, Greece; contact e-mail: zahari@iesl.forth.gr.

Fluorescence molecular tomography (FMT) has emerged as a powerful tool for monitoring biological functions in vivo in small animals, providing the means to determine volumetric images of fluorescent protein concentration. Using different probes tagged to different proteins or cells, different biological functions and pathways can be simultaneously imaged in the same subject. In this work we present a new-generation noncontact FMT system and applications both in phantoms and in vivo in mice. It incorporates a horizontal positioning, which ensures the best comfort and robustness during the measurements and easy interchange between reflection and transmission geometries. The surface geometry of the subject is calculated using a 3D camera. An optical scanner is employed for the delivery of excitation light providing increased versatility and adaptability to the requirements of specific experimental arrangements and making it possible to implement more than one laser source for true multiwavelength excitation and multispectral detection applications. These include tomographic imaging of more than one fluorescent probe, as well as complete mapping and removal of autofluorescence. The technique is based on the recording of tomographic data in multiple spectral regions with different excitation lights and on the application of a linear unmixing algorithm for separating the emission of the different fluorescent probes. We show results of two-color imaging from phantoms containing two different fluorophores, as well as Dsred- and GFP-fused cells in F5-b10 transgenic mice in vivo. Furthermore, results from phantoms exhibiting different background autofluorescence strengths, such as Intralipid and TiO_2-based gels, are presented and discussed.

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SILICON PHOTOMULTIPLIERS FOR VERY HIGH-RESOLUTION SMALL-ANIMAL POSITRON EMISSION TOMOGRAPHY AND POSITRON EMISSION TOMOGRAPHY/MAGNETIC RESONANCE IMAGING. Gabriela Llosa,[1] Nicola Belcari,[1] Gianmaria Collazuol,[2] Alberto Del Guerra,[1] Sara Marcatili,[1] Sascha Moehrs,[1] Claudio Piemonte.[3] [1]University of Pisa and INFN Pisa, Pisa, Italy; [2]Scuola Normale Superiore and INFN Pisa, Pisa, Italy; [3]IRST, Trento, Italy; contact e-mail: Gabriela.Llosa@pi.infn.it.

Silicon photomultipliers (SiPMs) developed at IRST in Trento (Italy) are being evaluated at the University of Pisa for their use as photodetectors in the construction of a PET tomograph for small animals. The expected volume spatial resolution of the device is below 1 mm^3. In addition, the possibility of operating SiPMs in a magnetic field makes them the optimum photodetectors for a combined PET/MRI scanner. The devices have 625 microcells in an active area of 1 mm × 1 mm, a breakdown voltage around 30 V, and a gain about 106. The characterization measurements of the first devices have yielded very encouraging results. New devices with reduced noise and increased active area will soon be available. In addition to single-pixel detectors, SiPM matrices are also under development. The first test structures, composed of four (2 × 2) SiPM pixel elements, have already been produced. The good results obtained with these devices will lead to the construction of matrices with a higher number of pixels. Tests have been performed with LSO crystals coupled to both SiPMs and SiPM matrices. Moreover, tests in a magnetic field have been carried out, and no degradation of the performance has been found. The results will be presented.

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TISSUE-SPECIFIC SPECTRAL ANALYSIS IN FLUORESCENCE IMAGING. Rosy Favicchio,[1] Anikitos Garofalakis,[2] Giannis Zacharakis,[2] Kai Schoenig,[3] Kleio Mamalaki,[1] Sifis Papamatheakis,[1] Jorge Ripoll.[2] [1]FORTH-IMBB, Heraklion, Crete, Greece; [2]FORTH-IESL, Heraklion, Crete, Greece; [3]CIMH, Mannheim, Germany; contact e-mail: rosy@imbb.forth.gr.

Optical imaging systems are governed by the properties of light where scattering, absorption, and fluorescence are the major processes involved and propagation through turbid media such as tissue is dependent on the optical properties of the sample. There are an ever-increasing number of biological applications using primarily optical imaging techniques to assess the localization and quantitation of fluorescent probes and, nowadays, many fluorescent proteins are available on the market with different excitation and emission characteristics. Detection of fluorescence in vivo is, however, affected by a variety of parameters: tissue autofluorescence, excitation, and emission wavelengths in addition to the fluorophore's intrinsic properties. To study the effects of tissue autofluorescence on signal detection and its dependence on the above-mentioned parameters we have utilized a system consisting of a CCD-mount spectrograph with an optical fiber to collect spectra of multiple fluorescent proteins in transgenic mice and HeLa cell cultures. Excitation was performed with an argon ion laser emitting at 457 nm, 488 nm, and 514 nm. The autofluorescence profiles underline the spectral characteristics of the different tissue types and the effect this has on fluorescence detection. Results from the spectral analysis of a variety of organs from control, DsRed, and GFP F5/B10 transgenic mice showed that fluorophore detection by optical systems is highly tissue dependent. Spectral data collected from different organs can provide useful insight into experimental parameter optimization (choice of filters, fluorophores, and excitation wavelengths) and spectral unmixing can be applied to measure the tissue dependency, thereby taking into account localized fluorophore efficiency.

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PROPOSAL AND CHARACTERIZATION OF A HYBRID POSITRON EMISSION TOMOGRAPHIC/SINGLE-PHOTON EMISSION COMPUTED TOMOGRAPHIC SCANNER. Pedro Guerra, Jose Luis Rubio, Juan Enrique Ortuno, Maria Jesús Ledesma-Carbayo, Georgios Kontaxakis, Andres Santos. Universidad Politecnica de Madrid, Madrid, Spain; contact e-mail: pguerra@die.upm.es.

The simultaneous tracing of several biological markers may allow us to visualize interrelated biochemical processes such as metabolic pathways. The design of a hybrid PET/SPECT can achieve this goal, showing the spatial distribution of a compound marked with a positron emission isotope and one or more radiopharmaceutical emitting gamma-rays with lower energy. We present here the design considerations of a low-cost small-animal PET/SPECT scanner that uses four identical rotating detectors based on YAP/LSO phoswich. Each detector includes detachable collimators to allow different system configurations (pure PET, pure SPECT, and combined PET/SPECT). Each detector block will include a H8500 photomultiplier, analog interface, ADCs, and digital electronics for pulse characterization and data streaming. In this work the performance of the system has been characterized through Monte Carlo simulations, trying to adjust the system parameters to achieve results similar to state-of-the-art independent PET and SPECT scanners. The estimated performance has been characterized in terms of spatial image resolution and sensitivity for both PET and SPECT acquisitions. In the combined mode, a spatial resolution of 1.4 mm in PET and 2.5 mm in SPECT is achieved, with a sensitivity of 0.6% for PET and 0.025% for SPECT.

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INITIAL EXPERIENCES ON LOW-FIELD PARAHYDROGEN-INDUCED HYPERPOLARIZATION. Bob C. Hamans,[1] Marco Tessari,[2] Sybren Wijmenga,[2] Arend Heerschap.[1] [1]Department of Radiology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands; [2]Institute for Molecules and Materials, Physical Chemistry, Radboud University, Nijmegen, the Netherlands; contact e-mail: b.hamans@rad.umcn.nl.

Hyperpolarization of spin systems in various (biological) compounds can enhance their (N)MR signal by several orders of magnitude,[1–3] thereby enabling large improvements in sensitivity. Techniques like dynamic nuclear polarization (DNP) and parahydrogen (pH2)-induced polarization (PHIP) have been reintroduced as means of preparing hyperpolarized media for in vivo applications. PHIP can be used to achieve strong proton polarization. Hetero nuclei like 13C and 15N can be hyperpolarized by using the order contained in pH2 by, eg, applying methods described in Goldman et al.[4,5] A simple and cheap setup for achieving pH2 enrichment up to 50% was used here for performing earth and low-field hydrogenations (ALTADENA). A setup for high-grade pH2 enrichment (> 95%) and high-field PHIP (PASADENA) was presented earlier.[6] For NMR experiments acrylonitrile (CH2CHCN) and Wilkinson's catalyst (Rh(PPh3)3Cl) were dissolved in deuterated chloroform (CDCl3) and frozen solid in a 5 mm NMR tube. The tube was subsequently vacuumed and pressurized with four bars of 49% pH2. Before NMR measurements the tube was rapidly thawed and vigorously shaken for 30 s. Hydrogenations were performed at earth field (50 μT), at NMR fringe field (≈0.2 mT), and in a Mu-metal shielded environment (< 5 μT). Immediately after hydrogenation the tubes were inserted in a 500 MHz spectrometer and detection at 1 H frequency was performed. A flip angle of 5.0° was applied repetitively; 512 spectra were acquired in 4m16s. Signal enhancement due to parahydrogenation was observed in the samples hydrogenated at earth and fringe field. A simple setup for low-field PHIP was realized, which can be used to optimize polarization transfer to hetero nuclei.

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INITIAL RESULTS OF THE EXPLORE VISTA SMALL-ANIMAL POSITRON EMISSION TOMOGRAPHY/COMPUTED TOMOGRAPHY. Juan José Vaquero, Javier Pascau, Santiago Redondo, Angel Udias, Mónica Abella, Manuel Desco. Hospital GU Gregorio Marañón; contact e-mail: juanjo@mce.hggm.es.

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The performance of a newly introduced PET/CT system for small-animal imaging (eXplore VISTA/CT, GEHC) is presented. An annular PET detector system based on phoswich scintillators and PS-PMTs and a microresolution x-ray scanner have been integrated in a unique gantry with adjacent FOVs that are and mechanically registered. This configuration permits easy acquisitions of both anatomical and functional images in a single machine using a unified protocol. The CT can be operated in different modes ranging from fast, low-dose (< 60 HU SD for 5 cGy), low-resolution (200 μm) for animal position or accurate attenuation correction of the functional image to high-resolution (50 μm) mode for accurate 3D reconstructions. Planar projection angiography is also feasible for dynamic studies with contrast. The CT is based on a microfocus x-ray tube and a solid-state in cone-beam geometry. Image reconstruction is done with a modified Feldkamp algorithm. Filtering of the x-ray beam, dual-energy acquisitions, and gating are also supported. The PET section relies on the explore VISTA system, which has been integrated together with the CT without any mechanical or functional modification, thus preserving all its intrinsic specifications (resolution = 1.6 mm FBP, sensitivity = 4.0% for 250–700 keV). Although when the system can be used as PET or CT, a new developed protocol handles the multimodality acquisition optimizing the experiment procedures and minimizing the time per animal in the scan. Resulting images come intrinsically registered, allowing an integrated and effortless display and analysis.

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HIGH-RESOLUTION CONFOCAL MICROSCOPY AND NANOSCALE DISTANCE MEASUREMENTS SHOW DIFFERENCE IN THREE-DIMENSIONAL CONFORMATION BETWEEN ACTIVE AND INACTIVE MOUSE BETA-GLOBIN LOCI. Mariette van de Corput, Tobias A. Knoch, Gert van Cappellen, Ernie de Boer, Frank Grosveld. Erasmus MC, Department of Cell Biology and Genetics, Rotterdam, the Netherlands; contact e-mail: m.vandecorput@erasmusmc.nl.

3C technology shows that in erythroid cells the DNAse I hypersensitive sites (HS) of the mouse beta-major globin locus are in close vicinity toward each other from which intervening sequences loop out. This clustering of the HSs is, however, not present in nonerythroid cells. We have developed 3D DNA fluorescence in situ hybridization method and combined this with high-resolution confocal laser scanning microscopy followed by image restoration by deconvolution to allow accurate nanoscale distance measurements within a chromatin region of 175 kb that contains the beta-major globin locus. Results show that within an actively transcribing locus the distance between the 5′and 3′ end of the locus is 502 nm ± 167 nm and inactive loci is 586 nm ± 258 nm. Frequency histograms show that the distribution of distance between the 5′ and 3′ end of the locus is more stable and distinctive in erythroid cells. In nonerythroid cells the distribution is broader, indicating a more randomly organized chromatin structure. Distances between the two globin alleles do not show preference of location toward each other in both cell types. In future, distance measurements across this entire chromatin region will elucidate the complete 3D structure of active and inactive m beta-globin loci.

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INTRINSIC RESPIRATORY GATING IN SMALL-ANIMAL COMPUTED TOMOGRAPHY. Julien Dinkel,[1] Soenke Heinrich Bartling,[2] Michael Grasruck,[3] Wolfram Stiller,[4] Wolfhard Semmler,[4] Fabian Kiessling.[2] [1]Department of Radiology, German Cancer Research Center, Heidelberg, Germany; [2]Junior Group Molecular Imaging, German Cancer Research Center, Heidelberg, Germany; [3]Siemens Medical Solutions, Forchheim, Germany; [4]Department of Medical Physics in Radiology, German Cancer Research Center, Heidelberg, Germany; contact e-mail: j.dinkel@dkfz.de.

Purpose: While currently all utilized means of gating in small-animal CT are based on extrinsic devices to derive a gating signal (cushion, laser), we developed and implemented a robust method for respiratory gating that works with the image data alone (intrinsic gating). **Materials and Methods:** Mice and rats with and without blood-pool contrast media were constantly scanned over 16 rotations consisting of 500 projections each in a flat-panel Volume-CT scanner. The relative z-positions of the center of mass of a constant region on the projection data that contained the diaphragm with adjacent structures were calculated. At every projection position the average of the z-positions from all other rotations was subtracted (normalization). Local maxima of the center of mass positions were used as gating references. With respect to this gating reference projections were selected and merged onto a new 360° data set for FDK reconstruction. An established extrinsic gating method based on a respiratory pillow was used as a comparison. **Results:** Extrinsic and intrinsic gating leads to the same image quality improvements in still images. Furthermore, extrinsic gating can also be used to calculate 4D time series of respiration. **Discussion:** It can be expected that this technique is a potent alternative to current methods of extrinsic gating in small animals because it is as reliable and robust as extrinsic gating methods but lacks the need for additional animal preparation and hardware. Furthermore, by utilizing a few more post-processing steps intrinsic cardiac gating might also become available.

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RETROSPECTIVE RESPIRATORY AND CARDIAC GATING IN A FLAT PANEL–BASED VOLUME-CT FOR MICE AND RATS. Soenke Heinrich Bartling,[1] Wolfram Stiller,[2] Michael Grasruck,[3] Wolfhard Semmler,[2] Fabian Kiessling.[1] [1]Junior Group Molecular Imaging, German Cancer Research Center, Heidelberg, Germany; [2]Department of Medical Physics in Radiology, German Cancer Research Center, Heidelberg, Germany; [3]Siemens Medical Solutions, Forchheim, Germany; contact e-mail: s.bartling@dkfz.de.

Purpose: Implementation and evaluation of retrospective respiratory and cardiac gating of mice and rats using a flat-panel Volume-CT prototype (fpVCT). **Materials and Methods:** Respiratory and cardiac gating was implemented by equipping an fpVCT with a small-animal monitoring unit. ECG and breathing excursions (pneumatic pillow) were recorded and two binary gating signals derived. Mice and rats were scanned continuously over 80 s after administration of blood-pool contrast media. Projections were chosen to reconstruct volumes that fell within defined phases of the cardiac/respiratory cycles. Chosen projections from all rotations were merged onto a new 360° by weighted interpolation. Then a modified FDK reconstruction was performed. **Results:** Multireader analysis indicated that in gated still images motion artifacts were starkly reduced and the diaphragm, tracheobronchial tract, heart, and vessels more sharply delineated in comparison to ungated images. From 4D series functional data such as respiratory tidal volume (mice: 0.21 ± 0.07 mL; rat: 1.52 ± 0.40 mL) and left cardiac ejection volume (mice: 0.02 ± 0.005 mL; rat: 0.17 ± 0.05 mL) as well as fraction (mice: 55 ± 6.9%; rat: 53 ± 10.4%) were calculated and matched well with values known from literature. Applied dose was 1.2 mGy/s air KERMA, resulting in an approximate exposure of 96 mGy per scan. Additional preparation effort per scan is low (< 2 min). **Discussion:** Implementation of retrospective gating in fpVCT improves image quality and opens new perspectives for functional cardiac and lung imaging in preclinical animal models. Applied dose is maintainable and even follow-up exams can be performed. In 4D time series the applied dose is fully utilized.

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PERFLUOROCARBON POLYMERIC CAPSULES AS CONTRAST AGENTS WITH TUNABLE SIZE AND ENHANCED STABILITY FOR ULTRASONOGRAPHY. Emilia Pisani,[1] Nicolas Tsapis,[1] Belfor Galaz,[2] Nicolas Taulier,[2] Erol Kurtisovski,[2] Olivier Lucidarme,[2] Jean-Claude Beloeil,[3] Brigitte Gillet,[3] Wladimir Urbach,[4] Elias Fattal.[1] [1]Université Paris-Sud, CNRS UMR 8612, Paris, France; [2]Université Paris 6-CNRS UMR 7623, Paris, France; [3]CNRS UPR 4301; [4]Ecole Normale Supérieure; contact e-mail: emiliaanna.pisani@fastwebnet.it.

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We present here an easy method for engineering versatile contrast agents with enhanced stability that can be used for ultrasonography. These contrast agents are capsules, prepared according to a modified emulsification-evaporation process, whose shell is made of poly(lactide-co-glycolide), a biodegradable and biocompatible polymer, and whose core is liquid perfluoro-octyl bromide. The method of preparation allows us to adjust the capsule size from 70 nm to 25 microns and the capsule thickness to radius ratio between 0.2 and 0.6. Ultrasonic properties were tested in vitro with a 50 MHz transducer: the signal to noise ratio can reach up to 15 dB for a 50 mg/mL suspension of 6 micron capsules and up to 6 dB for a 50 mg/mL suspension of 150 nm capsules. Capsule stability at 37°C in PBS was at least 4 hours. In vitro ultrasonic imaging of tubes containing capsules was achieved in both normal and tissue harmonic imaging (THI) modes. These results are very promising and future applications could include quantification of the perfusion of an organ or a tumor with ultrasonography. The encapsulation method could easily be scaled up for industrial applications.

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MULTITRACER IMAGING USING CDTE GAMMA-CAMERA FOR STUDYING MOLECULAR IMAGING FOR PLANT SCIENCE. Naoki Kawachi,[1] Shu Fujimaki,[1] Nobuo Suzui,[1] Satomi Ishii,[1] Noriko S. Ishioka,[1] Shimpei Matsuhashi,[1] Takahiro Satoh,[2] Tadayuki Takahashi,[3] Shinichiro Takeda,[3] Shin Watanabe,[3] Yasuhiko Iida,[4] Takashi Nakano.[4] [1]Plant Positron Imaging Group, Japan Atomic Energy Agency; [2]Department of Advanced Radiation Technology, Japan Atomic Energy Agency; [3]High Energy Astrophysics, ISAS-JAXA; [4]Faculty of Medicine, Gunma University; contact e-mail: kawachi.naoki@jaea.go.jp.

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We have developed imaging systems for plant science research, which images biological processes in living systems noninvasively, quantitatively, and repetitively. One is the positron-emitting tracer imaging system, which images the tracers of nutrients and pollutants in intact plants. In addition, for

the numerical analysis of plant physiological functions, tracer kinetics have analyzed with a simplified physiological model of test plants. On the other hand, we have developed a prototype of the multielement imaging system for plant study using a CdTe semiconductor detector, which has high-energy resolution. The feasibility of this system for gamma-ray emission imaging of radioactive multinuclide tracer was examined by imaging experiments with a plant. The distribution of the two sample tracers, technetium and thallium, fed to a tobacco plant was successfully visualized for each nuclide simultaneously. The presented imaging methods will yield plant molecular imaging, which visualizes dynamics of some competitive elements in intact plant, noninvasively and quantitatively.

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A UNSUPERVISED METHOD FOR THE SEGMENTATION OF RODENT WHOLE-BODY DYNAMIC POSITRON EMISSION TOMOGRAPHIC IMAGES. Renaud Maroy,[1] Raphaël Boisgard,[2] Claude Comtat,[1] Bertrand Kuhnast,[1] Frédéric Dollé,[1] Ramin Hamzavi,[3] Peter E. Nielsen,[3] Régine Trébossen,[1] Bertrand Tavitian.[2] [1]CEA/I2BM/SHFJ/LIME; [2]CEA/I2BM/SHFJ/LIME;INSERM U803; [3]University of Copenhagen, Copenhagen, Denmark; contact e-mail: renaud.maroy@cea.fr.

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Positron emission tomography is a useful tool for pharmacokinetics studies in rodents during the preclinical phase of drug and tracer development. However, rodent organs are small as compared to the scanner's intrinsic resolution. We present a new method, called local means analysis (LMA), for the segmentation of rodent whole-body PET images that takes these two difficulties into account by estimating the pharmacokinetics in the center of each organ. In whole-body numerical rat phantom simulations including 3 to 14 organs affected with physiological movements, LMA achieved a quite good segmentation quality and organ detection rate, while two other methods, k-means[1] and SCA,[2] failed to obtain a correct segmentation. LMA showed the best resistance to spillover. In each of a large set of preclinical images, six preselected organs were manually delineated (MD). LMA performed correctly, unlike k-means and SCA. The time activity curves (TAC) calculated with LMA also showed an excellent correlation with MD ([TACLMA = 1.029 × TACMD-7.742e-05, P<2.2e-16]). In addition, LMA was much faster, detected more organs, and extracted organs' mean TACs with a better reproducibility than MD. These results are in favor of small-animal dynamic PET scan segmentation with LMA and suggest that systematic use of LMA for the in vivo pharmacodistribution analysis could efficiently speed up the drug development process.

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1. Brankov, 2003.
2. Wong, 2002.

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SPECTRAL UNMIXING OF FLUORESCENT MARKERS AND HETEROGENEOUS AUTOFLUORESCENCE. Harald Hauglin, Harald Carlsen, Lene Gilen, Peter O. Hofgard, Miriam Zangani, Bjarne Bogen, Rune Blomhoff. University of Oslo, Oslo, Norway; contact e-mail: harald.hauglin@basalmed.uio.no.

We present a systematic method for improving in vivo fluorescence imaging by spectrally unmixing signals due to exogeneous target fluorophores from endogenous background autofluorescence. Spectral image stacks are acquired using multiple combinations of excitation and emission filters in a simple filter wheel–based fluorescence imager. Multivariate analysis of the autofluorescence response is used to determine spectral signatures and magnitudes of important autofluorescence components. This multicomponent description of autofluorescence in combination with spectral signatures of relevant exogeneous probes forms the basis of a constrained unmixing scheme. The method is demonstrated by tracking and unmixing fluorescence signals from DsRed and eGFP tagged tumor cells injected into nude mice.

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