poster

POSTER TOPICS

DEVELOPMENT IN IMAGING TECHNOLOGY

poster number: 001

A TARGETED FLUORESCENT IMAGING AGENT ALLOWS ENDOSCOPIC IDENTIFICATION OF DYSPLASIA IN BARRETT'S OESOPHAGUS

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Introduction: Molecular imaging of fluorescent probes in vivo has been hampered by a lack of inexpensive non-toxic, structurally stable probes, which can tolerate labelling without loss of activity, and be applied clinically using wide field-of-view fluorescence-capable endoscopes. We have hypothesised that specific alterations in glycosylation, in the sequence from normal oesophagus to adenocarcinoma (AC), could provide a progression biomarker, detectable by lectin-based fluorescence imaging. We have previously shown that glycan pathways undergo coordinated changes in expression, and that lectin microarrays could be used to identify probes that can detect some of those glycosylation changes in the progression sequence. Wheat germ agglutinin (WGA), a normal dietary constituent, is one of the lectins identified. Molecular imaging has been undertaken in Barrett's oesophagus (BE) because it is endoscopically accessible and has a dysplastic pre-cancerous stage, which if identified and treated, can prevent progression to AC.

Methods: Human biopsies (n=33) were incubated at the bedside with WGA-Alexa Fluor 680. The biopsy fluorescence was then measured using a Caliper IVIS 200 system. Oesophagectomy specimens, stapled shut along the gastric resection margin, were examined through the intact oesophageal lumen with an Olympus IVIS Lucera endoscope. WGA-Alexa Fluor 488 was applied through the endoscope instrument channel. The oesophagus was then opened longitudinally and re-imaged on the IVIS 200 system. Following imaging, the specimens were fixed and processed for histology. Specificity was confirmed using an N-acetyl glucosamine competition assay and by preincubation with neuraminidase. Statistics: Bonferroni correction applied to ANOVA calculations and a one-sided paired t-test were used to test for a decrease in signal in specificity assays. For *ex vivo* experiments a two-tailed t-test with a Mann-Whitney correction was applied for small sample size. Spearmen-rank correlation was used to measure correlation.

Results: Whole-biopsy WGA binding diminished ($P<0.0001$) in biopsies containing dysplasia and was due to specific binding (competition assay $P=0.03$, neuraminidase assay $P=0.01$). Whole organ imaging was performed following endoscopic application of WGA via the instrument channel and allowed imaging of otherwise undetectable dysplasia ($P=0.0002$). WGA binding was reduced in advanced cancer. The signal-to-background ratio (SBR) obtained was >5, where the signal from areas of high-grade dysplasia was >5-fold lower than the surrounding normal tissue. The mean SBR was 5.2±3.9 with a signal-to-noise ratio (SNR) of 30.3±15.1.

Conclusion: We have identified a molecular imaging probe for the clinical detection of dysplasia in the oesophagus. This probe generated a considerably greater SBR, compared to other large targeted molecular imaging agents in the literature, and could be imaged with high sensitivity and specificity, using clinically available fluorescence endoscopes. The next step is to use the technique for real-time guidance of histological sampling *in vivo*.

poster number: 002

SIMULTANEOUS IN VIVO STRUCTURAL AND MOLECULAR IMAGING OF APP23 MICE WITH A NOVEL FMT/MR HYBRID SYSTEM

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Introduction Hybrid imaging provides complementary biomedical information. In particular, FMT/MRI can visualize molecular activity, structure and function at high resolution. A novel FMT/MRI hybrid system has been used to characterize APP23 mice, a transgenic mouse model of Alzheimer's disease (AD). Structural information derived from MRI was combined with FMT measurements of the amyloid plaque load using the plaque-specific dye AOI987 [1].

Methods A novel insert for hybrid FMT/MR measurements inside a Bruker Biospec 94/30 MR scanner (Bruker Biospin MR, Ettlingen, Germany) has been developed. It integrates a surface coil for MR acquisition and a CMOS detector (CSEM, Switzerland) for optical acquisition. The sample is excited with a 671nm cw laser outside the magnet. (See abstract F.Stuker et al.). Experiments were performed in strict adherence to the Swiss Law for Animal Protection. Two 18 months-old APP23 mice were shaved on the head one day prior to measurement. They were anesthetized with 2.5% isoflurane in air/oxygen mixture (2:1) and placed on the heated FMT/MRI platform. Fish roe were placed on the shaved skull as geometrical reference points. At time point 0, AOI987 (0.1mg/kg, 0.01mg/ml) was administered via the tail vein. Simultaneous FMT and MRI measurements were performed at 1, 15 and 30 min post-injection. The optical signal at 680nm and 720 nm was collected from a 4.7x4.7cm2 ROI on the head using a 8x14 source excitation grid. MR images were acquired using a 2D FLASH sequence (TE/TR = 5ms/383ms, FOV = 2.2x2.1cm2, matrix size = 200x200, 8 averages) with 29 axial slices of 0.7mm thickness. MR data were denoised with a Wiener filter and segmented. Isosurfaces were determined to compute the top surface height map, which was interpolated to match the optical image pixel dimensions. The height map and the optical white light image were used for co-registration. Signals from fish roe and anatomical landmarks on the skull were interactively selected to compute an affine transformation between the two images. The co-registered surface was used in FMT reconstruction. Programming was done in Matlab (The Mathworks, Inc.), visualization in Volview (Kitware Inc.)

Results Simultaneous in vivo FMT/MR measurements are feasible. Artifacts from the camera power supply degrade the MR SNR - however, this can be accounted for by use of suitable MR sequences and subsequent denoising.Tthe MR image quality allowed accurate delineation of major brain structures at a nominal resolution of 110x100µm2 . The reconstructed AOI987 fluorescent signal revealed predominant dye accumulation in cerebral cortex. The AOI987 signal decreased over time due to fast elimination of non-specifically bound dye molecules.

Conclusions An in vivo application of a novel FMT/MR hybrid system was demonstrated on a mouse model of Alzheimer's disease. Future work on APP23 and wild type mice will verify the system's performance. The method can be extended to correlate brain structural and functional changes with plaque load on Alzheimer mice.

Acknowledgments: Dr. Esther Sydekum assisted with animal preparation. This work was supported financially by the EU-FP7 FMT/XCT project and by the Swiss National Science Foundation.

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poster number: 003

IMPROVED DETECTION OF CEREBRAL MICROBLEEDS WITH QUANTITATIVE SUSCEPTIBILITY MAPPING IN THE ARCABETA MOUSE MODEL OF CEREBRAL AMYLOIDOSIS

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Introduction: Cerebral microbleeds (CMBs) are radiological findings in patients with neurological disorders such as cerebral amyloid angiopathy and Alzheimer`s disease, and are indicative of an underlying vascular pathology. In this study, we investigated the feasibility of using quantitative magnetic susceptibility mapping (QSM), a novel technique based on gradient echo (GRE) phase data, for the detection of CMBs in the arcAß mouse (arcAß), a mouse model of cerebral amyloidosis [1]. For validation we compared QSM with histopathology. Furthermore, we investigated image quality and lesion conspicuity of QSM and compared it to conventional magnitude image, phase and susceptibility-weighted image (SWI) post-processing.

Methods: ArcAß and wild type controls (wt) at 16 month of age were scanned with a Bruker small animal MRI system operating at 400 MHz equipped with a cryogenic quadrature surface RF probe [2]. 3D velocity compensated GRE data were collected with TE/TR/FA=12/250/15° and a voxel volume of 60×60×60 µm³. Phase aliasing was resolved by using a 3D phase unwrapping algorithm [3]. Background phase contributions were eliminated using the projection onto dipole field technique [4, 5]. A triangular weighting function was applied to create a phase mask. SWI were then computed by fourfold multiplication of the phase mask with the corresponding magnitude image [5]. QSMs have been derived from phase data using a regularization procedure, minimizing the difference between the actual phase values and those computed on the basis of assumed values for the magnetic susceptibility [6, 7].

Results: Circular regions of high magnetic susceptibility were observed in the olfactory bulb, cortical and sub-cortical areas in the arcAß mice (n=4), but not in age-matched wt controls (n=6). Histopathological analysis of Prussian blue stained brain slices revealed structures that appeared as degenerated endothelial lining with hemosiderin deposits, indicative of CMBs. Phase, SWI and QSM images revealed a higher number of CMBs than magnitude images (15±3, 15±3 and 14±3 vs 12±3). The CNR of the CMBs were higher on phase, SWI images and QSM compared to magnitude images (9±2.5, 12.6±2.8, 10.6±1.1 vs 6.6±2.3). SWI, phase and magnitude show strong blooming of CMB area (0.131±0.144 vs 0.067±0.13 and 0.075±0.05 mm2 respectively) with SWI having the strongest overestimation of CMB extent. QSM corrects for the blooming effect with an estimated CMB area of 0.036±0.06 mm2, and shows significantly smaller variation in spatial extent and good correspondence with histopathological delineation of the CMBs.

Conclusions: QSM has an increased diagnostic sensitivity for the detection of CMBs and improved contrast compared to conventional GRE magnitude imaging, and depicts both the localization and spatial extent of CMBs with high accuracy. QSM may become a diagnostic important tool for studying CMBs in experimental models and in the clinic.

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poster number: 004

A COMPARISON OF IN VIVO FLUORESCENCE AND PET BODY-COMPARTMENT MODELLING OF THE TEMPORAL BIODISTRIBUTION OF DUAL-LABELLED CYCLIC RGD PEPTIDE

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Introduction *In vivo* fluorescence imaging has added an easy and economical modality to the rapidly growing field of molecular imaging. It offers the possibility to perform experiments analogous to bioluminescence imaging through fluorescent proteins or fluorophores, but at longer and more efficient wavelengths. A further distinction is the translational and research aspects enabling the imaging of fluorophore-labelled biologics and activatable reagents. One unique aspect limiting the sensitivity of *in vivo* fluorescence methodologies is the confounding effect of tissue autofluorescence, which can be addressed through the proper use of spectral imaging. However, like other molecular imaging modalities, reagent-based *in vivo* fluorescence imaging also has to contend with sensitivity and contrast problems due to non-specific signals and long wash-out times, both limiting the detection of specifically bound reagent and preventing accurate determination of uptake rates.

Methods To address this issue, we have developed a kinetic imaging modality, Dynamic Contrast Enhancement, or DyCE, that combines rapid imaging (up to 10 frames/s monochrome or 10 s/frame multispectrally) with an advanced data processing methodology. When combined, these allow determination of (1) the rates of change of the intensity of the fluorophore in each pixel of the image and (2) the rate of uptake and wash-out in the animal. By utilizing the uptake and wash-out rate information, a much higher contrast image of the accumulating fluorophore can be obtained in a much shorter period of time. In addition, body compartment data can provide information on the temporal distributions of a fluorescent agent during the experiment and can act as inputs for rate-of-change or body compartment models. To demonstrate the utility of DyCETM, an ???3 integrin-targeting ligand, c(RGDyK), was dual-labeled with 64Cu-DOTA and ZW-1 (NIR dye, abs. 800 nm) for in vivo fluorescence and PET imaging. The resulting probe, Na-ZW-1-Cys(64Cu-DOTA)-c(RGDyK), was synthesized and its chemical/biological properties were characterized. The initial biodistribution and dynamic PET imaging and fluorescence imaging studies were performed using U87MG tumor-bearing mice.

Results Sixty-min dynamic PET and fluorescence scans for the dual-labeled probe were implemented and a two-compartment model of pharmacokinetic analysis in tumor were applied with time activity curves derived from the series of dynamic images. A good correlation between PET and fluorescence modalities in probe concentration and spatial signal distribution were found.

Conclusions Although *in vivo* fluorescence imaging cannot be considered as quantitative as PET imaging, these findings suggest that dynamic fluorescence imaging is a useful tool for pharmacokinetic modeling and can potentially be used as a surrogate or initial screening for dynamic PET imaging in biodistribution studies.

poster number: 005

MOLECULAR PHOTOACOUSTIC MICROSCOPY USING NANOSCALED CONTRAST AGENTS

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Introduction Photoacoustic imaging is an emerging modality allowing the detection of biological structures with optical contrast and acoustical resolution[1]. Like conventional ultrasound, this modality is scalable with respect to the image resolution which enables imaging both on the cellular and the macroscopic level. Several groups have recently shown the feasability of photoacoustic molecular imaging in vivo. For enabling a more detailed understanding of the interaction of targeted photoacoustic contrast agents and cells, we developed a microscopic platform allowing photoacoustic imaging with cellular resolution.

Methods The imaging system (SASAM OPTO, Fraunhofer IBMT) is based on an inverted optical microscope (Olympus IX 81) combined with a high frequency ultrasound lens and a nanosecond pulsed Nd:YAG (? = 1064 nm) laser for signal generation. We used two nanoparticle types (Gold nanorods[2], Magnetite nanoparticles[3]) for showing the ability of the system to image small amounts of contrast agents bound to our cell models (SKBR3 and A549). Gold nanorods are generated by reduction of ionic gold in the presence of CTAB (cetyltrimethylammonium bromide) leading to the formation of gold colloid and further anisotropic growth of the particles. The synthesis of the magnetite nanoparticles is based on controlled hydrolysis of an aqueous ferric chloride solution and the polyamine spermine. Different heterobifunctional Polyethylenglycoles were used for coupling of the particles to biological ligands.

Results We used secondary fluorescent antibody and spectrometry to verify the successfull biological modification of the nanoparticles used as molecular contrast agents. In a first step, the sensitivity of the photoacoustic microscope was determined using phantom models with both nanoparticle types at different concentrations. In projection images of the samples having dynamic ranges of 30 to 40 dB, localized nanoparticles could be detected down to picomolar concentrations. The acquired invitro data showed the suitability of our platform for the sensitive detection of photoacoustic contrast agents with a resolution in the range of 3 µm. Since the modality intrinsically provides three dimensional data due to the acquisition of time resolved ultrasound signals, the functionalized nanoparticles could be localized in a 3D cell context.

Conclusions A microscopic photoacoustic imaging platform was established and its performance in term of sensitivity and resolution was characterized. We developed different types of nanoparticles and showed their detectability down to nanomolar concentrations in phantom models using our system. Further, 3D data allowing to assess the spatial distribution of nanoparticles bound to A549 cells could be acquired. On the macroscopic scale, molecular imaging is based on the generation of laser induced signals by agglomerations of defined cells expressing specific markers which enable the binding of targeted contrats agents. Our microscopic photoacoustic imaging platform allows studying this binding process - which is critical for specific imaging - on the cellular level. It therefore fills the gap between the biochemical aspects (contrast agent synthesis and coupling of ligands) of molecular imaging and the actual use as macorscopic diagnosis tool.

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OPTICAL CHARACTERIZATION OF BIOPSY SAMLES WITH COMPUTED TOMOGRAPHY

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Optical computed tomography has been applied in a variety of applications covering a wide range of scales from microscopy to mesoscopy and macroscopy [1]. Novel and exciting systems have been designed and realized for imaging in developmental biology, gene expression, disease monitoring, cancer treatment and others. The main advantage of Optical-CT is the reduced cost, ease of use and straightforward theoretical approaches. In principle it can be considered the optical equivalent of X-ray CT and it is applied to structures and samples where light absorption is the dominant interaction process. It has however, been shown to produce very valuable and qualitatively and quantitatively correct results in applications where the samples exhibit also significant light scattering. Such applications include Optical dosimetry in cancer treatment plans [2] and Optical Projection Tomography [3], even when the theoretical models used are based on simple photon backprojection algorithms using the Radon transform. Here we present a new system that takes these principles to the characterization of biopsy samples from skin cancer lesions.

The light source was a white LED array whith a diffuser placed in front of it. Diffuse light rather than a collimated laser beam was preferred for homogeneous sample illumination and for acquiring average values of the optical properties. The light was then guided onto a linear polarizer and after transmission through the samples it looses its linear polarization due to scattering. The samples were positioned on a rotational stage covering 360° of rotation. Light signals were detected by a CCD-Camera equipped with a 50mm f/28 objective and a polarizer which was adjusted either parallel or perpendicular to the polarization of the transmitted light. The whole experimental procedure was controlled with an in-house developed software and images were stored on a PC.

When the axes of the two polarizers were crossed only light transmitted through the sample was detected increasing thus the dynamic range of the method. During the experiment two measurements were acquired: i) a set of measurements with the sample every 5° and a full rotation of 360° and ii) a set of reference measurements without the sample. Analysis was performed using a filtered backprojection algorithm.

The reconstructions correspond to 3D attenuation maps of the samples, which can then be used for identifying the lesion, calculating its depth and characterizing its state (malignant or benign). A large number (n=20) of biopsy sample were measured, including atypical navi and malignant melamomas and the results are in excellent agreements with those provided by histology, demonstrating the potential of the technique.

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IMAGING PROTEASE ACTIVITY IN A GLIOMA MODEL WITH A BRAIN-FMT IMAGER

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Introduction: Tumor growth, angiogenesis, invasiveness and metastasis are main hallmarks that determine the malignancy of cancer. For all of them, remodeling of the extracellular matrix and the vascular or epithelial basal membranes is required and is orchestrated by several proteases (1), such as Cathepsin B, L, S and Plasmin. The main aim of this work is to develop a brain-fluorescence molecular tomography (FMT) setup that enables in vivo imaging of protease activity during the progression of a glioma model.

Methods: 2x105 GI261 cells were implanted in the right striatum. The activatable probe Prosense680 was used to assess protease activity (2). Measurements were performed over the time using a FMT setup adapted specifically for brain imaging. This new brain-FMT setup allows the combination of images recorded both in reflection and transmission mode in order to account for source profiles rendering reconstructions more robust.

Results: Using our recently developed brain-FMT imager images were recorded both in reflection and transmission mode. Their combination allowed to effectively account for source profile and thus enabled reliable three-dimensional reconstructions of the fluorophore concentration. The novel brain-FMT was applied to estimate protease activity levels during the development of the orthotopic GI261 glioma tumors using Prosenese680 as a reporter.

Conclusions: Determination of the protease activity over time during malignant processes constitutes a useful tool for follow-up studies of tumor progression and for evaluating the effect of experimental therapeutic treatments. Our brain-FMT imager shows great potential in efficiently and accurately quantifying the progression of orthotopically implanted tumors in the mouse brain. Current work includes incorporating anatomical maps as a priori information for improved reconstructions.

Acknowledgements: This research was supported by the E.U. FP7 Collaborative Project 'FMT-XCT'. A. Arranz acknowledges support from the Marie Curie Intra-European Fellowship program (FP7-PEOPLE-2010-IEF).

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poster number: 008

MOLECULAR IMAGING WITH PET-MRI IN THE ASSESSMENT OF BREAST TUMORS: PROOF OF CONCEPT

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Introduction: To demonstrate the feasibility of combined multiparametric PET-MRI for molecular imaging of breast tumors and to assess possible increase in diagnostic sensitivity and specificity.

Methods: 28 patients with breast lesions detected by mammography or ultrasound and classified as BIRADS 3-5 were included in this IRB approved prospective study. All patients were examined with dedicated ^{18}FDG-PET-CT and multiparametric MRI of the breast at 3T. Examinations were scheduled no longer than 7 days apart. MRI protocol included: 3D-^{1}H-magnetic spectroscopic imaging (MRSI) before application of contrast agent to avoid contamination of spectra, a diffusion-weighted sequence (DWI), a T2-weighted sequence and a combined contrast-enhanced high temporal and spatial resolution 3D-T1-weighted sequence before and after application of a standard dose Gd-DOTA. For PET-CT patients fasted at least 6 h before injection of approximately 300 MBq ^{18}F-FDG based on the patients weight. Scanning was started 45 min after injection. Blood glucose levels were <150 mg/dl. A prone PET dataset over the breasts was acquired using a positioning device allowing the same patient geometry as the breast MRI coil. CT data was used for attenuation correction. Co-registration of imaging data and image fusion were performed. PET-MRI was assessed for lesion morphology and EH-kinetics according to BIRADS, restricted diffusivity, increased Choline (Cho)-levels and 18FDG -avidity. An ADC threshold 1.25 x10-3mm2/s and a signal-to-noise ratio of the Cho resonance peak >2.55 were defined as a marker of malignancy. Lesions classified as positive when ^{18}F-FDG-uptake was greater than blood-pool activity. All lesions were histopathologically verified.

Results: PET-MRI achieved an excellent sensitivity of 95% and good specificity of 78% in the diagnosis of breast cancer. Diagnostic accuracy was 89%. The PPV was 0.9 (95% CI 0.7-0.97) and the NPV was 0.88 (95% CI 0.53-0.98).

Conclusions: Molecular imaging with PET-MRI enables an assessment of breast tumors with improved sensitivity, specificity and diagnostic accuracy.

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IN VITRO AND *IN VIVO* MOLECULAR IMAGING OF THE ESTROGEN RECEPTOR USING NOVEL ESTROGEN-RECEPTOR-TARGETED MRI CONTRAST AGENTS

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The estrogen receptor a (ER), discovered 50 years ago (1), is a member of the nuclear receptor family and serves as a key regulator in the female reproductive organs, including the mammary gland. ER is over expressed in ~70% of breast cancers and is a well-established histological prognostic marker for breast cancer. The presence of high level of ER serves also to select patients for hormonal therapy.

We have developed molecular imaging means for measuring ER expression level in vitro and in vivo, using novel ER-targeted contrast agents based on pyridine-tetra-acetate-Gd3+ chelate (PTA-Gd) conjugated to 17ß-estradiol (EPTA-Gd) and to tamoxifen (TPTA-Gd) (2). As a control we also tested a new non-targeted contrast agent composed of the paramagnetic center of the above two targeted probes - PTA-Gd. The investigations focused on the mechanism of the paramagnetic induced water enhancement and the binding affinity to the receptor in solution, as well as on the biological activity and MRI relaxivity of these new probes in ER-positive versus ER-negative human breast cancer cell cultures and tumors *in vivo.*

Both ER targeted probes showed in solution a high affinity to ER, in the micromolar range. EPTA-Gd and TPTA-Gd exhibited in various ER-positive cells strong and mild estrogen-like agonistic effects, respectively, augmenting cell proliferation and inducing the expression of specific genes. Both probes also increased the T1 and T2 relaxation rates of water in MDA-MB-231 human breast cancer cells transfected with ER as compared to the wild type-ER-negative cells of the same origin.

Imaging ER in vivo was investigated by monitoring contrast enhancement in orthotopic tumors of the MDA ER- positive and negative cells implanted on both sides of the same mouse. Furthermore, the pharmacokinetics of the new probes in the blood and their unique interactions in different tissues of the body were also monitored during the experiments. The dynamic datasets were analyzed pixel by pixel, employing principal component analysis (3). The results indicated specific interaction of EPTA-Gd with ER, enabling differentiation between ER-positive and ER-negative tumors. In contrast, the un-conjugated PTA-Gd agent acted as an extracellular probe and induced similar enhancement in both tumor types, indicating the same vascular perfusion capacity of the two types of tumors. In addition, we found that TPTA-Gd interacted selectively with components in muscle tissue and could not identify preferentially ER-positive tumors *in vivo*.

In summary, this work provides a basis for developing and applying novel MRI probes targeted to detect and localize ER-positive tumors *in vivo*.

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poster number: 010

A NEW MULTIMODAL PET/MRI PRECILINCAL IMAGING SYSTEM

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Introduction: Our aim was to develope a sequentially integrated magnetic resonance and a positron emission tomography imaging system. We present the first results, some test measurements of the system integration where PET and MR are working in a distance less than 10 cm sequentially.

Methods: We are presenting some preliminary specification measurement results for a new, compact, sequential PET/MR system for rodents. The PET/MR system was constructed on the basis of existing Mediso special permanent magnet 1 T MR subsystem minimizing external magnetic field and crystal-based PET detector technology. The Mediso nanoScan® PET/MR imaging system has 12 PET detector modules of 81x39 LYSO crystals (1.12x1.12x13mm), tightly packed (pitch 1.17mm, PF 92%) coupled to two 256-channel PSPMT's. To avoid inhomogeneities in the centre of the FOV, modular PET detector ring design was favored. PET is aligned axially in front of the MR component that has 1 Tesla field strength with 90 mm diameter and 60 mm high barrel FOV, specified with 100 micron areal pixel resolution. The center of the PET FOV is close to the MR magnet bore opening (distance: 6.425 cm). Animal is moved from the PET ring bore to the MR subsystem bore with a common animal bed (as in PET/CT devices). We have measured the PET ring performance with the working MR system using a 22Na point source and a 0.8-1.5 mm rods Micro-Derenzo phantom filled with 18F-FDG. We also present signal/noise ratio (S/N) of the MR subsystem before and after integration using a 35 mm diameter mouse coil.

Results: PET resolution in center FOV (2D-OSEM reconstruction) ranged from 0.97 to 1.34mm, sensitivity in the center was 7.40%. The MR subsystem S/N ratio was 714/mm3 before the integration. With working PET electronics and PET detector within the combined system MR S/N ratio was 664/mm3. Original MR subsystem specification is >500/mm3.

Conclusions: The nanoScan® imaging system fulfils the original PET and MR specifications. Cross-talk effects are not affecting imaging efficiency. The sequential Mediso PET/MR system presented here is a prompt, viable stand-alone solution providing high PET resolution and effective, flexible MR imaging in the same animal and imaging session with affordable throughput.

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A DEDICATED BREAST PET-CT SYSTEM FOR METABOLIC IMAGING OF BREAST TUMORS

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Introduction To demonstrate feasibility of a dedicated ^{18}FDG-PET-CT for metabolic imaging of breast lesions.

Material and Methods 103 breast tumors in 93 patients classified by mammography or ultrasound as BIRADS 4 or 5 were included in this IRB approved prospective study. Patients fasted at least 6 h before injection of approximately 300-700 MBq ^{18}F-FDG based on the patients weight. Scanning was started 45 min after injection. Blood glucose levels were <150 mg/dl (8.3mmol/l). All patients were subjected to 18FDG-PET-CT scanning using a combined PET-CT in-line system (Biograph, Siemens, Erlangen, Germany). PET data were acquired in the prone position similar to breast MRI to allow an optimal expansion of breast parenchyma. CT data was used for attenuation correction. PET scan was reconstructed applying a standard iterative algorithm [ordered-subset expectation maximization] into a 168 · 168 matrix. Dedicated breast ^{18}FDG-PET-CT was assessed for ^{18}FDG-avidity as well nodal status. Tumors within tissues of mild metabolic activity were classified as positive when ^{18}F-FDG uptake > blood-pool activity. Tumors within tissues demonstrating moderate or high physiologic activity were considered positive if the activity was greater than the adjacent physiologic activity. All tumors were histopathologically verified.

Results There were 33 benign and 70 malignant lesions. Mean histopathological tumor size was 28.8 mm (range 3-90 mm). 18FDG-PET-CT demonstrated a sensitivity of 94%, a specificity of 82% and a diagnostic accuracy of 90%. PPV was 0.92 (CI: 0.83-0.96) and NPV was 0.87 (CI: 0.71-0.95). The 6 false positive tumors were juvenile fibroadenomas and one chronic abscess. The 4 false negative tumors were all smaller than 1cm associated with very high physiologic activity of the breast parenchyma.

Conclusion Metabolic imaging of breast lesions with ^{18}FDG-PET-CT using a prone patient geometry is possible and allows a detection and assessment of breast tumors with a good sensitivity and specificity. False negative results seem to be influenced by tumor size (<1cm) and high physiologic background activity. Clinical Relevance Radiologist should be aware that lesions with high cellular density or inflammatory changes can mimic malignancy and lead to false-positive results in breast PET-CT.

poster number: 012

CUSTOMIZED AMINATED NANOPARTICLES FOR BIMODAL VISUALIZATION

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Purpose&Introduction: Creation, characterization, and comparison (with previously available designs) of a multimodal, functionalizable nanoparticle. Several previously unavailable design requirements necessitated the introduction of a new synhesis protocol: Long plasma half-life required a hydrodynamic target size of 35nm, small enough to evade fast reticuloendothelial uptake and large enough to avoid renal clearance. Stability requirements necessitated covalent cross-linking of the carbohydrate cage, nanomolar MR-detectability the creation of a superparamagnetic crystalline ferrite core, easy functionalization the introduction of sterically accessible amine groups.

Methods: Particles were precipitated from Fe2+ and Fe3+ (2:1 molar ratio) in water in presence of monodispersed 10kD dextran. Cross-linking was achieved with epichlorohydrin, amination with ammonia, purification with ultrafiltration, dialysis and ultracentrifugation. Particles and two commercial products (Endorem, Schering; Resovist, Guerbet) underwent core and hydrodynamic size determination (transmission electron microscopy, electron tomography, laser light scattering), amine group determination (incubation with fluorescin isothiocyanate), relaxivity measurement (1.5T, 3T, 7T, water and plasma, 20°C, 37°C), Fe2+/3+ ratio determination (Mössbauer spectroscopy), physicochemical stability assessment (zeta potential measurement, laser light scattering during thermal cycling 20°C-80°C). Biological compatibility was assessed by Resazurin and LDH assay on LLC-PK1 cells, internalization was measured for three cell lines (HAEC, HASMC, HT29).

Results: Core size was 4.8nm, hydrodynamic size 38.7nm. Size alternations upon cycling were <2%. Iron content was 1000-2000 atoms Fe per particle. The number of sterically accessible amine groups averaged 17. The cores showed cubic magnetite structure. R1 and R2 were 12.49 and 140.52 msec. Cellular viability was unchanged after incubation, no internalization was observed. Zeta potential was -30.7mV and -7.25mV before and after cross-linking.

Conclusions: We created a biocompatible, functionalizable, stable nanoparticle with smaller size and therefore potentially longer plasma half-life, yet comparable relaxivity to commercial particles detectable in nanomolar concentrations with MRI as well as fluorescence and electron microscopy. Attachment sites allow for functionalization with diverse ligands, and potentially multivalent interactions with the target.

poster number: 013

MURINE CARDIAC IMAGES OBTAINED WITH FOCUSING PINHOLE SPECT ARE BARELY INFLUENCED BY EXTRA-CARDIAC ACTIVITY

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Introduction: Ultra-high-resolution SPECT images can be obtained with multi-pinhole collimators particularly when the pinholes are focused to a specific scan volume. Here we investigate the influence of high tracer uptake outside the scan volume on the reconstruction of tracer distributions inside the scan volume. The application being studied is 99mTc-tetrofosmin myocardial perfusion scanning in mice.

Methods: Projections were simulated of a digital mouse phantom (MOBY) in a focusing multi-pinhole SPECT system (U-SPECT-II, MILabs, The Netherlands). Differently sized user-defined volumes can be selected, by translating the animal in 3D through the focusing collimators. Scan volume selections were set to (i) a minimal volume containing just the heart, acquired without translating the animal during scanning, (ii) a slightly larger scan volume as is typically applied for the heart, requiring only small XYZ translations during scanning, (iii) same as (ii), but extended further transaxially, and (iv) same as (ii), but extended transaxially to cover the full thorax width (gold standard).

Results: Despite an overall negative bias that is significant for the minimal scan volume, all selected volumes resulted in visually and quantitatively quite similar images. Differences in the reconstructed myocardium between gold standard and the results from the smaller scan volume selections were small; the 17 standardized myocardial segments of a bull's eye plot, normalized to the myocardial mean of the gold standard, deviated on average 6.0%, 2.5% and 1.9% for respectively the minimal, the typical and the extended scan volume, while maximum absolute deviations were respectively 18.6%, 9.0% and 5.2%. Averaged over 10 low-count noisy simulations, the mean absolute deviations were respectively 7.9%, 3.2% and 1.9%. In low-count noisy simulations, the mean and maximum absolute deviations for the minimal scan volume could be reduced to respectively 4.2% and 12.5% by scanning the exterior only shortly and focusing the remaining scan time at the organ of interest.

Conclusion: In focused multi-pinhole SPECT, reconstructed tracer distribution in the myocardium is influenced by activity in surrounding organs when a too narrow scan volume is used. When selecting scan volumes as typically, this problem is adequately suppressed. This approach is more effective than employing a narrow scan volume with an additional survey scan.

poster number: 014

MULTI-ANGLE SPECT/OPTICAL/CT/X-RAY IMAGING OF BONE USING NUCLEAR AND FLUORESCENT BISPHOSPHONATE PROBES

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Introduction: Advances in pre-clinical imaging have afforded researchers options in imaging modalities. Understanding of the strengths and limitations of each modality can help ensure success during in vivo studies. Here we present the use of a high resolution trimodal nuclear imaging system (PET/SPECT/CT) and a commercially available multimodal imaging system (Optical/X-ray/Planar Radioisotopic) equipped with an animal rotation system to arrive at a SPECT/CT and multiangle Fluorescence/X-ray representation of bone remodeling. Through the use of multiple reporters, limitations of each modality could be minimized and produce more reliable data. SPECT imaging was used to overcome the inherent depth penetration and changes in reporter brightness with local chemical environment, while optical imaging was used to overcome the short imaging window of nuclear imaging and while minimizing use of and exposure to radioactivity. Labeled biosphosphonates were utilized as an example of a probe which can be labeled with radiotracers and fluorophores. Biosphosphonates bind to calcium in bone and are used to treat osteoporosis through apoptosis of osteoclasts as well as diagnose fractures, arthritis, and osteosarcomas. Incorporation of bisphosphonates into bone was measured by SPECT and optical imaging and co-localized to structural images of bone by CT and planar X-ray.

Methods: Nude athymic mice were injected with 2 nmol of bisphosphonate-near infrared fluorophore conjugate (Osteosense 750) and 40 µCi/g body weight of methylene-diphosphonate-technetium-99m conjugate intravenously (MDP) by way of the retroorbital sinus. SPECT imaging was completed on the mouse 30 minutes after dosing on a trimodal PET/SPECT/CT system equipped with two cameras consisting of a 50x50x4 mm CsI(Na) crystal coupled to a 64 anode PSPMT. MDP uptake into bone was imaged and directly fused to a CT image. At 24 hours post dosing, the mouse was imaged with the Carestream MS FX PRO imaging system equipped with an animal rotation system to arrive at a 360 degree representation of planar optical reporter (Osteosense 750) and X-ray images.

Results: SPECT/CT tomographic reconstructions gave rise to a three-dimensional representation of bone structure and activity while a 360 degree representation of planar fluorescence and X-ray images provided a much more complete representation of bone structures than in single view imaging.

Conclusion: The described method presented as a useful method for nuclear and optical co-localization of signals in bone and may prove useful in non-invasive imaging of drug delivery and bone abnormalities.

poster number: 015

VHIST/VINCI - SCRIPTING WITH META-INFORMATION

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Introduction: We have integrated VHIST [1] into VINCI [2], our package for visualization and analysis of tomographical data. This enables us to natively create VHIST files which can store meta data of original file formats, filter operations, 'image arithmetics' and registration parameters [3]. In a current project, we take advantage of remotely controlling (scripting) VINCI and VHIST from several languages (Matlab and Python), allowing for automatization of routine operations in multi-modality imaging with improved handling of meta-information.

Methods: VHIST has been developed to document workflows in multi-modality imaging. The VHIST file format is PDF compatible and it can be displayed with any PDF viewer ('human readable'). In addition, VHIST files also contain structured information on each workflow step (embedded XML) suitable for automated processing. Applications include the comparison of workflow histories e.g. image data of one subject which has been aquired by the same scanner but which has been processed in a different way (type and order of filter steps; thresholding; application of masks; different types of normalization, different reconstruction parameters; different approaches when converting raw data) [4].Previous use cases involved the creation of VHIST files from shell scripts (by commandline tools from the VHIST distribution, e.g. after a workflow step that calibrates image data from an experimental scanner) and the interactive use of VINCI with VHIST logging enabled, e.g. when subtracting images and all meta-information should be conserved.

Scripting VINCI and VHIST expands these capabilities: it allows easy access to meta-information from image data and operations (patient or animal id, date of acquisition, radiopharmaceutical used, etc.) from high-level programming languages (Matlab, in addition to previous Python bindings). This is a suitable basis for bulk processing data in directory trees (e.g. pseudonymization with additional plausibility checks) or exploratory sessions that combine the flexibility of an interactive Matlab session with an optional workflow history. We use sockets and pipes for interprocess communication on all platforms where VINCI is supported (MS Windows, MacOS X, Linux, Solaris). Our Python and Matlab bindings mostly provide language-specific high-level support for XML-based messages with the VINCI process, originally a by-product of our framework for automated testing.

Results: We believe that in particular the new Matlab support of VINCI and VHIST will prove useful for processing complex tasks in a more automated fashion. First results with new capabilities for exploratory, interactive sessions (remotely controlling VINCI/VHIST from Matlab) have been encouraging.

Acknowledgements: We thank R. Krais, M. Hoevels, K. Herholz, J. Matthews, J. Seehafer for support and fruitful discussions.

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poster number: 016

DISSECTING THE MAMMARY MICROSTRUCTURE AND BREAST CANCER CELLULARITY USING PARAMETRIC DIFFUSION IMAGING

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Diffusion provides a powerful source of contrast in MRI. The changes in the self diffusion of water in tissues are usually attributed to variations in the micro/cellular structures in the underlying tissue. We have previously investigated water self diffusion in the different microenvironments of orthtopic human breast cancer tumors in animal models (Paran et al. NMR Biomed. 2004, 17:170-180 and Israel J Chem 2003, 43:103-114). In addition, we applied diffusion tensor imaging for tracking the mammary ductal trees and identifying blockage of the ducts by cancer cells (Eyal et al, ISMRM 2010, p. 362; Eyal et al, ISMRM 2008. p. 508). Here we describe advanced imaging protocols and image processing tools for obtaining maps of the various diffusion parameters, characterizing the normal mammary gland, as well as cancerous and benign breast lesions.

Images were acquired at 3 Tesla. MRI diffusion protocols included fat saturated axial diffusion weighted images with constant diffusion time (CT) and varying diffusion gradient strength integral, and with constant diffusion gradient strength integral (CG) and varying diffusion times, as well as fat saturated axial multi-directional (30) diffusion-weighted images at two b-values. The spatial resolution in all diffusion protocols was 1.9x1.9x2.0 (or 2.5) mm3 and their total duration was ~10 min. Pixel by pixel analysis of the diffusion weighted images and construction of maps of the various diffusion parameters were performed using fast operating proprietary software. Analysis of the multidirectional diffusion weighted images yielded vector maps and parametric maps of the diffusion tensor eigenvectors, (v1, v2, v3), and eigenvalues, (?1, ?2, ?3), respectively, as well as diffusion anisotropy indices. These maps enabled dissecting the architectural features of the fibroglandular tissue, separating the ductal/glandular from the connective fibrous tissue and identify cancer growth. Analysis of CT and CG experiments yielded parametric maps of estimated intracellular and extracellular volume fractions and intracellular and extracellular apparent diffusion coefficients. These maps enabled the cellular compartmentation and histopathological characterization of breast cancers: cells in malignant breast tissue exhibit a different morphological features, size and density than cells in the normal fibroglandular tissue and in most benign lesions.

In summary, the various parameters characterizing water self diffusion in the breast facilitate the ability to differentiate breast cancer lesions from normal breast tissue and from benign lesions.

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poster number: 017

AN ITERATIVE ALGORITHM FOR THE PROJECTION IMAGE ESTIMATION FOR AN MLA BASED OPTICAL DETECTOR

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Objective: To improve spatial resolution and image contrast of a microlens array (MLA) based light detector intended for in vivo optical molecular imaging.

Method: An iterative approach has been developed to estimate the projection image detected from a microlens array based light detector. Due to its multilens structure an immediate image is not available but needs to be calculated. In our previous study we employed an inverse mapping algorithm to calculate the projection images [1]. Because of an overlap of mapped elemental images from the individual lens units, this algorithm yields rather high image blurring and is limited in its spatial resolution. In this work we model the image formation process of this specific optical detector as a system matrix A, which maps a projection image X at a reconstructed image plane to detected raw data Y at the sensor plane. Therefore, the estimation problem is to find an X, which solves AX=Y. The optimization process is based on the compressive sensing concept which minimizes the l_1-norm of a discrete gradient transform of X, subjecting to AX=Y. The problem is numerically implemented in an iterative process with an alternating manner which contains a standard SIRT (Simultaneous Iterative Reconstruction Technique) step to keep AX=Y, and a steepest descent step to minimize the l_1-norm of the discrete gradient transform of X.

Results: To evaluate this approach a Derenzo-like pattern and a Siemens star phantom were studied. In comparison with the inverse mapping algorithm the presented iterative estimation demonstrates a significant improvement in image contrast. The spatial resolution is also improved to 11.8%, 13.2% and 13.5% for 25 mm, 30 mm and 35 mm distance, respectively.

Discussion: The precondition of this approach, as well as the previous one, is that the distance between the imaging object and the light detector is known. This precondition can be overcome as our optical detector is supposed to acquire data simultaneously with other imaging modalities, such as PET. Hence the distance information of a three-dimensional object surface could be extracted from the secondary modality. With known surface information this approach can be repeated for different distances to generate a 3-dimensional volume of the imaged object.

Reference: [1] D. Unholtz et al. Image Formation with a Microlens-Based Optical Detector: a 3D Mapping Approach, Applied Optics, 48/2009:D273-D279

IMAGING CANCER TREATMENT AND EVALUATION

poster number: 018

INFLUENCE OF HUMAN MESENCHYMAL STEM CELLS ON ADENOCARNOMAS CANCER CELL PROLIFERATION IN SUBCUTANEOUS AND LUNG MODELS

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Introduction:Human mesenchymal stem cells (hMSCs) are mostly studied for their potential clinical use in regenerative medicine. Recently, much attention in the field of cancer research has been paid to hMSCs. Indeed they have been found to have a central role in the pathogenesis and progression of tumours. Because there is evidence that tumours can be considered sites of potential inflammatory cytokine and chemokine production, these properties may enable MSC to home to the tumour environment.

Methods: In this study, we investigated the influence of hMSCs on the proliferation of TSA mouse adenocarnomas cell line stably transfected by Luciferase (TSA-Luc+) in Nude mice. Cancer cells were injected subcutaneously or by intravenous way to obtain lung tumours. After co-injection or sequentially injection of hMSC, we evaluated the tumour growth by bioluminescence imaging, the tumour volume evolution and we quantified the haemoglobin rate.

Results: Our results demonstrated that hMSCs decrease the tumour growth. But subcutaneous transplantation of TSA-Luc+ tumour cells mixed with hMSC resulted in greater tumour size than did transplantation of TSA-Luc+ cells alone. We then evaluated if hMSCs could modified tumour angiogenesis. We then evaluated the in vivo avß3 integrin expression on mouse tumours by 3D fluorescence imaging associated with RAFT-RGD coupled with Alexa700.

Conclusion: In conclusion, our results could explain the discrepancies found in the literature concerning the pro or anti-proliferative characteristics of hMSC and due to the timing at which MSCs are introduced into the tumour microenvironment may be an important consideration.

poster number: 019

DYNAMIC CONTRAST-ENHANCED ULTRASOUND AS ALTERNATIVE TO MRI FOR IMAGING ANTI-ANGIOGENIC TREATMENT RESPONSE IN EXPERIMENTAL BONE METASTASES

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Purpose: Evaluation of treatment response to systemic therapies in bone metastases according to RECIST guidelines concentrates on morphological MRI and CT imaging. However, recent studies indicated that imaging of vascular changes in experimental bone metastases upon treatment with anti-angiogenic tyrosine kinase inhibitors (TKI) can be assessed with dynamic contrast-enhanced MRI (DCE-MRI) before changes in lesion size occur[1,2]. Ultrasound (US) is, compared to MRI, a widely applicable, inexpensive and transportable real-time imaging method. We therefore conducted this first preclinical study of DCE-US for assessment of anti-angiogenic treatment response in experimental breast cancer bone metastases.

Methods and Materials: Nude rats bearing breast cancer bone metastases (n=20) were imaged using MRI and US on days 30, 32 and 35 after tumor cell inoculation. Treated animals (n=10) received the anti-angiogenic TKI sunitinib from days 30 to 35 and were compared to untreated controls (n=10). Using a 1.5T clinical MRI scanner (Magnetom Symphony, Siemens), T2-weighted MRI was performed to determine the volume of the soft tissue metastases (STM). For DCE-MRI, Magnevist (Schering) was infused intravenously. Data was analyzed according to a two-compartment model to calculate amplitude A (associated with relative blood volume) and exchange rate constant k_{ep} (representing vessel permeability). A clinical US unit (Acuson Sequioa 512, Siemens) was used to perform DCE-US. After injection of a microbubble contrast agent (SonoVue, Bracco), the respective signal intensity over time curves were analyzed to calculate peak enhancements (PE), areas under the curve (AUC) as well as wash-in and wash-out rates. At the end of the observation period on day 35, bone metastases from three representative animals per group were stained with antibodies against von Willebrand Factor (vWF) and smooth muscle actin (SMA) to determine mean vessel calibres (MVC) and visualize endothelial cells as well as pericytes, respectively. For statistics, the Wilcoxon-test was applied.

Results: No significant differences for STM volumes between treated and untreated animals were found during the observation period ($p>0.05$). Compared to controls, DCE-MRI revealed significantly decreased values for amplitude A on day 35 and exchange rate constant k_{ep} on days 32 and 35 for animals treated with sunitinib ($p<0.05$, respectively). Using DCE-US, significantly decreased values for PE, AUC and wash-out rates were determined in animals treated with sunitinib compared to controls on day 35 ($p<0.05$, respectively). Histological evaluation of bone metastases revealed significantly increased MVC and decreased positive area fraction for vWF in animals treated with sunitinib ($p<0.05$), while no changes were noted for SMA marker densities between groups ($p>0.05$).

Conclusions: DCE-US for non-invasive assessment of treatment response to the TKI sunitinib in experimental breast cancer bone metastases is feasible and findings could be confirmed by immunohistology. Due to its broader availability DCE-US might be a suitable alternative to DCE-MRI for capturing early anti-angiogenic effects.

References: [1]Bäuerle et al., Clin Cancer Res. 2010 Jun 15;16(12):3215-25 [2]Merz et al., Eur J Cancer. 2011 Jan;47(2):277-86

poster number: 020

QUANTITATIVE, *IN VITRO* 19F MRI MIGRATION ASSAY APPROXIMATES DENDRITIC CELL MIGRATION AFTER PATIENT VACCINATION

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Introduction: 19F Magnetic Resonance Imaging (MRI) for cell tracking is a relatively new field that builds on existing 1H MRI technology by adding 'hot spots' for 19F signal. Importantly, 19F MRI allows absolute quantification of cell numbers directly from image data [1]. We apply this technology to optimize migration of dendritic cells (DC) after vaccination in melanoma patients. Currently, migration of these DC to relevant lymph nodes in vivo is poor [2], although increasing numbers of migratory DC correlates with improved therapeutic outcome [3]. However, optimization is extremely difficult with current clinical cell tracking techniques, and financial and logistic concerns. On the other hand, there is no suitable in vitro substitute to measure cell migration that sufficiently replicates clinical conditions, in particular the large cell numbers used in vaccines. Hence, we developed a 19F MRI-based assay, where clinically-relevant numbers of labeled DC (10^5-10^7) migrate in a 3D scaffold that closely mimics tissue conditions. On comparing these results with our clinical DC migration data, we found that this novel in vitro assay is predictive of clinical DC migration.

Methods: Primary human DC were cultured and labeled with 111In before intradermal injection and scintigraphy [4]. For the in vitro migration assay, DC were 19F-labeled [5], loaded in a collagen scaffold or tissue with a chemokine gradient, and placed vertically in a 7T Clinscan Bruker system. 19F acquisitions were carried out for 10 hours to (1×1×10 mm^3 matrix; TR/TE=600/2.94ms; 500 averages; elliptical k-space sampling).

Results: Our 19F labeling is well-tolerated by DC [5]. We found that decreasing the number of cells in the cell layer in vitro resulted in a higher percentage of migratory cells, ranging from 0-4% for 0.5-10x106 DC. Similar numbers were obtained in patients: 1-4% of the DC reached the draining lymph nodes. Furthermore, the percentage of migratory cells increased as the total number of cells dropped from 10 to 5 to 0.5 million both in vitro in tissue samples and in vivo. Histological evidence suggests that overcrowding at the injection site hinders emigration. Pretreatments of the injection site were also tested.

Conclusion: Cell migration is challenging to measure quantitatively in vivo; however its optimization is necessary for the clinical success of DC vaccination. Our 19F MRI-based migration assay acts as an in vitro substitute for clinical optimization of DC migration. We have shown that the in vitro results match patient data, and that this novel assay can be developed to predict and optimize DC migration in vitro, cheaply and effectively. The assay is readily adaptable to different cell types, tissue samples, chemokines, pretreatments or other factors.

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poster number: 021

IS DIFFUSION-WEIGHTED MRI A USEFUL TOOL FOR THE ASSESSMENT OF BENIGN, INVASIVE AND NON-INVASIVE BREAST TUMORS?

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Introduction To evaluate the diagnostic value of diffusion-weighted imaging (DWI) in the diagnosis of breast tumors and to show if apparent diffusion coefficient (ADC)-values correlate with benign, invasive and non-invasive tumor types and grading at 3 Tesla.

Methods 176 breast tumors detected with ultrasound, mammography and/or contrast-enhanced MRI were subjected to DWI. A 3-Tesla MR-imager with a dedicated breast coil was used for data acquisition. A diffusion-weighted sequence with b-values of 50 and 850 sec/mm^2 was performed and ADC-maps were calculated. ADC-values were measured in the tumors by two experienced breast radiologists in consensus. The threshold for malignancy was set at 1.25x10^{-3} mm^2/sec. All tumors were histopathologically verified and graded using the TNM-system. Histopathology revealed 44 benign and 132 malignant lesions, including 100 invasive ductal (IDC), 12 invasive lobular (ILC) carcinomas, 7 non-invasive carcinomas (DCIS) and 12 other types of malignant tumors. Sensitivity, specificity, diagnostic accuracy and NPV/PPV were calculated. Welch's t-test and Games Howell post hoc test were performed to calculate and correlate the mean ADC values of malignant and benign tumors, as well as the different malignant tumor types and grading.

Results DWI reached a sensitivity of 91% (PPV 0.94, CI 0.89-0.97), a specificity of 84% (NPV 0.75, CI 0.62-0.85) and a diagnostic accuracy of 89% for benign-malignant-discrimination. The mean ADC-values were 1.57x10^{-3} mm^2/sec±0.40 for benign and 0.97x10^{-3} mm^2/sec±0.24 for malignant tumors. Sensitivity was 98% for IDC and 92% for ILC, but only 57% for non-invasive tumors. Mean ADC-values for the malignant tumor types were 0.93x10^{-3} mm^2/sec±0.18 for IDC, 0.91x10^{-3} mm^2/sec±0.27 for ILC and 1.2x10^{-3} mm^2/sec±0.26 for non-invasive tumors. ADC-values were significantly lower in IDC and ILC than in benign tumors ($p<0.001$), while no significant difference between benign and non-invasive tumors could be found ($p=0.061$). ADC values did not correlate with grading.

Conclusion DWI is highly accurate in the differentiation of invasive carcinomas and benign tumors, but limited in non-invasive carcinomas.

poster number: 022

EVALUATION OF HUMAN DENTAL PULP STEM CELLS AS THERAPEUTIC DELIVERY VEHICLES FOR MALIGNANT GLIOMA: A HIGH RESOLUTION MAGNETIC RESONANCE (MR) IMAGING APPROACH.

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Introduction: Malignant gliomas are by far the most common type of brain tumor in the western world. Current treatment focuses on resection of the tumor, followed by adjuvant radio- and chemotherapy. Yet the prognosis of these patients is still extremely poor mainly due to its strong infiltrative growth pattern. Consequently, resection is almost always followed by re-growth of tumor cells residing in adjacent regions of normal brain. Therefore, the need for an alternative treatment approach in which the glioma cells are specifically targeted forms an open challenge. Recent progresses in stem cell research revealed that mesenchymal stem cells (MSCs) show a natural tropism for tumors and their metastasis. Furthermore, low expression levels of human leukocyte antigen (HLA) makes these cells immunoprivileged. This remarkable combination of properties forms the basis of current study in which MSCs derived from human dental pulp are evaluated as glioma-specific delivery vehicles for therapeutic agentia.

Methods: Human dental pulp stem cells (hDPSCs) were loaded with a commercially available MRI contrast agent (Endorem®) and uptake was assessed by MR-imaging and transmission electron microscopy (TEM) and subsequently quantified by atomic absorption spectrophotometry (AAS). Tetrazolium salt (MTT) assays were performed to assess the effect of this labeling technique on the cellular metabolism. Tumorgeneity of the cells was assessed by in vivo transplantation into the brain of immunocompromised mice. The optimal labeling fraction was determined by MRI - analysis. In vivo visualization and migration potential was assessed by MRI.

Results: Present study investigated the labeling capacity of hDPSCs and its effect on cell viability. Based on MR-imaging, ultrastructural analysis and AAS, our results demonstrated efficient hDPSC labeling by combining the commercially available (FDA approved) ferum oxide Endorem® with PLL as a transfection agent. Furthermore, hDPSCs did not form tumors following stereotactic injection into the brain. MRI-analysis demonstrated a dose-dependent relationship between the fraction of labeled cells and the decrease in T2 relaxation times. Even a low concentration of 15 ug/ ml of iron resulted in sufficient in vivo contrast for cell visualization.

Conclusions: This study provides evidence that hDPSCs can be successfully labeled with commercially available SPIO particles when combined with PLL as a transfection agent. SPIO labeling was not found to be deleterious to hDPSCs. When transplanted into the brain, hDPSCs don't show any sign of tumorgeneity and can therefore be regarded as potential new delivery vehicles for therapeutic agentia into the brain. Further research regarding specific migration towards glioma is ongoing.

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poster number: 023

LONGITUDINAL PET IMAGING WITH 68GA-AMBA IN THE FOLLOW UP OF DASATINIB TREATMENT OF PROSTATE PC-3 TUMOR-BEARING MICE

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Introduction**:** Imaging biomarkers able to assess the most relevant molecular events in cancer are highly suitable to detect early responses and potentially predict treatment outcome. AMBA is a bombesin analog with high affinity for gastrin-releasing peptide receptors (GRP-R), up-regulated in many cancers, including prostate, breast and lung. GRP-R is a promising target for molecular imaging and targeted cancer therapy, as on binding of an agonist ligand, the receptor-ligand complex is internalized in the cells. The aim of this study was to assess the ability of the DO3A-chelated AMBA labelled with ^{68}Ga in monitoring the response to Dasatinib treatment in prostate PC-3 tumour bearing mice.

Methods**:** Five weeks old NCR athymic nude mice were inoculated with five million PC-3 cells in the right flank. Tumour volumes were measured twice a week starting four days after cells inoculation. A PET scan with both ^{18}F-FDG and ^{68}Ga-AMBA was performed immediately before the beginning of the treatment. Animals were then divided in two groups (n=8 each for control and treatment); the treatment group was administered for four weeks with Dasatinib (50 mg/kg p.o. daily, 5 days on/2 days off) and for subsequent 30 days at a dose of 25 mg/kg. ^{68}Ga-AMBA scans were performed two and four weeks after the 50 mg/kg treatment and four weeks after the 25 mg/kg one. A second ^{18}F-FDG scan was performed four weeks after the higher treatment dose.

Results**:** Dasatinib treatment at 50 mg/kg inhibited the tumour growth in all the animals of the group, while tumours of the control group continued growing exponentially. ^{18}F-FDG tumours uptake was lower respect to ^{68}Ga-AMBA one, both before and after the treatment. Moreover, ^{68}Ga-AMBA uptake changed along treatment, while ^{18}F-FDG uptake was comparable before and after treatment. The different trends highlighted in the change of %ID/g of ^{68}Ga-AMBA uptake after Dasatinib administration could be predictive of a different response to the treatment. The extension of the treatment, switching the dose to 25 mg/kg, let the tumours restart growing almost exponentially and did nearly not affect the ^{68}Ga-AMBA slope trends.

Conclusions**:** Molecular imaging with ^{68}Ga-AMBA can accurately allow the visualization of prostate tumours in PC-3 tumour bearing mouse model. Moreover, the targeted radiotracer has a higher uptake (~ twofold) respect to ^{18}F-FDG. The experimental Dasatinib doses used for treatment cannot allow to draw a conclusion on the predictive value of ^{68}Ga-AMBA in the response to treatment with the chemotherapy compound. New experimental conditions should be tried to increase the dynamic range of the animal response to produce a correlation between the treatment response and the ^{68}Ga-AMBA uptake.

poster number: 024

HISTOLOGICAL VALIDATION OF ULTRA-HIGH RESOLUTION FOCUSING PINHOLE SPECT FOR IMAGING THE INTRATUMORAL DISTRIBUTION OF A HUMAN ANTIBODY IN A MOUSE TUMOR MODEL

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Introduction Increasing the intratumoral availability of anti-cancer antibodies is an important determinant in preclinical cancer therapy research. However, the in vivo visualization of the intratumoral distribution of these constructs in mouse cancer models is very demanding due the small size of the tumors. In this study a sub-half-mm resolution SPECT system with focused collimation, the U-SPECT-II, was used to explore the intratumoral distribution of an antibody directed towards Epidermal Growth Factor Receptor (EGFR), a key receptor in tumorigenesis.

Methods Human monoclonal EGFR-targeting antibodies were radiolabeled with In-111 and injected into a mouse xenograft model expressing high EGFR levels. Whole body and local tumor SPECT images were acquired at 1 hour and 48 hours after injection. Tumors were snap-frozen after the last SPECT experiment, cryosectioned and stained for EGFR expression using immunohistochemistry. The stained histological slices were stacked and registered in 3D to the SPECT image.

Results Global SPECT imaging showed that at 10 minutes after injection, activity was predominantly present in the blood and the heart. At 48h activity was mainly located in the tumor with some residual traces in heart and kidneys. High resolution SPECT imaging at 48h showed that the In-111-labeled EGFR-targeting antibody was taken up heterogeneously in the tumor with much less activity in necrotic regions. Immunohistology confirmed that regions showing little SPECT activity also exhibited lower EGFR expression and EGFR patterns correlated very well with SPECT patterns.

Conclusions The study clearly showed that the U-SPECT-II system generates images with sufficiently high resolution to visualize the heterogeneous distribution of antibodies in sub-compartments of small tumors in mice. The focused multi-pinhole setup provides researchers with a powerful tool to further optimize anti-cancer medication.

poster number: 025

ULTRASOUND MOLECULAR IMAGING FOR EVALUATION OF SOLID TUMORS

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Contrast ultrasound is an emerging imaging technique for imaging tumor progression, both in a clinical and research setting. The advent of microbubble ultrasound contrast agents has expanded the use of ultrasound into molecular and functional imaging. In particular, targeted microbubbles are now being utilized as molecular contrast agents for molecular imaging of angiogenesis in basic science and the drug discovery industry.

We present a novel microbubble-based ultrasound contrast agent that can be used for quantitative ultrasound imaging of tumor vascular function and molecular imaging. We have developed a method of coupling ligands against molecular markers of angiogenesis to the surface of the microbubble agent using biocompatible conjugation chemistries. We have validated ligands against VEGFR2 and alpha-v beta-3 integrin that can be translated to human use. Microbubble adhesion to recombinant VEGFR2 or alpha-v beta-3 integrin, or stimulated endothelial cells, was first assessed in vitro using a flow chamber. Both agents were subsequently assessed in mouse models of colon, bladder, prostate, and breast cancer. The VEGFR2 targeted agent was used in canines to assess the feasibility of guided prostate biopsy.

We found that a 27 kDa ligand based on human VEGF showed extremely high specificity for VEGFR2 both in vitro and in vivo. Likewise, a pentameric RGD-containing peptide was able to mediate microbubble adhesion to alpha-v beta-3 integrin, but only when conjugated in high copy number to the microbubble surface. Out of eight companion canines enrolled in the study with suspected prostate disease, two were diagnosed with prostatic adenocarcinoma by biopsy. In both animals, cancer was confirmed in biopsy samples taken from regions of contrast agent uptake, with no verified cancer in non-enhancing regions. No contrast agent uptake was observed in animals with benign disease.

We have constructed two ultrasound molecular imaging agents targeted to molecular markers or angiogenesis. Both are species-independent and suitable for use in multiple research species, as well as translation to clinical use. We validated uptake of both agents in various mouse models of subcutaneous and orthotopic cancers, and demonstrated specificity for the intended molecular targets. We are in the process of evaluating the VEGFR2- agent for use in targeted prostate biopsy in a large animal model, and preliminary results are encouraging. Ultrasound molecular imaging agents may have numerous applications in both clinical medicine and pre-clinical research, and the development of translatable agents represents a key milestone to enabling routine use of this promising technology.

poster number: 026

NEW 18F-LABELLED INHIBITORS OF MATRIX METALLOPROTEINASES AS IMAGING PROBES FOR PET IMAGING

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Introduction. Matrix Metalloproteinases (MMPs) are a family of zinc dependent endoproteases that degrade proteins in the extracellular matrix (ECM) and have a key role in tissue remodeling in normal (angiogenesis, tissue repair) as well as pathological conditions (cancer, stroke, neurodegeneration). There is a growing body of evidence indicating that different pathological states are characterized by a specific pattern of MMP activity, such that this pattern may be regarded to as a sort of signature of a given pathology. The level of MMPs expression and activity in malignant cancers is generally higher than in normal or premalignant tissues, with maximum activity occurring in areas of active invasion at the tumor-stroma interface. In oncology, the role of different members of the MMP family is under intense scrutiny. Gelatinases (MMP-2 and MMP-9) are well-established to sustain all stages of tumor progression, including proliferation, adhesion, migration, angiogenesis, and evasion of the immune system. On the other hand, other metalloproteinases (such as MMP-8 and MMP-26) seem to have anti-tumour properties. There is a growing consensus on the fact that the visualization in the body of the activity of a selected panel of MMPs would be a very valuable tool for the staging of tumors and the choice/calibration of the therapy. Amongst the available imaging techniques, nuclear medicine techniques offer the greatest sensitivity and the possibility to obtain true metabolic/molecular images, provided that suitable radiolabelled imaging probes are available.[1]

Methods. We have developed a number of MMP inhibitors (MMP-I) that can be radiolabelled with 18F for Positron Emission Tomography (PET) imaging. These inhibitors are based upon biphenylsulfone scaffolds, with carboxylic acid as the ZBG. PET images of healthy and xenografted with U87MG glioblastoma mice (athymic nude) were obtained by using the YAP-(S)PET system. Anesthetized (isofluorane) mice were injected i.v. with [18F] MMP-I (250 ±50) uCi in 0.15 mL volume.

Results. The fluorinated MMP-I showed a good affinity and selectivity against gelatinases, with best IC50 values against MMP-2 in the order of 10 nM. Rather surprisingly, the selectivity of fluorinated derivatives somewhat increased with respect to the precursors. Compounds could be radiolabelled with 18F with a RadioChemical Yield (RCY) suitable for preclinical studies and very good purity (> 90 %). The biodistribution of the most potent compounds was evaluated by PET imaging in mice. It was found to be largely dominated by the lipophilicity of the compound and by its quite strong interaction with blood proteins (especially albumin). Preliminary molecular PET images of MMP activity in a glioblastoma model subcoutaneously grafted in mice showed a weak accumulation of the tracer into the tumor.

Conclusions. PET imaging of MMPs through 18F labelled inhibitors is feasible. A substantial improvement is expected if sequestration of the tracer by blood components is minimized.

Acknowledgments. This work is supported by the Regione Piemonte (Italy) as part of the Converging Technologies BIO_THER project and part of the PIIMDMT project.

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poster number: 027

IN VIVO BIODISTRIBUTION AND PROSTATE CANCER DETECTING PROPERTIES OF AN ANTI-PSMA MONOCLONAL ANTIBODY ADMINISTERED AS WHOLE MOLECULE OR SCFV

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Introduction: Advanced, aggressive and metastatic forms of prostate carcinoma have presently no decisive therapeutic solutions. Therefore, new treatment modalities must be evaluated to complement more conventional therapeutic regimens. Immunology-based interventions have been proposed as a promising curative chance that may effectively attack postoperative minimal residual disease and distant metastatic localizations of the tumor. The key to this approach is the identification of antigens with high expression on tumor cells, but reduced or absent expression on normal tissues. In the specific case of prostate carcinoma, two biomarkers are promising targets of immunotherapeutic approaches, namely the prostate Specific Membrane Antigen (PSMA) and the Prostate Stem Cell Antigen (PSCA). PSMA is a type-II integral membrane protein of 100 kDa with a short 19 amino acid cytoplasmic tail, predominantly localized to the epithelial cells of the prostate gland. The low expression of PSMA in normal prostate epithelial cells increases several fold in high-grade prostate cancers, in metastatic and in androgen-insensitive prostate carcinoma. The importance of PSMA in cancer detection and therapy is further illustrated by the finding that it is also expressed in tumor neovasculature but it is absent on normal endothelial cells. Using an in vivo fluorescence imaging approach, we aimed at assessing and comparing the biodistribution and cancer detecting properties of an anti-PSMA monoclonal antibody (mAb) administered in mice bearing human PSMA positive or negative prostate tumors, either as a whole molecule or in its scFv (single chain fragment variable) format.

Methods: Anti-PSMA mAb D2B and the derived scFv were labelled with Alexa680 fluorophore using a commercially available kit. In vivo experiments were performed using 6- to 8-wk-old male immunodeficient mice, which were inoculated subcutaneously with both PC3 cells (a prostate tumor cell line that not express PSMA) and the PSMA-transfected counterpart, at different sites. Upon intravenous inoculation of mAb or scFv, mice were imaged with a MX2 time-domain (TD) system (ART), using a 670 nm laser and a 693LP filter; the spatial resolution/scan step was fixed at 1 mm, exposure time was 0.5 seconds and laser power was automatically adjusted for each scansion.

Results: Inoculation of the whole mAb was followed by a strong uptake at the site of the PSMA-positive tumor; signal progressively increased to reach a peak at 48-72 hours, but was still detectable after 7-10 days. Nonetheless, a relevant non-specific accumulation of the mAb could also be visualized at the site of the PSMA-negative control tumor injection in particular during the first 24-48 hours, thus markedly reducing the specificity of tumor identification. Conversely, the scFv revealed a very rapid kinetics with a striking and highly specific uptake only at the site of the antigen-positive tumor. Notably, signal peaked at just 1-3 hours to progressively fade during the following 3 days.

Conclusions: Production of scFv can represent an important evolution of diagnostic mAb for in vivo cancer imaging and detection. Moreover, optical techniques provide an assessment and validation step that is prodromic to clinical transfer allowing the identification of potential molecule candidates to be labelled with radionuclides.

poster number: 028

IN VIVO EVALUATION OF DEN-INDUCED LIVER TUMOR MOUSE MODELS

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Introduction: Liver tumors are of special interest in the oncological field of research for several reasons. From the clinical point of view, liver tumors are among the ten most often causes for tumor deaths in the US [1], and still, are very difficult to detect as, e.g. positron emission tomography (PET) lacks specific tracers, thus best results are obtained using magnetic resonance imaging (MRI) [2]. From a preclinical point of view, reliable detection and differentiation of liver tumors is of special interest to evaluate new PET-tracers and to possibly reduce the number of animals in the bioassay for drugtesting. In this experiment, we evaluated MRI protocols to assess the detection limits of liver lesions in mice and monitored growth of individual tumors in vivo.

Methods: Two mouse models of liver tumors were evaluated; each group consisted of 6 animals. For the first model, tumors were initiated by a single dose of diethylnitrosamine (DEN) at age of 2 weeks. In the second model, tumors were initiated at age of 6 weeks with subsequent treatment with phenobarbital (PB), following an initiation - promotion protocol. MRI was performed with a 7T dedicated small animal scanner (ClinScan, Bruker Biospin GmbH, Ettlingen, Germany). Anatomical imaging was performed using a T2-weighted 3d turbo-spin echo. During imaging sessions, animals were anesthetized with 1.5% isoflurane in medical oxygen. To improve image quality, MRI acquisitions were respiration-gated to minimize breathing artifacts (SAInstruments, Stony Brooks, NY, USA).

Results: Tumors of both groups appeared as hyperintense lesions in T2-weighted images with a minimal diameter of approximately 1 mm. First tumors appeared 19 - 20 weeks after DEN-toxification in both groups. Assuming exponential tumor growth, tumors of group 1 (without PB-treatment) grew faster than tumors of group 2. Tumor volumes as well as total number of tumors per mouse were very heterogeneous throughout both groups. Tumor phenotypes were differentiated with histological stainings.

Conclusion: We could show that in vivo monitoring of liver tumors non-invasively in mice is feasible, once tumors reached a minimal size of 1 mm in diameter. Based on these protocols, we provide values to estimate total tumor size after DEN-treatment, which may allow evaluating possible therapy response early. As second important result, these protocols can be used to evaluate possible PET tracers in combined PET-MRI sessions, aiming at in vivo non-invasive tumor phenotyping.

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poster number: 029

MOLECULAR IMAGING FOR EARLY DETECTION OF MELANOMA METASTASIS AND RESPONSE TO THERAPY

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Introduction: Currently melanoma is considered one of the highest metastatic potential malignant tumors, with poor prognosis in advanced stages. Available therapeutic strategies guarantee positive results in a low percentage of patients (<25%). We have recently demonstrated that a short interfering RNA (siRNA) can effectively contrast antiapoptotic action of the FKBP51 protein, a marker of malignancy and radioresistance of melanocytes. Multimodal Molecular Imaging has become widely used in the oncological research. Aim of this study was to evaluate the efficacy of preclinical PET/CT and Ultrasound Biomicroscopy (UBM) for early detection of melanoma metastatic lesions in mice models of melanoma and to monitor the response of this tumor type to an innovative therapy.

Methods: 8 nude mice, Balb/c NOD SCID IL2gamma (null) strain, 4-5 weeks old, received an iv injection of 1,5x106 melanoma cells (SAN) in 100 µl of PBS. 4 mice 15 and 24 days post SAN injection received iv siRNA therapy (Group 1), and 4 mice had no therapy (CTRL Group). All mice were weekly monitored with UBM to detect hepatic lesions, and were analyzed with ^{18}F- fluorodeoxyglucose (FDG) at day 27 post melanoma cells injection.

Results: Group 1 showed no or rare liver metastasis at UBM examination, and a low ^{18}F- FDG uptake limited to small lungs area liver (lung SUV Mean 0.75, lung SUV Max 0.77, liver SUV Mean 0.37; liver SUV Max 0.40). CTRL Group showed diffuse metastatic lesion both in liver and lung and a high 18F- FDG uptake in lung and liver (lung SUV Mean 1.13, lung SUV Max 0.88, liver SUV Mean 1.14; liver SUV Max 1.2).Imaging results are correlated with macroscopic post mortem lung and liver findings.

Conclusion: Multimodality Molecular Imaging is able to monitor non invasively the progression of melanoma metastatic disease and therapy outcome. PET/CT and UBM allow a early detection of liver and lung metastatic lesions, representing a great tool to establish early therapy and preventing melanoma progression.

poster number: 030

DEVELOPMENT OF A RED FLUORESCENT LABELED AGENT FOR ASSESSING AVB3 INTEGRIN EXPRESSION *IN VITRO* AND *IN VIVO*

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Introduction: Integrins, a family of transmembrane glycoproteins consisting of two a and ß?subunits, mediate cell-to-cell and cell-to-extracellular matrix interactions, predominantly via interaction with an RGD recognition sequence in their ligands. The integrin avß3 in particular appears to play a critical role in tumor growth, metastasis and angiogenesis. The aim of this study was to develop a red fluorescent avß3 targeted agent to non-invasively image and quantify tumor-associated integrin expression in vitro and in vivo.

Methods: A small peptidomimetic avß3 antagonist was synthesized, coupled to a red fluorescent dye, and tested for specificity by binding to avß3-expressing murine breast adenocarcinoma 4T1 and leukaemic monocyte macrophage RAW 264.7 cell lines as determined by flow cytometry and fluorescence microscopy. The pharmacokinetic profile was assessed in mice by measuring plasma fluorescence at different times after intravenous injection with the agent (2 nmoles), and tissue biodistribution was determined in vivo and ex vivo using Fluorescence Molecular Tomography (FMT). In vivo quantification and specificity of tumor signal was confirmed in 4T1 tumors orthotopically implanted in nude mice by evaluating the effect of in vivo competition with unlabeled ligand.

Results: The red-shifted avß3 imaging agent (649 nm excitation/665 nm emission) bound to integrin-expressing 4T1 and RAW 264.7 cells (0.25 uM for 15 min), and specificity of this binding was confirmed by effective blockade with unlabeled integrin antagonist (25 uM for 5 min before adding labeled agent). In vivo, the agent showed a short plasma half-life (t1/2 = 10 min), and in vivo and ex vivo biodistribution assessment showed high signal within the bladder, suggesting predominant renal clearance. Tumor signal peaked at 3-6 hours, decreasing thereafter. In vivo specificity of binding within the tumors was confirmed by blockade with excess unlabeled integrin antagonist (100 nmoles), injected 5 min before the imaging agent (tumor signal 28.57 +/- 5.90 versus 7.38 +/- 1.81 pmoles , respectively, p=0.002 at 6h imaging time).

Conclusions: A red fluorescent-labeled integrin antagonist selectively targets avß3 integrin, allowing both in vitro use as well as non-invasive in vivo fluorescence tomographic imaging and quantification of tumors for assessing cancer progression and treatment monitoring.

poster number: 031

NO CORRELATION BETWEEN GLUCOSE METABOLISM AND APPARENT DIFFUSION COEFFICIENT IN DIFFUSE LARGE B-CELL LYMPHOMA: A PET/CT AND DW-MRI STUDY

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Introduction: Positron emission tomography/computed tomography (PET/CT) and diffusion-weighted magnetic resonance imaging (DW-MRI) share similar applications in the field of clinical oncology. PET/CT with the use of 2-deoxy-2-[^{18}F] fluoro-D-glucose (^{18}F-FDG) tracer provides quantitative information regarding cellular glucose metabolism. The maximum standardized uptake value (SUVmax) is commonly used to evaluate tumor glucose metabolism, which is a biomarker for clinical diagnosis of tumor malignancy, disease recurrence, and metabolic response to therapy [1]. Cancer is not only characterized by pathological metabolism, but also higher cellularity; therefore, restriction of water diffusion has been found to be a common feature of tumors. DWI with apparent diffusion coefficient (ADC) mapping provides information on tissue cellularity, tortuosity of extracellular space, and cell membrane integrity. ADC value has been applied to distinguish benign from malignant lymph nodes [2, 3]. Recent studies have shown that DWI is a valuable imaging modality for detecting metastasis and cancer relapse, and it has also been used to assess treatment response in various malignancies including lymphoma [4, 5]. In order to compare the clinical usefulness of ADC and SUVmax, it is necessary to clarify whether a correlation exists between these two parameters. The aim of this study was to explore the correlation between SUVmax and mean ADC value based on each pathological lymph node in untreated patients with diffuse large B-cell lymphoma (DLBCL).

Methods: Fifteen pre-therapy patients with histologically proven DLBCL underwent integrated PET/CT (Discovery STE 16, GE Healthcare, Milwaukee, WI, USA) and MRI (3 Tesla MR System, Siemens Trio-Tim, Erlangen, Germany) examinations within two days. The PET, CT, and DW images including ADC map were fused using the AW Volume Share™ workstation. Tumor glucose metabolism was evaluated by the SUVmax and mean SUV (SUVmean) on the PET/CT images. The mean ADC value was measured directly on the parametric ADC maps.

Results: In total, 28 lymphoma lesions with best match PET/CT and DWI were identified and evaluated. The mean SUVmax and SUVmean were 16.8 and 11.1, respectively; the mean ADC value was 0.74 x 10^{-3} mm^2/s. There was no correlation between the mean ADC and the SUVmax or SUVmean.

Conclusion: SUV determined from PET/CT and ADC value measured from DWI are different indexes for the diagnosis of tumor malignancy, they may provide complimentary functional information of tumor tissue.

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NEW DOTA-CONJUGATED ANTIBODY FOR CANCER DIAGNOSIS

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Introduction: The treatment of cancer is being revolutionized by the development of targeted therapeutic biologics. Translational pharmaco-imaging represents a key step in the evaluation of these new targeted anti cancer therapies. Thus, SPECT (Single Photon Emission Computed Tomography), or PET (Positron Emission Tomography) are both powerful techniques using a large variety of radionuclides, including ^{111}In (? emitters) for SPECT or ^{68}Ga (positron emitter) for PET. However, in order to prevent the release of the radioactive metal in the organism by either transmetallation or transchelation, it is essential to strongly trap the radiometal by ad hoc ligands allowing the formation of very stable complexes. [1] Polyazacycloalkanes are chelating agents particularly adapted to such radiometal cations as they form kinetically and thermodynamically stable complexes. These molecules can furthermore be functionalized by the insertion of a grafting function that target amino-acid for covalent protein binding.

Method: A new macrocyclic reagent (DOTAGA-anhydride, bearing an anhydride function able to react with primary amines) was used for DOTA conjugation of trastuzumab (Herceptin®), a monoclonal antibody used in breast cancer therapy. [2] DOTA trastuzumab conjugate was synthesized by a direct reaction between DOTAGA-anhydride and trastuzumab, and the efficiency of the process was evaluated by mass spectrometry.

Results: DOTA-conjugated antibody was metallated with ^{111}In under optimized conditions (labeling yield >75%) and with high specific activity (>700 MBq.mg^{-1}). DOTA trastuzumab conjugate was thus evaluated in vitro by saturation assays using two human breast cancer cell lines (SK BR 3 and HCC1954), and in vivo in subcutaneous BT474 tumor-bearing mice.

Conclusion: Together, these results demonstrate the efficiency of our new DOTA-synthesis and conjugation processes for biologics radiolabeling to assess biodistribution, anti-cancer efficacy and related toxicity of anti-cancer drugs by in vivo non-invasive imaging.

Acknowlegement: Support was provided by the CNRS, the University of Burgundy, the Conseil Re´gional de Bourgogne, and OSE´O. This work was completed within the framework of PharmImage® project.

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poster number: 033

SYNTHESIS OF FLUORESCENTLY LABELED AND SULFHYDRYL MODIFIED PH SENSITIVE POLYMERS FOR DEVELOPMENT AS SIRNA DELIVERY AGENTS

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Introduction: RNA interference (RNAi) has emerged as an important tool for gene therapy. RNAi directed against the chemoresistance protein stathmin reduces its level and increases the sensitivity of malignant glioma cells to specific types of chemotherapeutic agents, holding promise for improved malignant glioma treatments. However, for small interfering RNA (siRNA) effectiveness depends on escape from endosomal compartments once internalized. Furthermore, dissociation of siRNA from polymer carriers in cytoplasm is a critical step for efficient siRNA delivery. At present, efficient delivery of siRNAs remains a major barrier to their clinical application. In this study, a novel polymer, phenylalanine-grafted poly(L-lysine iso-phthalamide) (PPLI) was examined for its ability to deliver siRNA to glioma cells.

Methods: Conjugation of PPLI with Alexa Fluor 647 was performed to examine the mechanism of PPLI internalization prior to undertaking siRNA delivery. For delivery of siRNA, we introduced a disulfide bond between carrier PPLI and siRNA to allow for effective dissociation of siRNA from PPLI in the reductive conditions of the cytoplasm.

Results: PPLI is effective at mediating endosomal disruption in over 95% of cells studied. For delivery of siRNA, A PPLI-siRNA conjugate against stathmin silences its expression both in vitro and in vivo. Furthermore, our results indicate that the conjugate is highly effective at inhibiting tumor growth when combined with the nitrosourea chemotherapy agent carmustine in a subcutaneous malignant glioma model.

Conclusions: PPLI is effective at mediating endosomal pore formation which presents as a promising new vehicle for the safe and efficient delivery of siRNAs and potentially other nucleic acids for therapeutic purposes.

poster number: 034

DYNAMIC CONTRAST-ENHANCED MRI IN A RODENT TUMOR MODEL; USING TIME RESOLVED IMAGING OF CONTRAST KINETICS (TRICKS) FOR HIGH SPATIAL AND TEMPORAL RESOLUTION SCANS

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Introduction: Dynamic contrast-enhanced (DCE) MRI has become an important tool for radiologists to stage tumor malignancy and response to therapeutics. Microenvironmental factors play an important role in tumor perfusion, and are studied extensively in preclinical settings concerning drug development and treatment response assessment. In small animal imaging, however, the requirements for high spatial and high temporal resolution can provide challenges. Studying the microenvironment in rat tumors requires very high spatial resolution which increases scantime, however DCE studies in rats require a very high temporal resolution (Tres) due to the fast physiological processes, such as a 350 bpm heart rate. The purpose of this study was to evaluate the use of a keyhole acquisition technique, used in clinical settings for angiography, (TRICKS), in acquiring high spatial and temporal resolution DCE-MRI scans in rat tumors.

Methods: 4 lewis rats bearing subcutaneous CA20948 tumors were imaged in a in a clinical 3T scanner using a custom-built 5cm birdcage coil. Next to T2, T2*, PD weighted images and a T1 map, TRICKS was optimized for the current tumor model (TR/TE 10/2ms, FA 12º, FOV 5cm, 116x116 matrix, Tres 4.5s). Time to peak (TTP), maximum enhancement (Emax) and area under the curve (AUC) were derived from the signal intensity - time curves using Matlab.

Results: To optimize the Tres for accurate AIF estimation we looked at the time it takes to reach the peak concentration in an artery. The maximum signal intensity of the arteries is reached within 15 seconds after contrast injection. From that we established that the desired Tres is less than 5 seconds. TRICKS allows us 0.2x0.2x1 mm voxel size at a desirable 4.5 second temporal resolution, with a FOV of 5 cm. The high spatial and temporal resolution allows us to accurately capture the spatially heterogeneous nature of the tumor by quantifying the TTP, Emax, and AUC.

Conclusion: The clinical keyhole acquisition technique 'TRICKS' is very suitable for low temporal resolution high spatial resolution DCE-MRI, necessary for angiographic and perfusion studies in animal models. TRICKS thus offers the capability to distinguish areas with different perfusion speed and enhancement for a 3D volume large enough for big rodent tumors.

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ENHANCEMENT OF OPTICAL *IN VIVO* DETECTION OF TUMORS WITH ANIMAL ROTATION

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Introduction:Non-invasive in vivo optical imaging has shown its utility for identifying and monitoring disease processes in live mice. Despite the strength of the method, its inherent weakness is the low depth penetration of emitted photons. Even in the near-infrared, a researcher would not expect to visualize fluorophores in depths greater than 1 cm. As a result, detection of signal often requires the animal to be positioned at an optimal orientation; one in which the origin of the fluorophore is positioned such that it minimizes the amount of diffusive tissues photons are required to pass through. To assist this very important concept of capture, a novel animal rotation device was developed to rotate a mouse to precise positions, thus minimizing the amount of tissue between the light source and the detector. Here we describe several experimental examples of optical signal capture optimization from tumors through the use of animal rotation. More specifically, animal rotation improved fluorescence quantitation of tumors and enabled visualization of small tumors with low-level emissions and narrow ranges of detection. These weak emitting tumors may have gone completely undetected through standard positioning.

Methods: In the first study, ovarian cancer stem-like cells labeled with Kodak X-sight 761 Nanospheres were injected subcutaneously at the flank ($3x10^6$ cells) and intra-peritoneally ($3x10^6$ cells) into athymic nude mice. The Carestream In-Vivo Imaging System MS FX PRO equipped with a Multimodal Animal Rotation System (MARS) was used to obtain X-ray and, concurrently, near-infrared fluorescence (NIRF) images at every 10° of a complete 360° rotation of a mouse. The second study used a male athymic nude mouse was injected with 2 x 10^6 human colorectral cancer cells subcutaneously in the right flank. After 17 days, the mouse received an intravenous injection of a near-infrared fluorophore label upon development of a mature tumor with a noticeable necrotic core. NIRF and X-ray images were taken at every 10° of a complete 360° rotation the mouse 30 hours after injection of fluorophore conjugate.

Results: In study 1, NIRF emissions produced reliable capture for the longitudinal quantification of the engrafted subcutatneous and two major intraperitoneal tumors out to 21 days post inoculation of tumor cells. Very small, low intensity satellite tumors could also be visualized within the peritoneum. These tumors had very narrow ranges of visualization of twenty to forty degrees, could not be visualized from a prone position, and would have gone undetected without multiple angle imaging. In study 2, the optimal angle of detection of the only tumor was at a 120° clockwise rotation of the mouse.

Conclusion: In this study, animal rotation demonstrated its utility in improving visualization and quantitation of fluorescently labeled tumors and allowed for localization of small satellite tumors that would have otherwise gone undetected in prone, lateral, or supine positioning.

poster number: 036

TUMOR VISUALISATION BY MRI IN A MOUSE MODEL OF BONE METASTASIS IN PROSTATE CANCER

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Introduction: Injection of prostate cancer cells in mouse tibia is used as a model of bone metastasis. We investigated, if MRI of the mouse tibia can be used for follow up of cancer cell growth.

Methods: Human androgen-independent prostate cancer cells were injected in the tibia of immunodeficient nude BALB/c mice. Nine weeks later, MRI of the left tibia was performed on a 7 T MRI system equipped with a 6 mm ID solenoid coil. A set of six FLASH slices was acquired along the tibia (in plane resolution 60x60 µm, slice thickness 500 µm). MR images were evaluated for cell growth and cell types inside the tibia. Modification in bone structure was estimated by the signal void.

Results:Twenty animals were examined in total. Two animals could not be evaluated in MRI due to low signal noise ratio. According to results of histology 50 % of the animals developed bone metastasis. Bone marrow stretches typically over the whole inner lumen of the bone. Optically, bone marrow appears in medium brightness with dark spots. In contrast, tumor tissue visualizes brighter and homogeneous in the center of the tumor. MRI and histolo-gy agreed in six cases of tumor growth (positive) and another 9 cases were no tumor growth (negative) was seen. However, there were three cases with tumor growth as confirmed by histology, which was not seen in MRI.

Conclusion:MRI can be used for follow up of tumor growth in the mouse model of prostate cancer metastasis. All cases without tumor growth could be identified. Currently, 10 % non interpretable results are due to low signal noise ration. 66 % of the samples with tumor growth were identified. 33 % false negative results might be caused by partial volume effects based on the slice thickness. Further activities will concentrate on reduced slice thickness and increase SNR.

poster number: 037

MULTI-MODAL MOLECULAR IMAGING OF ANTI-ANGIOGENIC TREATMENT STRATEGIES OF EXPERIMENTAL GLIOMAS

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Introduction: Glioblastomas are highly vascularized tumors. Hence, anti-angiogenic treatments are an attractive option. However, they are limited by the potential evolution of the tumor cells towards infiltrative, angiogenically independent cells, co-opting the pre-existing vasculature, and leading to recurrence of the cancer. Development of imaging methods to non-invasively characterize the tumor vasculature in terms of vessel size, density and neo-angiogenesis and the tumor proliferation is essential to assess the effect of anti-angiogenic treatment.

The goal of our study was to describe changes in tumor vasculature and proliferation upon Bevacizumab treatment (anti-VEGF neutralizing antibody) using positron emission tomography imaging (PET) and magnetic resonance imaging (MRI).

Methods: Nude rats (n=12) were intracranially implanted with human glioblastoma biopsy samples. Three weeks after tumor implantation, [C-11]methionine (MET) and [F-18]fluorothymidine (FLT) PET as well as diffusion-weighted imaging (DWI) and steady state contrast enhanced (SSCE) MRI for vascular imaging were performed. Half of the rats were then treated with weekly injection of 10 mg/kg of Bevacizumab for 3 weeks. Imaging was performed again after treatment. Tumors were then analyzed by immuno-histochemistry.

Results: Three weeks after implantation tumors could be observed on the T2*-weighted MR images but were barely visible on the PET scans. MRI analyses showed an increase of the vessel density and the cerebral blood volume. Three weeks later treated and non-treated rats showed an increase of FLT and MET uptake. Accumulation of both tracers was however less intense for the treated group compared to the non-treated group (MET, p=0,035; FLT, p=0,190). In both groups total blood volume and vessel size were increased at week 6 compared to week 3, whereas vessel density was decreased. Microvessel blood volume was decreased in treated rats compared to the non-treated rats (p=0.013), whereas mean vessel size was increased (p<0.01). Immuno-histochemical analyses confirmed imaging data.

Conclusions: Bevacizumab treatment failed to prevent formation of dilated vessels but development of new small vessels was decreased. Good correlation was observed between MET uptake, calculation of vessel size and density using the MRI scans and histological observations. These data indicate that multi-modal imaging by PET and MRI reveals important information on anti-angiogenic treatment strategies in gliomas.

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poster number: 038

IMPROVEMENT OF TEMOZOLOMIDE CHEMOTHERAPY USING VIRALLY DELIVERED ANTI-MGMT SHRNA

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Introduction: Despite treatment combining surgery, radiation-, and chemo-therapy, most patients with malignant glioma have a very poor prognosis. Addition of temozolomide (TMZ) to radiation therapy is now the standard therapy for the treatment of glioblastoma. However, the effectiveness of alkylating agents such as TMZ is limited by tumor over-expression of the DNA repair protein O6-methylguanine-DNA methyltransferase (MGMT). Inhibition of MGMT is therefore a promising strategy to increase efficiency of chemotherapy. The goal of our study was to use the high specific and efficient RNA interference pathway in order to modulate MGMT expression and increase TMZ therapeutic effect in resistant glioblastoma.

Methods: Two anti-MGMT and one control shRNA sequences were cloned into a lentivirus backbone. Lentiviruses were used to infect a human glioblastoma cell line (LN18) presenting a high expression of MGMT. Control of the MGMT expression level and resistance of the modified cells toward TMZ were assessed using western blot, growth and clonogenic assays were used to. Nude mice subcutaneously xenografted with cells depleted or not in MGMT were treated with daily injections of TMZ. Tumour sizes were measured before, after 7 and after 12 days of treatment. FLT-PET scans were performed before and after 7 days of treatment.

Results: A specific inhibition of the MGMT expression was observed at the protein level using the two anti-MGMT shRNA sequence (90% and 95% of inhibition). Reduction of the TMZ LD50 was observed after down-regulation of the MGMT protein in culture. Tumour growth inhibition and reduction of FLT uptake was observed following TMZ treatment for the xenografts with low MGMT expression in contrast to xenografts with high MGMT expression.

Conclusions: Specific inhibition of MGMT protein could be obtained through the use of virally delivered shRNA, leading to improvement of the TMZ therapeutical effect. Anti-MGMT shRNA gene therapy could be used in combination with TMZ chemotherapy in order to improve the treatment of resistant glioblastoma.

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poster number: 080

TUMOR REOXYGENATION FOLLOWING INHIBITION OF THE MAPKINASE PATHWAY: UNDERLYING MECHANISMS AND RADIOSENSITIZING EFFECTS

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Introduction. It is well recognized that tumor hypoxia is a critical determinant for response to radiotherapy. In the current study, the chronic effects of different MAPK inhibitors were monitored in vivo using EPR (Electron Paramagnetic Resonance) oximetry in experimental tumors and the window of reoxygenation was exploited in order to sensitize tumors to radiation therapy. The regrowth delay assay was correlated with ADCw measured by DW-MRI at 11.7T in tumors 24h after irradiation.

Methods. FSa II (Fibrosarcoma) tumor bearing C3H mice were injected daily with either vehicles or a MAPK inhibitor (Sorafenib or Gefitinib at 45 mg/kg/Day; PD-0325901 or Farnesyl Thiosalycilic Acid (FTS) at 20 mg/kg/Day). Local pO2 was estimated using in vivo EPR oximetry (1.1GHz).

Results. Sorafenib, Gefitinib, PD 0325901 and FTS treated mice dramatically modified FSaII tumor pO_2 upon one day post-injection. The window of reoxygenation was identified at day 2 and used for the rest of the experiments. O_2 consumption, assessed by X-band EPR, decreased in treated tumors for all MAPK inhibitors tested. A rough estimate of tumor blood flow, assessed by patent blue staining, showed a high increase for Sorafenib, but no modification for the 3 other inhibitors. The increase in tumor pO_2 was therefore shown to be the result of two major factors: (i) an increase in blood flow for Sorafenib only, which could be explained by the anti-angiogenic effect of Sorafenib (and confirmed by CD105 antibody staining that suggests a normalization effect); and (ii) a decrease in O_2 consumption for all MAPK inhibitors, likely to be due to an alteration of the mitochondrial activity observed using the Mitotracker probe in Sorafenib treated tumors. This effect could be due to the observed GSH depletion which could affect mitochondrial activity. The therapeutic relevance was assessed by a regrowth delay assay following a 20 Gy irradiation (X-rays) at day 2 after treatment with Sorafenib. The tumor radiosensitivity was enhanced by a factor of 1.5 in the treated and irradiated group compared to X-rays alone. Moreover, a group including clamped tumors (deprivation of oxygen at the time of irradiation) and Sorafenib did not show any enhancement of response, which definitely proves the involvement of an oxygen effect. No cellular death after 2 days of treatment with Sorafenib was observed using DW-MRI and TUNEL assay, which excludes a major effect of cellular death on the observed reoxygenation. Finally, ADCw measured at day 3 (24h post irradiation) was significantly increased in the Sorafenib treated group, suggesting that ADCw could be an early marker of tumor response after inhibition of the MAPK pathway.

Conclusion. This study constitutes the first demonstration of the tumor reoxygenation following MAPK pathway inhibition.

poster number: 081

CLINICAL EVIDENCES OF GM3 (NEUGC) GANGLIOSIDE EXPRESSION IN HUMAN PRIMARY AND METASTATIC BREAST CANCER USING THE 14F7 MONOCLONAL ANTIBODY LABELED WITH 99MTC

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Introduction: The relevance of certain gangliosides in tumor growth and metastatic dissemination has been well documented; reasons exist for considering these molecules as potential targets for cancer immunotherapy and diagnosis. GM3 (NeuGc) ganglioside is particularly interesting due to its restrictive expression in normal human tissues. According to immunohistochemical studies and biochemical methods, they have strongly suggested its over-expression in human breast tumors. Nevertheless, the lack of a direct evidence of this antigenic display in human breast cancer has kept the subject controversial. On the other hand, concomitant studies have been shown that NGcGM3 ganglioside-mediated 14F7 mAb-induced cell death was accompanied by cellular swelling, membrane lesion formation, and cytoskeleton activation, suggesting an oncosis-like novel phenomenon (1).

Methods: For the first time, we described herein the ''in vivo'' detection of GM3(NeuGc) ganglioside in human breast primary and metatatic tumors using a radioimmunoscintigraphic technique with 14F7 monoclonal antibody (mAb), a highly specific anti-GM3(NeuGc) ganglioside monoclonal antibody, labeled with 99mTc (2). We performed two clinical studies. In the first one, a prospective Phase I/II clinical trial, including women diagnosed in stage II breast cancer, the 14F7 monoclonal antibody accumulation in tumors at doses of 0.3 (n=5), 1 (n=5) and 3 mg (n=4) was evaluated. In the second study we performed a Phase II clinical trial using 1 mg of 14F7 labeled with 99mTc, including 17 women in stage IV metastatic breast cancer, the 14F7 mAb accumulation in the primary tumor and its metastasis was evaluated.

Results: Noteworthy, in the first clinical trial the immunoscintigraphic study showed antibody accumulation in 100% of patients' tumors for the 1 mg dose group. In turn, the radioimmunoconjugate injected at doses of 0.3 mg or 3 mg of the antibody, was uptaken by 60 and 33.3% of breast tumors, respectively. In the second clinical trial, including metastatic breast cancer women there was an immunoscintigraphic sensitivity of 62.5 %. The evaluation of the toxicity by alteration of physical examination, and clinical laboratory studies (including HAMA tests) showed that the radioimmunoconjugate administration did not provoke any alteration at the tested dose levels.

Conclusion: We might reasonably conclude that 14F7 mAb administered by the intravenous route for tumor-imaging studies is safe and is able to detect breast primary and metastatic tumors. We are reporting that tumor associated GM3(NeuGc) ganglioside can be recognized 'in vivo' by antibodies. These results could support further therapeutic approaches using NGcGM3as a molecular target for antibody-based cancer immunotherapy.

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poster number: 082

IN VIVO DETECTION AND QUANTIFICATION OF PHOTOACOUSTIC SIGNAL FROM NANOPARTICLES USING A COMBINED PHOTOACOUSTIC MICRO-ULTRASOUND SYSTEM

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Introduction We have developed a photoacoustic (PA) micro-ultrasound imaging system which inherently coregisters structural and functional molecular information and can be used to image contrast agents in vivo. Here we describe the assessment of two types of intravenously-administered nanoparticles used as non-targeted (gold nanorods, GNRs) and targeted (single-walled carbon nanotubes, SWNTs) contrast agents in tumors in nude mice.

Methods A photoacoustic imaging system (Vevo LAZR, VisualSonics) was operated whereby light was generated by a tunable laser (680 - 970 nm) and delivered through fiber optic bundles integrated into a linear array transducer (LZ-550, fc = 40 MHz). 2D and 3D scans of a subcutaneous hindlimb tumor were performed before, during and after tail-vein injection of a bolus of GNRs (Nanopartz, Loveland, CO) at 800 nm or single-walled carbon nanotubes conjugated with arginine, glycine and asparagine (which are targeted to integrins) (Stanford University, Palo Alto, CA) or saline in a nude mouse at several wavelengths from 680 to 970 nm. The average PA signal in the 2D image of the tumor before, during and after the bolus was measured and compared. Pre- and post-bolus 3D scans were compared to provide an average photoacoustic value for the tumor both before and at several time points after injection of the nanoparticles.

Results For the gold nanorods, the average photoacoustic signal in the 2D scan performed during the bolus increased to a maximum of approximately 55% at 15 seconds. After approximately 2 minutes, the photoacoustic signal dropped back down to close to baseline. For the single-walled carbon nanotubes, the average photoacoustic signal during the bolus increased steadily to 10% approximately 3 minutes after bolus injection. Analysis of the 3D scans revealed a significant difference in signal after 35 minutes at 750, 800, 850 and 970 nm and the difference increased after 2 hours and persisted above the baseline (to a maximum of 26%) above baseline after 5 hours. The average photoacoustic values for the control tumor changed little (+/- < 5% of baseline) over the same time course as the SWNT animal. An increase in signal was observed for the SWNT-RGD injected animal above control at all time points with the exception of the first time point at 680 nm.

Conclusions We have demonstrated that the Vevo LAZR platform is capable of detecting signal from two different nanoparticle-based contrast agents in subcutaneous tumors in nude mice. The possibility of visualizing nanoparticles in vivo in real-time to understand cellular and molecular processes highlights the potential of this novel imaging system to be an in vivo molecular modality.

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MICRO-SPECT TO QUANTIFY RENAL UPTAKE OF RADIOPEPTIDES AND MONITOR KIDNEY FUNCTION

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Introduction: Recent improvements in spatial resolution and sensitivity of small animal SPECT(/CT) cameras have opened new possibilities in preclinical research, including serial imaging of the same animal to monitor disease progression or long-term therapy effects. Furthermore, accurate quantification of uptake of radioactivity is feasible, e.g. in kidneys.Peptide receptor radionuclide therapy (PRRT) with radiolabelled peptides targeting specific receptors is used to treat cancer patients. Renal reabsorption of radiopeptides labelled with ß-emitters may lead to long-term nephrotoxic effects. We used microSPECT to study the mechanisms of this renal retention and methods to reduce it. Here, an overview of SPECT quantification of renal uptake of different radiopeptides in mice and follow-up of kidney function after PRRT in rats is given.

Methods: Wild-type and kidney-specific megalin deficient mice were imaged 3 h and 24 h after injection of 40 MBq/2 nmol 111In-labelled peptides using the multi-pinhole NanoSPECT/CT camera. 111In-labeled octreotide, octreotate, neurotensin, minigastrin and exendin were studied in the same mice with 3-6 week intervals. Also, three groups of Lewis rats were imaged: controls and therapy rats receiving 460 MBq/15 µg 177Lu-DOTA-octreotate (177Lu-octreotate), without or with coinjection of 400 mg/kg lysine (n=4) to reduce renal retention of the radiolabeled peptide. At day 4 post therapy (p.t.) renal uptake of 177Lu-octreotate was quantified. At days 90 and 140 p.t. 99mTc-DMSA SPECT-scans were acquired to quantify renal reabsorption capacity. Dynamic dual isotope SPECTs using 50 MBq 111In-DTPA and 50 MBq 99mTc-MAG3 were performed between 100-120 days p.t. to monitor glomerular filtration and tubular secretion, respectively. Uptake of radioactivity was quantified and expressed as percentage of injected dose (%ID).

Results: The renal uptake of all tested radiopeptides in megalin-deficient mice was reduced to 25-60% of that in wild-type mice. The renal retention of 177Lu-octreotate in rats receiving lysine was reduced to 55% of the unprotected group. Follow-up of kidney function revealed an inverse correlation with absorbed kidney dose. 99mTc-DMSA uptake in control rats was 13.7 ± 1.4 %ID/kidney, which dropped to 6.4 ± 2.4 %ID/kidney in unprotected treated rats. In treated rats that received lysine renoprotection, uptake was similar to controls: 12.5 ± 1.2 %ID/kidney. Renography using 99mTc-MAG3 and 111In-DTPA resulted in a time-activity curve with a peak 2-6 min after administration (~15% and 6% ID/kidney, respectively), followed by a decline of uptake. Both radiopharmaceuticals demonstrated reduced peak activity in unprotected treated rats. In lysine-protected rats, uptake of 111In-DTPA was not significantly affected, but uptake of 99mTc-MAG3 was significantly reduced. These scintigraphic results were in agreement with biochemical parameters such as urinary protein and urea/creatinine serum levels as markers of renal function.

Conclusion: MicroSPECT proved to be an accurate tool to quantify uptake of radiopharmaceuticals in animals over time. Here we showed that the multi-ligand receptor megalin, expressed on renal proximal tubules, plays an important role in the reabsorption of radiopeptides. Co-administration of the basic amino acid lysine interfered with the renal retention of 177Lu-octreotate and protected against kidney damage as demonstrated by dual-isotope dynamic SPECT.

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ENMD-1198 IS CYTOTOXIC TO TUMOR CELLS WHILE IT PROTECTS BONE IN A MOUSE MODEL FOR METASTATIC BONE DISEASE

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Introduction: Due to the complex nature of bone metastasis new treatments should simultaneously target tumor cell proliferation, angiogenesis, immunological alterations and halt the cycle of bone metastases. In this study we describe the in vitro and in vivo efficacy of a combination therapy targeting these major processes involved in metastatic growth in bone. To do so we combined low dose 'metronomic' cyclophosphamide (CTX), the 2-methoxyestradiol derivate ENMD-1198 and the bisphosphonate risedronic acid.

Methods *in vitro* experiments. ENMD-1198 cytotoxicity was assessed using an MTS assay. The effect on bone resorption was assessed with a ^{45}Ca release assay [1]. Vascular effects were assessed using metatarsal vascular outgrowth assays [2,3]. *In vivo* experiments. The treatment effects of ENMD-1198 and ENMD-1198 based combination therapies were performed using intraosseous injection with the bone specific MDA-BO2-Luc cells. Follow up of tumor growth was done with bioluminescent imaging. Radiography and MicroCTwere used for the quantification of osteolysis.

Results:ENMD-1198 is cytotoxic to tumor cells, it showed both nti-angiogenic and vascular disruptive properties and protected the bone against tumor-induced osteolysis. We confirmed our *in vitro* data *in vivo*, showing the beneficial effects of ENMD-1198 and ENMD-1198-based combination treatments of metastatic breast cancer in bone both on tumor progression and on survival with long term ENMD-1198 treatment. We confirmed the *in vivo* relevance of the ENMD-1198 protective effect on bone both with X-ray radiographs and micro-CT. We combined ENMD-1198 treatment with low dose 'metronomic' clophosphamide and risedronic acid, leading to a mild increase in treatment efficacy.

Conclusions: ENMD-1198 treatment of osteolytic bone metastases has profound *in vitro* effects on both tumor growth and osteolysis. ENMD-1198 based combination treatments can control tumor growth, however the tumor continues growing after treatment is stopped indicating a strong cytostatic effect. Due to the potent cytostatic and anti-angiogenic effects of ENMD-1198, only a weak additional value of having CTX treatment could be observed. ENMD-1198 has strong anti-resorptive properties and the addition of risedronic acid lead only to a mild improvement of the overall treatment outcome. ENMD-1198 is a promising new compound in the field of metastatic bone disease affecting all processes that are crucial during development and growth of bone metastases.

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BEVACIZUMAB TREATMENT INCREASES APOPTOTIC CELL DEATH INDUCED BY IRINOTECAN DEMONSTRATED BY 99MTC HIS-ANNEXIN A5 IMAGING IN COLO205-BEARING MICE

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Introduction: Colorectal tumors are dependent on angiogenesis for growth and VEGF is a key mediator of tumor angiogenesis (1). Antiangiogenic drugs can induce a transient normalization of the tumor vasculature and can thus potentiate the activity of co-administered chemotherapy (2). The efficacy of anti-human VEGF antibody (bevacizumab) with or without irinotecan was evaluated in a human colorectal cancer xenograft model by 99mTc His-annexin A5 SPECT.

Methods: Colo205-bearing mice received a single-dose (ip) of bevacizumab (5mg/kg) 2, 4 or 6 days prior to administration of a single-dose of irinotecan (100mg/kg) or 0.9% NaCl. Microvessel density (MVD, von-Willebrand factor immunostaining), collagen covered tumor vessels (Masson's Trichrome staining), pericyt coverage (a-smooth muscle actin immunostaining) and tumor hypoxic fraction (pimonidazole staining) was determined at the three different time points following treatment of bevacizumab. Protein levels of VEGF-A in the tumor lysates and serum were determined by Elisa. To investigate the possible synergistic effects of the combination therapy, the apoptosis-detecting radiotracer 99mTc His-ann A5 was injected (iv, 0.5 mCi) in mice 12, 24 and 48 hours after start of the irinotecan treatment and to control mice (n=3 in each time group). MicroSPECT imaging was subsequently performed 3.5 hours after injection of the radiotracer. The results were correlated to histological analysis for apoptosis (caspase-3 activation).

Results: Normalization of the tumor vasculature was observed four days after receiving a single-dose bevacizumab. At this time point, MVD decreased significantly, a-smooth muscle actin and collagen covered vessels were increased compared to control tumors. Hypoxic fraction was slightly reduced 4 days after treatment with bevacizumab. Levels of VEGF-A in tumour lysates decreased significantly after a single-dose bevacizumab ($p < 0.05$), compared to controls, at all three time-points. In serum, VEGF-A levels were slightly increased compared to controls. SPECT analyses demonstrated the highest significant increase in tumoral 99mTc His-ann A5 uptake 4 days after bevacizumab treatment and 24h after irinotecan administration (233 ± 25% injected dose/ tumor weight/body weight, $p < 0.05$) compared to each monotherapy demonstrating a synergistic effect of both therapies. Quantitative 99mTc-ann A5 tumor uptake correlated well with the number of apoptotic cells as determined by caspase-3 immunostaining ($R2= 0.81$, $p < 0.05$).

Conclusion: Four days after administration, VEGF inhibition by bevacizumab normalizes the tumor vasculature in the Colo205 model, leading to an improved cytotoxic effect of irinotecan. The ability to measure chemotherapy-induced cell death in a noninvasive way may help clinicians to determine most optimal combination treatment schedule of anti-VEGF treatment with chemotherapy.

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COMBINED NIRF-OT AND OCT FOR CHARACTERIZATION OF TUMOURS IN SKIN CARCINOGENESIS

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Introduction**:** Diagnosis in skin carcinogenesis is achieved by invasive biopsies. One important criterion for the discrimination between pre-invasive and invasive tumour stages is the integrity of the basement membrane. At the invasive stage, the basement membrane is degraded by matrix metalloproteinases (MMPs). As non-invasive modalities, optical coherence tomography (OCT) can deliver morphological information of the basement membrane status (3), whereas near-infrared-functional optical tomography (NIRF-OT) allows the assessment of active MMPs in vivo. (1) Therefore**,** the aim of this study was to characterize the aggressiveness of different carcinoma types in mouse xenografts by combining NIRF-OT for assessing MMP-activity with OCT for morphological analysis of the basement membrane.

Methods**:** The aggressiveness of subcutaneous and intradermal HaCaT-ras A5-RT3 skin carcinoma xenografts in nude mice (2.0*106 cells per injection) was compared. Active MMPs were assessed in vivo by NIRF-OT (FMT2500LX, PerkinElmer) 6 hours after injection of 2 nmol of MMPSence 750 FAST (PerkinElmer) per animal. Micro computed tomography (µCT, CT Imaging GmbH, Erlangen, Germany) and FMT data were fused using Amide (sourceforge.net) and allowed quantification of active MMPs. Tumours were excised and measured ex vivo via the OCT system at the Institute for Semiconductor Electronics (2). Data were validated on tumour sections in vitro (MMP-activity by in-situ zymography (EnzCheck Gelatinase/Collagenase Assay Kit, Molecular Probes), basement membrane by immunostaining to collagen IV).

Results**:** A higher MMP-activity was measured in subcutaneous compared with intradermal tumours. This was confirmed by the higher gelatinase/collagenase-activity in sections of subcutaneous tumours. The penetration depth reached by the OCT-system was not high enough to allow a clear discrimination between tumour and surrounding dermal stroma. The measurement will be re-done using a clinical OCT system with higher penetration depth. Conclusion**:** NIRF-OT indicates a higher MMP-activity in subcutaneous than in intradermal tumours. Further studies are directed to analyze MMP-activity and basement membrane status including pre-invasive tumour stages.

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IMAGE-GUIDED SURGERY FOR TUMOR TARGETED ELECTRO-CHEMOTHERAPY

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Introduction: In most cancers tumor resection is the first therapeutic indication before chemotherapy or radiotherapy on condition that the tumor is removable. The patient survey largely depends on the primary tumor resection exhaustiveness and also possibly on metastasis removal or adjuvant chemotherapy. In the present work we developed a micro-metastases model very close to the clinical situation in which metastases appear secondarily to primary tumor resection. This experimental model offers the advantage to be finely controlled since both primary tumor growth and metastases invasion are monitored with non invasive multimodal imaging (bioluminescence, 3D fluorescence and CT). The model was used to evaluate a new therapeutic approach combining image-guided tumor location and electrochemotherapy (ECT) i.e. the use of electric pulses (EP) to enhance bleomycine uptake.

Methods: BALB/C mice were injected with luciferase positive adenocarcinoma cells (TS/Apc-pGL3) into the left kidney capsule. Seven days later (D8) the tumorous kidney was removed. Six days later (D14) multimodal imaging (bioluminescence, 3D fluorescence and CT) allows the evaluation of metastases peritoneal invasion. Mice were then randomized in 3 groups (n = 5) (G1: ECT (Bleo+electro); G2: Bleo Control; G3: EP Control) and all mice received an i.v. injection of RAFT-(cRGD)4-AlexaFluor700 for tumor fluorescence imaging the following day. At day 15, mice from G1 and G2 received an i.v. injection of low amount of Bleomycin (5mg/kg in 0.15M Nacl) whereas G3 received EP only. Five minutes later, mice underwent an abdominal surgery under the field of view of the portative Fluobeam®700 (Fluoptics) for image-guided metastases location and counting. For G1 and G3, each spotted metastasis was immediately subjected to EP. After surgery recovery, metastases progression or regression was followed by non invasive bioluminescence twice a week until mice sacrifice for ethical considerations.

Results: Mice from G2 (Bleo Control) quickly presented severe metastases progression. At day 19 the tumor burden was 324% of day 14. As a consequence mice from this group were killed at day 20. At the same time, G 1 (ECT) displayed a strong tumor regression (48% of day 14) corresponding to 15% of G2 tumor burden. Metastases invasion was mildly slowed down for G 3 (EP Control) in which the tumor burden at day 19 decreased to 73% of day 14. From day 25 some of the mice from G1 and G3 had to be killed but if considering the surviving mice, at day 28 tumor burden in G1 was still 2-fold smaller than in G3.

Conclusions: We developed a micro-metastases model in which metastases appear secondarily to primary tumor resection. Primary tumor development and metastases invasion were finely characterized by multimodal imaging. Then we evaluated a new therapeutic approach combining image-guided tumor location and electro-chemotherapy. We demonstrated the strong effectiveness of the ECT treatment to slow down metastases invasion.

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COMPARATIVE ANALYSIS OF IN-VIVO AND EX-VIVO MICRO COMPUTED TOMOGRAPHY IMAGING FOR ASSESSING TUMOUR VASCULARISATION IN XENOGRAFT TUMOUR MODELS

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Introduction: The visualisation of tumour blood vessels and quantification of relative blood volume (rBV) are essential for assessing treatment responses to anti-angiogenic therapies. In experiments using tumour xenografts, anti-angiogenic effects are generally evaluated by calculating micro vessel density (MVD) using immunohistochemistry (IHC). This method has several limitations; for example, the 3-D architecture and the vessel functionality (rBV) are not fully considered. To overcome these shortcomings, we investigated the potential of in-vivo micro computed tomography (µCT) and high-resolution 3-D µCT for the visualisation and quantification of tumour vascularisation.

Methods: Five different tumour models (A431, A549, Calu-6, MDA-MB-231, MLS) were analysed, which differed in vessel maturation and density. Following intravenous administration of a radiopaque contrast agent, tumours were imaged in-vivo using µCT (CT Imaging, Germany). After in-vivo scanning, mice were perfused with Microfil, a lead-containing radiopaque contrast agent which polymerises intravascularly. The tumour was then extracted, formalin-fixed and scanned in a high-resolution µCT scanner (SkyScan, Belgium) with a maximal spatial resolution of 2-4 µm. Histological validation was performed by CD31 and alpha-smooth muscle actin (SMA) staining. After defining a tumour isolating region of interest the rBV in tumours was determined by the rule of proportion from the brightness of the tumour after blood pool contrast agent injection, a large blood vessel (100% rBV) and the tumor before injection (0% rBV).Additionally, tumour vascular architecture was visualized using 3-D rendering software (MeVisLab).

Results: Histological analyses showed that nearly all CD31 positively stained vessels with a diameter greater than 6µm were filled with Microfil. In-vivo µCT analysis of vascularisation in tumour models with large and highly matured vessels (CD31-positive, SMA-positive), such as A549, enabled the detection of blood vessel branches up to the 3rd order. In comparison, using vascular casting and ex-vivo high-resolution µCT, blood vessel branches up to the 7th order could be visualized. Using high-resolution µCT, tumours such as A431, which had small and immature vessels (CD31-positive, SMA-negative) showed visualization of radom assembled fine vessels without hierarchy.In-vivo µCT data on rBV ranged from 2.2% to 6.2% and were comparable to histological MVD (ranging between 2.4 and 4.2 %). In contrast, rBV determined from high-resolution µCT vascular casts demonstrated higher values, ranging from 4.8% to 9.2%.

Conclusions: An imaging protocol, based on in-vivo µCT and high-resolution ex-vivo µCT after vascular casting was developed. This accurately determined the rBV of tumour xenografts and visualized the 3-D morphology of tumour blood vessels (with diameters larger than ~6µm). We showed that high-resolution 3-D µCT imaging of tumour vessel casts enabled the analysis of the micro-architecture of tumour vessels and may be an exquisite tool to study the effects of pro- and anti-angiogenic stimuli on vascular development. While rBV determined by in-vivo µCT and histology were comparable, higher values were obtained from high-resolution µCT imaging. We are currently analyzing whether in-vivo µCT and histology underestimate the rBV, or whether ex-vivo µCT overestimates it. Differences may originate from vascular perfusion during the casting process or from suboptimal thresholding in high-resolution µCT analysis.

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FIRST CLINICAL APPLICATION OF 99MTC LABELLED LONG-ACTING AGONIST OF GLP-1 (EXENDIN-4) IN ENDOCRINE TUMORS DIAGNOSIS

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Introduction: Overexpression of glucagonlike peptide-1 receptors in tumors is target for future imaging and therapy. GLP-1-receptor scanning offer a new approach for successful localization of small or not detectable by other methods insulinomas. Aim of the study: The aim of study was to present first successful experiences of our unit with new tracer - [Lys40(Ahx-HYNIC-99mTc/EDDA)NH2]-Exendin-4.

Material and method: [Lys40(Ahx-HYNIC-99mTc/EDDA) NH2]-Exendin-4 receptor scintigraphy was performed in 9 patients: 7 suspected to be insulinomas, one with nesidioblastosis and one with malignant insulinoma suspected as recurrence. Moreover the study was performed in 3 patients with medullary thyroid carcinoma (MTC) -one with metastases to the liver and 2 suspected as recurrence. All images were acquired with a dual-head, large field of view E.CAM gamma camera with low-energy high resolution (LEHR) collimators. Whole-body scans were performed in 6 time points using the same procedure. Additionally SPECT 6 hours after injection the tracer was performed in all cases.

Results: In 5 from 7 cases the uptake of [Lys40(Ahx-HYNIC-99mTc/EDDA)NH2]-Exendin-4 in pancreas was found. In case of one residual patient from this group hypoglycemia was caused by exogenous injections of insulin by patient. In case of second patient no lesion in GLP-1 scintigraphy was found. In women suspected as recurrence the small uptake in the place of removed head of pancreas was found. In patients with MTC: one has the pathological accumulation of the uptake in the liver, in one suspected as recurrence similar uptake in thyroid scintigraphy with the use of Tc99m and I131was found, in one recurrence of MTC was assessed.

Conclusion:[Lys40(Ahx-HYNIC-99mTc/EDDA)NH2]-Exendin-4 scintigraphy offers a new diagnostic possibility in case of localization of hardly detectable small insulinomas and may be also useful to assess recurrence and avancement of the disease in patients with medullary thyroid cancer. Further clinical trials should be conducted.

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DW-MRI AS AN ALTERNATIVE TO DCE-MRI FOR ASSESSMENT OF DRUG-INDUCED VASCULAR CHANGES IN EXPERIMENTAL BONE METASTASES

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Introduction Dynamic contrast-enhanced (DCE)-MRI is often employed to assess functional parameters of vascularization in primary tumors or metastases [1]. Diffusion-weighted (DW)-MRI emerges as an important technique to characterize tumors, e.g. cell density and tissue perfusion, without the need for contrast media application. Here, we investigated whether DW-MRI was useful as an alternative to DCE-MRI to determine vascular changes in experimental bone metastases after systemic therapy with an integrin inhibitor.

Methods Human breast cancer cells (MDA-MB-231) were injected into the superficial epigastric artery of nude rats resulting in bone metastases development in the right hind leg [2]. Rats treated daily for 25 days with the small molecule integrin inhibitor and control rats were imaged using DW-MRI and DCE-MRI prior the onset of therapy and after treatment. DCE-MRI was performed on a 1.5T MR scanner using 0.1 mmol/kg Gd-DTPA according to the pharmacokinetic model of Brix (amplitude *A*, relative blood volume; exchange rate constant k_{ep}, vessel permeability and perfusion) [3]. DW-MRI was performed using following b-values; 0, 50, 100, 150, 200, 600 and 1000 s/mm^2. The apparent diffusion coefficient (ADC) was calculated before and after the therapy from (i) whole set of b-values; ADC_{total}, (ii) low b-values (0, 50, 100 s/mm^2); ADC_{low} and (iii) high b-values (600, 1000 s/mm^2); ADC_{high}. Data acquired by DCE-MRI and DW-MRI after the treatment were normalized to those obtained before the onset of therapy. Finally, immunofluorescent analysis of representative animals was performed using the following antibodies: anti-collagen IV (basement membrane) and anti-SMA (pericytes). Difference between the groups was tested using the two-sided Wilcoxon test.

Results Relative values for amplitude *A* decreased in treated rats compared to controls (89% T/C; p=0.05) whereas exchange rate constant k_{ep} showed an increase (180% T/C; p=0.05) as assessed by DCE-MRI. From DWI, relative ADC_{low} values were significantly increased in the treatment group at the end of the observation period (148% T/C; p<0.05) compared to controls. However, relative ADC_{high} and ADC_{total} values were not significantly different between the two groups at the corresponding time point (108% and 117% T/C, respectively). Immunoflurescent analysis confirmed vascular remodeling in bone metastases after therapy compared to untreated controls.

Conclusion Vascular remodeling upon integrin inhibition was observed by DCE-MRI and confirmed by immunofluorescent analysis. Using only low b-values sensitive to perfusion (and to a lesser extent diffusion) [4,5] for ADC calculation, DW-MRI revealed significantly increased tissue perfusion after therapy, confirming the findings obtained with DCE-MRI. We conclude that DW-MRI is a valuable alternative to DCE-MRI for assessment of drug-induced vascular changes in bone metastases.

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IMAGING OF THE ENDOTHELIN-A-RECEPTOR EXPRESSION IN MURINE THYROID CANCER XENOGRAFTS BY OPTICAL IMAGING

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Introduction: Endothelin (ET) receptor dysregulation as a parameter of angiogenesis has been described in a number of pathophysiological processes, including cardiovascular disorders, renal failure and cancer. A number of human cancer cell lines, including thyroid cancers, show an upregulated expression of ET-A receptors, influencing tumour growth and aggressiveness. Labelled receptor ligands offer the possibility to noninvasively assess ET receptor distribution in vivo using dedicated imaging systems. Optical imaging (OI) is a powerful technology for the preclinical study of diseases at the molecular level. In this study, a fluorescent dye labelled biomarker, targeted to ET-A receptors, was used for the evaluation of target expression in papillary thyroid carcinoma xenografts using optical imaging tools.

Methods: Subcutaneous and orthotopic xenograft models of papillary thyroid carcinoma cell line K1 were established in CD-1 nude mice. A fluorescent dye-labelled analogue of the known ET-A receptor ligand PD 156707 was designed[1] and applied in fluorescence reflectance imaging (FRI) and fluorescence mediated tomography (FMT) examinations. The binding of the tracer to ET-A receptors on tumour sections was evaluated by microautoradiography using ^{125}I-ET-1 as competing receptor ligand.

Results: In optical imaging experiments using FRI and FMT techniques, a high fluorescence signal was visible in the tumour region after 24 hrs. Significant reduction in signal intensity was observed after predosing with lead compound PD 156707. Biodistribution studies after 48 hrs confirmed a significantly lower fluorescent signal in tumour tissue when animals were predosed. Autoradiography proved specific receptor binding of the designed tracers in vitro, especially emphasizing the high receptor density in the border region of the tumour. These findings yet confirm the results of FMT imaging, where also a stronger fluorescent signal in the tumour margin was found.

Conclusions: Papillary thyroid carcinoma xenografts are a feasible model for the detection of endothelin receptor expression as a benchmark of tumour angiogenesis. Optical imaging techniques in combination with labelled endothelin receptor ligands can be useful tools for the evaluation of target expression in tumour lesions. Optimization of tracer performance may offer the possibility for a practicable targeted molecular imaging approach.

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EVALUATION OF TUMOR TREATMENT WITH DOCETAXEL IN MICE USING 18F-FLT- AND 18F-FDG-PET AND DIFFUSION WEIGHTED MAGNETIC RESONANCE IMAGING

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Introduction: In this study we investigated tumor proliferation, functional status and growth of a human colon adenocarcinoma cell line (HCT116) in mice by [18F]FLT/[18F]FDG-PET and Diffusion-weighted magnetic resonance imaging (DWI) using the cytostatic drug docetaxel as treatment over a period of seven days.

Methods: Measurements were performed on a microPET scanner and a 7 T small animal MRI system. 107 HCT116 cells were inoculated into female SWISS nude mice (n=16). A visible tumor appeared after ten days. After baseline imaging, docetaxel (15 mg/kg) was injected intravenously the following day. Further PET and MR imaging was performed on day 2, 5 and 7 after administration of docetaxel. For the PET scans mice were administered with ~13 MBq [18F]FLT or [18F]FDG. The uptake times for the static PET scans were 90 min for [18F]FLT and 55 min for [18F]FDG, with a 10 minute static scan then acquired. DWI was performed in sagittal direction (b= 150, 250, 400, 600, 800, 1000 sec/mm2, TE= 112 ms, TR = 5000 ms, Δ= 41 ms, δ= 20 ms). Coregistration and analysis of the MR and PET images were performed using Inveon Research Workplace.

Results: On the baseline imaging day, mean tumor size for both, control and docetaxel treated groups, was 0.29±0.15 cm3. Tumors in the control group grew throughout the study (1.10±0.37 cm3 on day 7) whereas tumors in the docetaxel treated group showed growth arrest (0.40±0.22 cm3 on day 7). The PET measurements of the [18F]FLT control group showed a slightly increased tumor uptake over the measurement period (from 4.73±0.61 %ID/cc to 5.09±2.02 %ID/cc on day 7). In contrast, the docetaxel treatment group showed a significantly (p=0.02) increased [18F]FLT uptake (from 4.68±0.60 %ID/cc to 6.07±0.36 %ID/cc on day 7). The [18F]FDG control group showed a decreased tumor uptake (from 3.80±1.34 %ID/cc to 3.26±0.28 %ID/cc on day 7) after an increase in tumor uptake on day 2 (4.29±0.62 %ID/cc) revealing a significant (p=0.03) decrease between day 2 and day 7. The docetaxel treatment group showed decreased tumor uptake (from 3.63±0.80 %ID/cc to 3.04±0.84 %ID/cc on day 7). The DWI revealed a mean baseline apparent diffusion coefficient (ADC) value of 0.69±0.10*10-3 mm2/sec with the control group showing no significant change in ADC values over the measurement period. In contrast the ADC values of the treated animals increased significantly (p=0.02) after administration of docetaxel (from 0.66±0.1*10-3 mm2/sec to 0.82±0.12*10-3 mm2 /sec on day 7).

Conclusions: Docetaxel treatment causes the promotion of microtubule polymerisation leading to cell cycle arrest that could explain the increased [18F]FLT uptake. It also causes apoptosis and cytotoxicity which could lead to the increased ADC and the decreased [18F]FDG uptake[1]. ADC maps revealed an inverse spatial correlation to the [18F]FLT/[18F] FDG uptake demonstrating the complementary relationship between diffusivity in necrotic regions and metabolism of proliferating cells.

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INTRAOPERATIVE FLUORESCENCE IMAGING OF PERITONEAL DISSEMINATION OF OVARIAN CARCINOMAS. A PRECLINICAL STUDY

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Introduction: Ovarian cancer is a leading cause of death in women [1]. Current management of advanced ovarian cancer is based on complete cytoreductive surgery and chemotherapy. The poor outcomes of this cancer which is directly related to the amount of diseased tissue left in situ call for an increase in surgical aggressiveness. In the past, the majority of groups have fixed a cut-off value of 2 cm to define what was called 'optimal' cytoreduction. The target has currently moved towards complete removal of visible implants [2]. However, the methods of assessment of residual disease and the concept of complete cytoreduction suffer from limitations, mainly in terms of visualization of the remaining disease [3,4]. The aim of this study was to validate the importance of fluorescence (AngioStamp® fluorescent agent) in the peroperative detection of human ovarian adenocarcinoma cells and to determine its efficiency in detecting infra millimetric tumour metastases using Fluobeam® as reader system.

Methods: A fluorescent RAFT-(cRGD)4 tracer molecule (AngioStamp®) was used. The tracer is based on a biomarker, which has a very high affinity for the avß3 integrin, which is overexpressed in a large ratio of cancer cells and neovessel endothelial cells during angiogenesis. Near infrared optical window wavelengths (650-900 nm) were used to avoid potential absorption and autofluorescence problems [5]. Infrared fluorescence was visualized with Fluobeam®, an open fluorescent imaging system that could potentially be used in peroperative conditions in the future.

Results: This novel technique allowed the specific detection of residual tumor deposits and inframillimetric metastases, smaller than 500 µm, which were resected under fluorescent guidance. AngioStamp® was able to detect all types of cell lines, derived from human ovarian adenocarcinomas. We evaluated the effective co-localization of this molecule with tumor sites (primary tumors and metastases), and demonstrated that this molecule effectively targets most of the human ovarian tumors as well as the associated tumoral neoangiogenesis. We confirmed that targeting of tumors was possible, even after chemotherapy treatment of the animals, which is highly relevant, as surgery in clinical trials is currently performed after 3 lines of chemotherapy. Finally the effectiveness of AngioStamp® for the detection of various human ovarian adenocarcinomas was assessed on 10 different human fragments of tumor, implanted subcutaneously in nude mice. All implanted tumor fragments were visualized by AngioStamp®. [6]

Conclusions : We validate the use of AngioStamp® for peroperative detection of inframillimetric ovarian adenocarcinoma tumors in experimental trials and provided preclinical information before designing a clinical trial.

Acknowledgements: Grant support: The Midi Pyrenees Region, the GRICR (Institut Claudius Regaud), the ANR (Biodendridot) and La Ligue Française contre le Cancer Midi-Pyrénées ».(1) Jemal A et al, 2009. CA Cancer J Clin 2009; 59:225-49. (2) Desfeux P et al. Gynecol Oncol 2005; 98:390-5. (3) Gutowski M, et al Clin Cancer Res 2001; 7:1142-8.(4) Nguyen QT, et al. Proc Natl Acad Sci U S A 2010; 107:4317-22.(5) Sancey L, et al. Int J Pharm 2009; 379:309-16.(6) Mery E et al , Gynecol Oncol 2011

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THE EFFECTS OF ANESTHESIA AND MRI CONTRAST AGENTS ON ORGAN AND TUMOR DISTRIBUTION OF SPECT TRACER OCTREOSCAN.

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Introduction: Multi-modality imaging approaches offer the advantage of complimentary information. MRI, with aid of contrast agents, provides detailed anatomical and functional tumor characteristics and can be complementary to SPECT, which we used to image tumor uptake of Octreoscan, a somatostatin analog labeled with Indium-111. However, it is important to ensure that there are no negative interactions of the animal handling protocols used during the scanning sessions. Radioactive exposure makes it desirable to perform MRI first. We therefore studied the effect of the MRI anesthesia protocol and MRI contrast agents on tumor internalization and distribution of Octreoscan.

Methods: The somatostatin receptor-positive rat pancreatic CA20948 tumor cell line was used to perform in vitro and in vivo experiments. In vitro tests were performed to study the influence of MRI contrast agents. Gadovist or Magnevist (0.7-70 mM) was added prior to, and during tumor-cell incubation with Octreoscan in a 6-well plate. In vivo studies were performed on Lewis rats bearing subcutaneous CA20948 tumors. Gadovist was administered intraveneously five minutes before injecting Octreoscan (3MBq/10µg). Effects of MRI anesthesia (medetomidine) were compared to SPECT anesthesia (isoflurane) during the in vivo circulation of Octreoscan. After 1h of circulation, all animals were euthanized and dissected, and then tumor and organ radioactivity was measured in a γ-counter.

Results: In vitro results show 60% ($p<0.01$) and 83% ($p<0.01$) less uptake tumor cell uptake when Gadovist or Magnevist (70mmol) respectively were added before, or at the start of Octreoscan internalization. At lower doses of contrast agents more Octreoscan internalized. Adding contrast agents later during the internalization process resulted in higher cellular internalization of Octreoscan. In vivo, administration of Gadovist showed no negative effect on tumor-uptake in comparison to the control group, however, significantly higher amount of radioactivity was found in kidneys and receptor-positive organs. Octreoscan uptake was higher in some organs when medetomidine anesthesia was used, but there was no significant change in tumor uptake.

Conclusions: Under in vitro conditions presence of Gadovist or Magnevist reduces cell internalization of Octreoscan . In vivo Gadovist does not affect tumor uptake of Octreoscan. Gadovist does affect octreoscan distribution in organs and tissues other than the tumor with a very pronounced effect in the kidney. The use of medetomidine anesthesia results in a higher amount of radioactivity in the blood and several organs compared to isoflurane anesthesia. The effect seen with medetomidine anesthesia is likely caused by effects of medetomidine on heart rate and blood pressure [2]. In combined modality studies using (contrast-enhanced) MRI and or different anesthesia protocols one should be aware of the effects anesthesia and MRI contrast agents have on the uptake of peptides in tumors and organs.

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CHARACTERIZATION OF EXPERIMENTAL GLIOMAS BY MULTIMODAL MOLECULAR IMAGING

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Introduction: Malignant gliomas are the most common primary intracranial neoplasms in humans. In view of the poor prognosis of this tumor it is important to carefully monitor the progress of this disease and early response - or non-response - to therapy. Molecular imaging allows the non-invasive longitudinal characterization of disease specific alterations in vivo. This technology is increasingly used for assessing cancer-associated molecular markers as the basis for implementation of disease-stage specific therapies. The aim of this study was to assess the growth dynamics of human gliomas in a mouse model by using different molecular imaging modalities.

Methods: Imaging of subcutaneous and intracranial growing and stably luciferase expressing human U87 and Gli36 gliomas in nude mice was performed employing bioluminescence imaging (BLI), fluorescence resonance imaging (FRI), positron emission tomography (PET), and computed tomography (CT). Molecular targets for imaging comprised expression levels of luciferase, activity of matrix metalloproteinases (MMP; MMPSense680), glucose metabolism (F18-FDG), and amino acid transport (C11-methionine). Some animals were followed after treatment with the chemotherapeutic agent temozolomide.

Results: Repetitive BLI of luciferase expressing intracranial gliomas revealed an exponential growth. The identity of the tumor could be confirmed by C11-methionine PET and histology. Active MMPs were detectable in gliomas by FRI of MMPSense680. Temozolomide treatment resulted in a decrease of the tumor size as measured by caliper and yielded a reduced biological tumor activity as assessed by luciferase BLI. This reduction of tumor metabolism could be quantified by F18-FDG PET analysis. Comparison of optical imaging with PET parameters demonstrated that optical imaging is less capable of reflecting inhomogeneities within a tumor with regards to differentiation of viable tissue from central necrosis of larger tumors.

Conclusions: Optical imaging is a valuable, relatively inexpensive and rapid technology to non-invasively analyze growth dynamics of experimental gliomas. It should be pointed out that tomographic optical methods including scatter and attenuation correction still have to be developed. PET adds important tomographic and quantitative molecular information which can be translated directly into human application.

poster number: 096

PRECLINICAL IMAGING OF PROTEIN SYNTHESIS: COMPARISON OF 68GA-DOTA-PUROMYCIN AND 44SC-DOTA-PUROMYCIN

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Introduction: Multiple attempts to measure protein synthesis *in vivo* have been made during the last decades, using mostly radiolabeled amino acids, with limited or unsatisfactory success. The difficulties in utilizing radiolabeled amino acids for *in vivo* determination of protein synthesis are due to the wide variety of possible metabolic pathways of amino acids[1]. Puromycin has played an important role in our understanding of the eukaryotic ribosome and protein synthesis. It has been known for more than 40 years that this antibiotic is a universal protein synthesis inhibitor that acts as a structural analog of an aminoacyl-tRNA (aa-tRNA)[2]. Applying pharmaceutical concentrations, premature proteins are produced. Puromycin, in low concentrations, binds specifically to the C-terminus of full-length proteins. The purpose of this study was to compare ^{68}Ga-DOTA-Puromycin and ^{44}Sc-DOTA-Puromycin as suitable radioconjugates for *in vivo* determination of protein synthesis.

Methods: DOTA-Puromycin was synthesized following our design. ^{44}Sc was obtained from ^{44}Ti/^{44}Sc-generator as described previously by Filosofov *et al.* in 2010[3]. ^{68}Ga was obtained from ^{68}Ge/^{68}Ga-generator as described previously by Zhernosekov *et al.* 2007[4]. In both case the purified generator eluate was directly used for labeling of DOTA-Puromycin at 95°C for 20 minutes. Radioconjugates were purified using C-18 cartridges. For µPET-studies 20-25 MBq of purified, labeled DOTA-Puromycin was administered to tumor bearing rats and animals were scanned for 45 minutes (^{68}Ga) and 2 hours (^{44}Sc) dynamically.

Results: µPET- images of tumor bearing rats showed significant higher tumor uptake for ^{68}Ga-DOTA-Puromycin compared with ^{44}Sc-DOTA-Puromycin. Images with both DOTA-Puromycin conjugates showed clearly visible tumor outlines. Tumor/reference ratios (T/nT) have been significantly higher for ^{68}Ga-DOTA-Puromycin (T/nT = 78:1) than for ^{44}Sc-DOTA-Puromycin (T/nT = 44:1).

Conclusions: In respect of preparation time for labeled DOTA-Puromycin (75 minutes), ^{44}Sc with its $T_{1/2}$ = 3.97 hours would be the radionuclide of choice. Regarding the biological properties and imaging resolutions ^{68}Ga-DOTA-Puromycin seems to be superior over ^{44}Sc-DOTA-Puromycin for determination of protein synthesis via PET. Since both radioconjugates are suitable for imaging of protein synthesis, the choice of the used radionuclide can be based on the availability of the radionuclides.

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poster number: 097

IN VIVO VISUALISATION OF MACROPHAGE ACTIVITY IN TUMOUR LESIONS ALLOWS FOR ESTIMATION OF THE MALIGNANT POTENTIAL OF THE DISEASE

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Introduction Characteristic features of malignant tumours like local invasion, neoangiogenesis and finally metastatic spread are crucially dependant on the interaction with tumour-recruited host cells. Amongst those functionalised cells, macrophages are one of the major fractions. Tumour associated macrophages (TAM) have been shown to promote tumour progression and metastasis, found e.g. at sites of basement-membrane breakdown, enabling the tumour cells to locally egress into surrounding stroma. TAM density and activity correlates with poor prognosis in malignant diseases.Macrophage activity is reflected by the local expression of S100A9. This study should confirm the possibility to visualise TAM activity in tumours in-vivo, using Optical Imaging (OI) and a S100A9-specific fluorescent tracer.

Methods An antibody, specifically binding S100A9, was labelled with Cy5.5 for in-vivo OI. Labelled, rabbit-derived IGG served as control for unspecific label distribution in single (label Cy5.5) and parallel (Cy7) injection settings.Balb/c mice were inoculated with 4T1 murine breast cancer or MOS murine osteosarcoma and lesion size was determined daily. OI was performed tumour size dependant 24h after injection of the fluorescent probes. For correlation of imaging results, immunohistochemistry of tumour sections was performed and tumour cells were analysed for S100A9 expression using ELISA.

Results Neither 4T1 nor MOS cells showed significant S100A9 production in ELISA. In fast-growing 4T1 tumours, Anti-S100A9-Cy5.5 injection resulted in fluorescence signal which was regularly about two-fold higher compared to IGG-Cy5.5 (2356.8 vs. 1283.2; $p<0.005$). At same size, slow-growing MOS accumulated less Anti-S100A9-Cy5.5 than 4T1 (1655.3 vs. 2356.8), reflecting lower macrophage activity. Perfusion effects could in this context be ruled out by parallel injection of IGG-Cy7 and determination of resulting, not significantly diversifying signals for both tumour entities. Histology confirmed in-vivo imaging results regarding TAM density. In vivo competition for the target could be excluded by parallel injection of both tracers without alteration of the acquired signal, compared to single injection.

Conclusion OI allows for specific in-vivo visualisation of macrophage activity in primary tumour lesions and therefore for estimation of their malignant potential as represented by the local growth.

poster number: 098

ASSESSMENT OF THE ERYTHROPOIETIN RECEPTOR STATUS BY NON INVASIVE IMAGING USING A NEWLY GENERATED NEAR-INFRARED PROBE

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The discovery of erythropoietin receptor (EpoR) transcripts in cancer and endothelial cells has led to controversial discussions about the use of recombinant human erythropoietin (rhuEpo) for treatment of cancer-associated anemia (1, 2). Functional EpoR in the tumor tissue might promote cancer growth and thus have a negative clinical outcome in patients receiving rhuEpo (3). Especially the longitudinal in vivo-analysis of the EpoR status in tumors is of great importance for elucidating the putative role of erythropoietin in cancer. Thus, the aim of this study was to develop and validate an EpoR-specific NIR-probe (Epo-Cy5.5) for in vivo-analysis of EpoR expression in subcutaneous human lung cancer xenografts by fluorescence molecular tomography. Methods: Epo-Cy5.5 was generated by coupling Cy5.5 hydrazide (GE Healthcare) to rhuEpo. In vitro binding was tested using the EpoR-positive NSCLC cell lines A549 and H838, the EpoR-negative cell line H2030 and EpoR/EGFP-overexpressing HeLa cells. In vivo specificity of Epo-Cy5.5 was tested by competition analyses in nude mice bearing A549 and H838 xenografts. Micro computed tomography (µCT, CT Imaging GmbH, Erlangen, Germany) and FMT data (FMT, FMT2500LX, PerkinElmer) were fused for quantification using Amide (sourceforge.net) and allowed determination of biodistribution. Specificity of Epo-Cy5.5 was further validated on tumor cryosections. Results: Biodistribution analyses for 50 h after injection demonstrated a rapid clearance of Epo-Cy5.5 from the circulation. A high accumulation was observed in liver and kidneys with maximal levels at 7 h post-injection, followed by a decline, indicating renal excretion. Almost constant accumulation of Epo-Cy5.5 was found in bone marrow and tumors, indicating specific receptor binding. The probe allowed the discrimination between H838 with higher EpoR-expression (89.54 nM ± 15.91) and A549 tumors with lower EpoR-expression (60.45 nM ± 14.59, at 25 h, $p < 0.05$). Specificity was demonstrated by the significantly reduced accumulation of Epo-Cy5.5 in the tumor when adding unlabelled rhuEpo ($p < 0.05$ at 4, 7 and 24 h). In vitro-validation confirmed specific binding of Epo-Cy5.5 to the tumor cells, correlating with their EpoR expression level. Binding was also observed on endothelial cells. Vessel density and Epo-Cy5.5 binding to endothelial cells were comparable. Conclusion: Epo-Cy5.5 allows the longitudinal analysis of EpoR expression in tumors and thereby to investigate the influence of erythropoietin on EpoR expression, tumor growth and angiogenesis. This study may provide the basis for in vivo-analysis of the EpoR status in cancer patients using a similar nuclear imaging probe.

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poster number: 099

MONITORING GROWTH OF PROSTATE CANCER XENOGRAFTS IN MICE BY COMBINATION OF PET AND OPTICAL IMAGING

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Introduction: Gastrin-releasing peptide receptor (GRPR) is over-expressed in several types of cancer including prostate cancer. ^{68}Ga-labelled bombesin-based GRPR antagonist RM1 has shown excellent tumour targeting properties in a PC-3 xenograft mouse model (Mansi et al., Clin Cancer Res 2009;15). Purpose of this study was to evaluate if ^{68}Ga-RM1 PET would be suitable for non-invasive monitoring of tumour growth.

Methods: Androgen independent PC-3 human prostate cancer cells, expressing either luciferine gene (PC-3M-Luc, Caliper) or red fluorescent protein gene (PC-3-RFP, Anticancer) were implanted s.c in immunodeficient mice. Tumour growth was evaluated once a week for 5 weeks 1) by external caliper, 2) with bioluminescence or fluorescence based optical imaging and 3) by ^{68}Ga-RM1 PET. After the last PET imaging, mice were sacrificed and ex vivo biodistribution of ^{68}Ga-RM1 was evaluated by measuring excised tissues with a gamma counter.

Results: Optical imaging and *ex vivo* ^{68}Ga-RM1 correlated well with tumour volume (correlation coefficient 0.79 - 0.99). However, no correlation was seen in case of *in vivo* ^{68}Ga-RM1 PET and tumour volume. We suspect that partial volume and/or spill-over effects might have invalidated PET imaging measures. Of special interest is that, the uptake of ^{68}Ga-RM1 in PC-3M-Luc tumours was significantly higher than in PC-3-RFP tumours ($P = 0.021$).

Conclusion: In this study design, the optical in vivo imaging proved to be superior for monitoring tumour growth. Further studies are in process to clarify GRPR levels in PC-3M-Luc and PC-3-RFP xenografts.

poster number: 100

IMAGING ANALYSIS AND QUANTIFICATION

ABILITY OF 1H MAGNETIC RESONANCE SPECTROSCOPIC IMAGING (3D-1H-MRSI) TO DIFFERENTIATE BETWEEN OF BENIGN AND MALIGNANT BREAST LESIONS: PRELIMINARY RESULTS

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Purpose: To asses the ability of 3D-^{1}H-MRSI to differentiate between benign and malignant breast lesions.

Material and Methods: 85 patients with a breast lesion detected by either mammography or breast ultrasound were included in this prospective IRB approved study. All patients were underwent 3D-^{1}H-MRSI of the breast unsing a dedicated breats-coil and the following sequence parameters: PRESS pre-selection; TR/TE=750/145ms; Voxel size: 10 x 10 x 10 mm; acquisition time 11min. 8 Patients had to be excluded due to insufficient quality of the examination because of motion artefacts. SNR of Cho was calculated using JMRUI and self-written software. MRSI voxels were assessed for elevated choline-levels and an SNR threshold of >2.55 was set to identify malignancy. All lesions were verified by histopathology.

Results: 50 malignant and 27 benign lesions were confirmed in 77 patients. 3D-^{1}H-MRSI I had a sensitivity of 88% and a specificity of 93%. NPV was 0.81, PPV was 0.96 and diagnostic accuracy was 90%.

Conclusion: 3D-^{1}H-MRSI allows differentiation of benign and malignant breast lesions with a good sensitivity and excellent specificity at clinically feasible scan-times.

poster number: 039

ENERGY-DEPENDENT RESOLUTION IN 3D CERENKOV IMAGING

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Introduction: F-18 FDG is the most widely used radiotracer for clinical diagnostics in oncological care. Detection of F-18 FDG distribution in the body has traditionally required use of a PET scanner, costly instrumentation which often is accompanied by a CT scanner for anatomical reference. Preclinically, optical imaging has emerged in the past year to show great promise in imaging radiotracer distribution in vivo by detecting the Cerenkov radiation emitted by the charged radiotracer decay products. While the charged particles travel faster than the speed of light in a medium (such as tissue), photons in the visible band are emitted via the Cerenkov effect, and these photons can be detected by optical means. Research in preclinical optical detection of in vivo radiotracers has proliferated recently, demonstrating that information derived from 2D optical images is commensurate with 3D PET. Given that the path length or 'range' of the charged particle at relativistic speeds is dependent on the kinetic energy of the emitted particle, we will show that 3D reconstruction resolution of radiotracers shares the energy dependence on the emitted charged particles. We will also show that optical tomographic imaging in vivo can reliably reconstruct emission sources in organs of high F-18 FDG uptake.

Methodology: Monte Carlo simulations of positron and electron paths were modeled for Fluorine-18 and Yttrium-90, respectively. The mean kinetic energy of the positron from F-18 is 249 keV and the electron from Y-90 is 933 keV. 6.8 µCi of F-18 FDG and 10 µCi of Y-90 were placed in separate 1.5 mm diameter vessels that were inserted in a mouse-shaped tissue phantom in two separate experiments. The phantom was imaged in the IVIS Spectrum multispectrally for tomographic analysis. 206 µCi of F-18 FDG was injected in a naïve mouse and imaged in IVIS Spectrum with 580, 600, 620, 640, and 660 nm filters. A structured light image was acquired for surface reconstruction. Multispectral optical tomography reconstruction was performed. The mouse was subsequently imaged in a microCT instrument for structural validation.

Results: Monte Carlo simulations show that the spatial distribution of Cerenkov photons emitted due to F-18 decays is narrower than that of Y-90. Reconstructions performed on tissue phantom data show a FWHM of 1.5 mm for the F-18 FDG source, and FWHM of 4.8 mm for Y90, as expected from Monte Carlo studies. For the in vivo experiment in which a bolus injection of F-18 FDG was administered to a mouse, surface emission 2D image data shows signal in the heart region. 3D reconstructions of F-18 FDG distribution verify uptake in the heart when registered to CT.

Conclusions: Spatial resolution of the tomographic reconstructions for tracer distribution is dependent on the kinetic energy of the emitted charged particle from the isotope decay. The in vivo spatial distribution of radioactive tracers for molecular imaging in small animals can be achieved through optical tomography methods.

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ACCURACY OF MESOSCOPIC EPIFLUORESCENCE TOMOGRAPHY FOR THE ASSESSMENT OF THE SIZE OF GFP/RFP-FLUORESCENT TUMORS

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Introduction: Optical imaging is used in pre-clinical studies for tumor detection since it provides a high sensitivity for molecular markers. However, its major limitations are the low spatial resolution and the low capacity of quantification due to tissue absorption and scattering, especially in the range of visible light. These restrictions can be solved by tomographic approaches like reflectance-based mesoscopic epifluorescence tomography (MEFT). MEFT has recently been demonstrated to reconstruct fluorescence distributions in the visible light by tomographic means with a high depth sensitivity and accuracy in scattering phantoms(1) and ex vivo(2). Consequently, the aim of our study was to test the ability and accuracy of MEFT in determining the tumor size in vivo compared with micro computed tomography (µCT) and ultrasound (US).

Methods: Six nude mice with subcutaneous HCT 116-GFP-RFP xenografts were measured by epi-illumination at the MEFT tomography system, µCT and US after 14 days post-injection. MEFT-data was processed in 2D and reconstructed in 3D. MEFT and µCT-data were correlated with US-data, the latter being taken as reference method. Pearson correlation coefficients (r^2) were determined. For further validation of in vivo-fluorescence data, these were compared with ex vivo-fluorescence data obtained from cryotome sections(3).

Results: A high correlation and congruency was observed between reconstructed MEFT- and US-data (GFP: r2=0.96, $p<0.05$; RFP: r2=0.97, $p<0.05$) as well as µCT- and US-data ($r^2=0.93$, $p<0.05$). In contrast, 2D fluorescence reflectance imaging of GFP and RFP overestimated (6 times) the tumor volume as measured by US. In addition, reconstructed in vivo MEFT-data showed a high correlation with ex vivo-cryotom measurements (GFP: $r^2=0.996$, $p<0.05$; RFP: $r^2=0.999$, $p<0.05$).

Conclusions: 3D reconstructed MEFT-data showed a much higher accuracy in determining the size of superficial tumors in vivo compared to 2D reflectance imaging, which overestimated the size. Hence, besides US and µCT, MEFT is a very suitable technique for longitudinally monitoring the size and growth of superficial tumors.

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A SEMI-AUTOMATIC METHOD FOR CO-REGISTRATION OF SMALL ANIMAL OPTICAL TOMOGRAPHIC IMAGING WITH OTHER IMAGING MODALITIES

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Introduction The exploitation of the full range of Fluorescence Diffuse Optical Tomography (fDOT) capacity would benefit from co-registration of 3D molecular fluorescence images with images from other modalities for calibration. Thanks to the surface reconstruction technique that implemented into optical imaging system, we developed a semi-automatic co-registration method for fDOT with other modalities, based on automatic identification of the coordinates of the fiducial markers (FM).

Methods A white light image and an optical surface image acquired by laser pattern that covering both the subject and the FM are used for FM's coordinate detection. Regions of Detection (ROD) are assigned onto predetermined positions around the FMs in the planar white light image. We apply successively a median filter, a threshold and a Gaussian filter to minimize the noise and center the gradient intensity change of the FM. The coordinates of the local maximum intensity (MI) in each ROD are attributed as the (x, y) of the FM. Since the two images are concatenated in the same orientation and the same pixel size, the coordinates of these two images are exactly related. The intensity value in (x, y) in the optical surface image gives the z value of the FM. We obtain the coordinates (x, y, z) of the FM for optical image. Then, a rigid transformation is applied to co-register the coordinates of the FM in optical images and in a second modality such as PET.

Results Our method was applied to a fDOT-PET co-registration of mice bearing tumor xenografts of MEN2A cancer cells injected with probes tagged with the [18F]FDG and the near-IR fluorescent dye Sentidye (Fluoptics, France). The position of the fDOT image on the mouse photograph is known *a priori*, it is enough for fDOT-PET co-registration to co-register the mouse photograph with the PET image. From the co-registered images, the signals of the FM from the PET images overlap perfectly with those from the mouse photograph. To evaluate quantitatively the accuracy of our co-registration method, we calculated the Fiducial Registration Error (FRE) and compare the average FRE of our MI based method with the results of the manual co-registration (MC). The FRE value for our method is 0.26mm (std=0.06mm) in optical images and 0.25 mm (std=0.12mm) in PET image, which is in the same range of the manual registration: 0.28mm (std=0.05mm) in optical images and 0.22 mm (std=0.09mm) in PET image. Student t-test shows no statistically significant differences between MI and MC ($p=0.51$ and $p=0.55$ for optical and PET images respectively). It means that our semi-automatic co-registration method is as accurate as an experienced human observer. Results allow concluding that the Sentidye vascular probe is present both in the tumor and the surrounding vessels.

Conclusions After only one initial definition of the ROD for the given mouse holder, our method detects automatically the coordinates of the FM, allowing automatic co-registration of the fDOT image with other modalities. Our method can be generalized to the co-registration of any optical imaging system equipped with a surface reconstruction technique.

poster number: 042

A BIOPHYSICAL TRANSPORT MODEL FOR PREDICTING THE PASSIVE TARGETING OF LIPOSOMES IN SOLID TUMOURS

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Introduction: The passive targeting of macromolecules in solid tumours is typically described by the enhanced permeability and retention (EPR) effect. This phenomenon is attributed to leaky vasculature and a lack of functional lymphatic vessels in solid tumours. The EPR effect is the rationale behind the development of numerous types of large molecular weight drug delivery vehicles, including polymeric nanoparticles, micelles and liposomes. The aforementioned tumour transport properties also results in an increased rate of trans-vascular fluid convection and inefficient lymphatic drainage which causes elevated tumour interstitial fluid pressure (IFP). Macromolecular weight agents transport predominantly by convection, and therefore IFP is likely linked to the EPR effect; However, a theoretical framework which describes the link between the passive targeting of macromolecules and IFP has yet to be presented and validated. We have developed a convection driven biophysical transport model which can predict the spatio-temporal distribution of macromolecules based on the underlying fluid dynamics in solid tumours. We have validated our model using quantitative, non-invasive, in vivo imaging of the passive targeting of liposomes in solid tumours using computed tomography (CT).

Methods: A biophysical transport model which describes the transvascular and interstitial transport of fluid and macromolecules was developed based on the physical principles of fluid mechanics. A CT liposome contrast agent, which is comprised of iohexol encapsulated in a lipid shell, was administered intravenously into 5 mice bearing human cervical carcinoma tumours (ME180). The whole body distribution of liposomes was measured every 24 hours using CT imaging for up to a week. The imaging data sets were used to quantify the average concentration of liposomes in the solid tumour (a typical EPR metric) and plasma concentration as a function of time. Additionally, CT measurements where also made to determine tumour geometry, interstitial volume fraction and vascular volume fraction which are important input parameters of the model. The transport model was fit to measurements. The goodness of fit and predicted IFP were used to determine if validate the model. Predicted IFP was compared with gold standard invasive wick in needle pressure measurements.

Results: The coefficient of determination was used as a good of fit metric and was > 0.9 for all measurements. The range of predicted average IFP was 3.1 to 7.9 mmHg. The 95% confidence interval for the predicted IFP for each animal was in the range of wick in needle measurements (4-21 mmHg). Additionally the model predicted several published observations of the passive targeting of macromolecules, including: (1) tumour area under the curve (AUC) increases linearly with plasma AUC; and (2) the spatial distribution of macromolecules is predominantly along the periphery of the tumour volume.

Conclusion This study demonstrated the feasibility of using a biophysical transport model to predict the passive targeting of liposomes in tumours. The long term goal is to offer a useful tool for predicting liposome drug delivery, relating the spatio-temporal distribution of liposomes to quantitative measures of the transport microenvironment, and assist in the development, application, and assessment of patient specific cancer interventions.

poster number: 043

EFFECTS OF IOXITHALAMATE ORAL CONTRAST AGENTS ON THE QUANTIFICATION OF PET/CT ONCOLOGY STUDIES

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Introduction: Ioxithalamate (Telebrix gastro, Guerbet) is an oral contrast agent that is used for gastrointestinal CT examinations, allowing for improved delineation of the gastrointestinal tract. Contrast agents may result in incorrect attenuation correction when using a (low dose) CT and thereby affect the calculation of standardized uptake values (SUV). Therefore, oral contrast agents are diluted, so that their effect on attenuation correction and SUV is considered to be low enough [1]. However, this should be verified for each PET/CT system, image reconstruction software and contrast agent used [2]. The aim of the present study was to assess to which extent ioxithalamate influences the quantification of PET/CT studies.

Methods: Nineteen low dose CT scans of the NEMA NU-1994 image quality phantom containing one cylindrical insert were acquired on the Gemini TF PET/CT (Philips Medical Systems). The insert was filled with various concentrations of ioxithalamate (range 1-50%). In addition, this phantom was scanned with 4 kBq/ml [^{18}F]FDG in both the background of the phantom and the insert, and no (0%, used as a reference) or clinically relevant concentrations of ioxithalamate (4.4, 6.1 and 7.8%) in the insert. All studies were acquired using the same procedure (2 min/bed) and standard clinical reconstruction parameters were used. Regions of interest were drawn in the insert and in the background area of the phantom.

Results: The μ-values obtained from the μ-maps within the insert ranged from 0.100 up to 0.187 cm^{-1} for 1-50% of ioxithalamate. For clinically relevant concentrations of ioxithalamate, μ-values of the insert showed an overestimation of 17 to 27%, which caused an SUV overestimation in the insert of 12 to 23%. The SUV in the background area showed an overestimation up to 2.6%, also when positioned close (~10mm) to the insert (1.9%).

Conclusion: The Gemini TF PET/CT linearly scales the μ-values for attenuation correction with oral contrast concentration. In line with [3], effect on SUV is estimated to be about ~12-23% for clinically relevant and diluted concentrations of ioxithalamate at or near the location of the contrast agent, but does not affect quantification in areas distant from oral contrast containing areas. In patient studies the use of diluted oral contrast agents may affect SUV for lesions in or at the border of the bowel, but likely not for those located outside the bowel (>5mm)

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poster number: 044

EVALUATION OF THE UTILE FOUR-CLASS ATTENUATION-CORRECTION TECHNIQUE FOR HEAD-AND-NECK PET/MR USING PATIENT DATA

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Introduction Accurate photon attenuation correction is essential for quantitative PET/MRI. Still, accurate, fast, and reliable attenuation correction is one of the challenging areas in combining PET with MR. In head-and-neck imaging, image segmentation approaches rely on the classification of image voxels into air, adipose tissue, soft tissue, and cortical bone. Ultra-short echo time (UTE) sequences have been proposed for separation of cortical bone and air [1], while the Dixon technique has been applied to distinguish between soft and adipose tissue [2]. Recently, a new UTE triple-echo (UTILE) MR-sequence combining UTE sampling and gradient echoes for Dixon water/fat separation in a single 3D radial acquisition was proposed [3].

Methods A triple-echo UTE sequence with TE1/TE2/TE3/TR = 0.09/1.09/2.09/4.1 ms was used to measure three simultaneous images at 3 T. Air masks were derived from the phase information of the image measured at TE1, cortical bone was segmented using the magnitude images acquired at TE1 and TE3. Soft-tissue/adipose-tissue discrimination was achieved using a three-point Dixon decomposition. The segmented tissues were assigned predefined linear attenuation coefficients to generate an MR-based attenuation map. UTILE data was acquired for a patient who received a head/neck ^{18}F-FDG-PET/CT examination. We compared the attenuation maps derived from MR and CT, respectively, as well as the reconstructed PET data using these attenuation maps.

Results Using the UTILE MR-sequence, we achieved an acquisition time for the head-and-neck region of 214 s with 1.75 mm isotropic resolution. The MR-based attenuation map after Radon transformation shows a very high linearity and correlation with the one derived from the CT scan (R^2 = 0.97826, $p < 10^{-6}$). Similar high correlation was observed for the reconstructed PET data (R^2 = 0.98105, $p < 10^{-6}$). 93.4%/96.9% of all voxels have been correctly classified (compared to the reference CT) after rigid/non-rigid bone-optimized registration. The maximum deviation of mean PET activity in correctly classified tissue classes is -0.15 SUV (bone tissue).

Conclusion The UTILE MR-sequence allows for generation of MR-based attenuation maps for head-and-neck imaging, yielding high correlation of attenuation maps and PET reconstructions to the reference method. Bone segmentation exhibits room for improvement in complex regions such as the paranasal sinuses. For whole-body imaging, lung parenchyma needs to be considered as an additional tissue class in the segmentation.

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BLOOD VESSEL STENOSIS QUANTIFICATION IN CT AND MR DATASETS BY VIRTUAL ELASTIC SPHERE FITTING

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Purpose: To implement a tool for objective quantification of blood vessel stenosis in CT, dual energy CT (DECT) and MR angiography data sets and to evaluate improvements of accuracy and reproducibility compared to manual caliper measurements.

Method and Materials: Our newly developed tool requires start and end points of a blood vessel of interest, based on which the vessel lumen is segmented, through which a virtual elastic sphere is fitted subsequently. The locally adjusting diameter of this elastic sphere along the vessel course is tracked and minimal and reference diameters are determined to compute a stenosis score according to the commonly used NASCET criteria. After verifying the accuracy of the tool with vessel phantoms, it was applied to measure diameters of carotid arteries in µCT data set of ApoE -/- mice that had undergone partial ligation of the left carotid artery. All experiments were approved by the animal care institutions. Furthermore, the tool was applied to measure stenosis scores in review board-approved prospectively acquired contrast enhanced dual energy CT (DECT) and MR angiography data sets of carotid artery stenosis patients. Consistency with manual stenosis scoring was evaluated and reproducibility of semi-automated and manual scoring was compared. A novel method of automated DECT-based discrimination between iodine-enhanced blood and calcifications was implemented and compared to a method for standard CT.

Results: Diameters of vessel phantoms were correctly determined with sub-pixel precision using the semi-automated tool. For µCT angiographies of ApoE -/- mice, our tool revealed significant differences between diameters of normal and injured carotid arteries ($P<0.01$). For MR and CT patient data sets, time-efficiency (36 vs. 104 seconds per carotid artery) and reproducibility ($P<0.01$) were significantly improved compared to manual measurements. Automated and manual stenosis scores correlated strongly ($P<0.001$), showing the consistency of the method with manual measurements. DECT-based tissue discrimination did not generate significantly different scores due to the small number of patients.

Conclusion: Our virtual elastic sphere tool is widely applicable, efficient to use and improves reproducibility over manual stenosis scoring.

poster number: 046

INTERACTIVE SYSTEM FOR EXPLORATION OF MULTI-MODAL RAT BRAIN DATA

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Introduction: In pre-clinical research, the combination of structural (MRI, CT, ultrasound), functional (PET, SPECT, specialized MRI protocols) and optical (BLI, FLI) imaging modalities enables longitudinal and cross-sectional studies in living organisms. The goal of this work is to develop software for interactive exploration of heterogeneous multi-modal data in follow-up studies.

Methods: To enable comparison and integration of follow-up multimodal data, image registration is required, where we differentiate inter-modal registration and intra-modal registration to detect changes over time. To combine different modalities rigid registration is used to compensate for any rotation or translation that exists between different datasets. To detect the changes in different regions of the brain over the life-cycle (deformation, tumor growth, etc.), the different time-points are elastically/non-rigidly registered to each other. For each elastic registration, the deformation field and the correspondent determinant of the Jacobian (detJac) are calculated. When comparing 2 different time-points the information provided by the deformation field can be used: without distorting the original data one can automatically pin-point the exact same region/voxel in both datasets and understand what deformation the brain suffered from one time-point to another in all directions. Analyzing the detJac one can find whether a specific region of the brain suffered any local compression or expansion.With the registration results for any possible combination of data at hand, one can easily choose what to visualize and compare side by side: same subject-same modality-different time-points, different subjects-same modality-same time-points, same subject-different modalities-different time-points, different subjects-different modalities-same time-point, etc. In the proposed method the registration was performed using elastiX© [1] and the visualization interface was built using MeVisLab™.

Results: The proposed approach was first tested using multi-modal follow-up data of 1 male Wistar rat (Harlan-Winkelmann). It was scanned repeatedly (every 2 months from 10 to 20 months of age) under 2% isoflurane anesthesia using a horizontal bore 11.7T Bruker BioSpec MRI scanner. Diffusion tensor imaging was used to calculate fractional anisotropy, mean diffusivity and eigenvalue maps; a multislice multiecho experiment was performed to calculate T2 maps; both datasets were acquired with identical geometry and spatial resolution. T2*maps were acquired with a multi gradient echo sequence, angiography scans with a FLASH-2D TOF sequence with or without saturation of venous blood. It was used to identify and follow in time a spontaneous brain tumor growth, later identified *ex vivo* as a melingeom. The automatic linking of the same ROI/voxel in non-rigidly registered datasets and the use of the detJac to search for asymmetries in brain deformation allowed for a more accurate comparison of follow-up data.

Conclusions: In this work we describe the first step taken to build an interactive and intuitive to use exploration system for multi-modal longitudinal and cross-sectional studies. In the future, quantification tools will be added to the platform and an intensive validation will be performed with multi-modal life-span rat brain data.

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poster number: 047

PERFORMANCE COMPARISON BETWEEN ITERATIVE RECONSTRUCTION OF MICRO-CT DATA WITH TOTAL VARIATION REGULARIZATION AND FILTERED BACKPROJECTION USING PHANTOM EXPERIMENTS

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Introduction: The most widespread used algorithms to reconstruct micro-CT data are based on filtered backprojection (FBP). These algorithms are relatively fast and work well on high quality data, but cannot easily handle data with missing projections or considerable amounts of noise. In this work, we compare FBP of high-resolution micro-CT data to iterative reconstruction with and without total variation regularization using phantom experiments.

Methods: A dedicated micro-CT performance phantom was developed. The phantom contains three sections: a blank, a contrast, and a wire section. The blank section is an acrylic cylinder measuring 30mm in diameter and 40mm in length. The contrast section has the same dimensions as the blank section but contains 5 different material inserts (6mm diameter) to cover a broad range of electron densities and which can easily be interchanged. The wire section contains two 50µm tungsten wires placed in air cavities perpendicular to each other. Wires can be easily changed to cope with different resolution requirements.The three sections were acquired on a SkyScan 1178 scanner into a 512x640 matrix with a 166µm pixel size. Exposure time was set to 200ms. Two scans were acquired for each section: a complete data set containing 666 projections, and a reduced data set containing 166 projections over 360 degrees. The six acquired data sets were reconstructed using FBP with different cut-off frequencies ranging from 0.10 to 1.00, using a Simultaneous Algebraic Reconstruction Technique (SART) using 1 up to 10 iterations, and using SART with total variation regularization (TV-RSART), also using 1 up to 10 iterations. Using A Medical Image Data Analysis Tool (AMIDE), contrast-to-noise ratios (CNR) were measured using the contrast and blank sections, and the full-width half-maximum (FWHM) was estimated using the wire section.

Results: Compared to FBP, better FWHM could be obtained with TV-RSART at the equivalent CNR when using the complete data set. Using TV-RSART a FWHM of 504±47µm was estimated at a CNR of 6.2. Using SART the same FWHM (504±49µm) could be obtained at a CNR of 3.8. At a CNR of 6.2, a significantly larger FWHM of 575±34µm was measured when FBP was applied. The CNR (averaged over all cut-off frequencies) decreased significantly from 6.1±0.7 to 4.1±1.1 when a reduced data set was reconstructed with FBP, while the FWHM did not change significantly and averaged 657±218µm. Using SART, the CNR (averaged over all iterations) increased significantly from 2.8±0.5 to 3.6±0.5 when a reduced data set was reconstructed. However, the FWHM also increased significant from 492±1µm to 700±5µm when reconstructing the reduced data set. Although, using TV-RSART the CNR (6.1±1.3) did not change significantly when reconstructing the reduced data set, the spatial resolution increased significant from 511±18µm, using the complete data set, to 664±28µm when reconstructing the reduced data set.

Conclusion: Compared to FBP, better FWHM can be obtained at equivalent CNR with TV-RSTART. Using a reduced data set TV-RSART could maintain the same CNR, however, at the expense of the spatial resolution.

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METHODOLOGY FOR THE VALIDATION OF QUANTITATIVE DCE-MRI WITH 3D-HISTOLOGY

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Introduction: In literature, many different DCE-analysis methods are used to characterize tumour tissue[1]. To validate these methods histology is the gold standard and exact co-localization between histology and MR images is therefore a prerequisite. This co-localization is complicated by deformation and shrinking of the tissue during histological processing. To overcome this problem, a method is developed to validate DCE-MRI parametric maps of tumour images, based on exact co-registration of MRI with 3D-histology.

Methods: Pancreatic tumours (CA20948 tumour) were grown in Lewis rats and the animals were imaged in-vivo with MR. Next, the tumour was dissected and an intermediate ex-vivo MRI of the tumour was acquired. Then the tumour was embedded in paraffin, cut into thin slices, stained with haematoxylin and eosin, digitized and stacked in a 3D volume using a rigid registration scheme. These 3D-histology stacks were registered to DCE-MRI images in a two-step process, using third-order non-rigid B-splines with a mutual information metric[2]. First the histology was registered to the ex-vivo MRI and then the ex-vivo MRI was registered to the in vivo DCE-MR images. This way co-localization between DCE-MRI and 3D histology was achieved. Both quantitative parameters, based on the two-compartment pharmacokinetic model[3,4], and semi-quantitative parameters such as area under the curve (AUC), wash-in and wash-out rate, and maximal enhancement (Smax) were calculated. For the evaluation, regions of interest (ROI's) consisting of vital and non-vital tumour tissue were outlined in histology and transformed to the DCE-parametric maps.

Results: The vital and non-vital regions show significantly different ($p<0.05$) mean values for all parameter maps calculated with the two-compartment model as well as for semi-quantitative measures. For example, mean±sd for quantitative parameter a was 0.8-04±0.5e-04 and 6.4e-04±4.3e-04 for the vital and non-vital region respectively. The mean±sd for AUC was 42.5e+03±9.9e+03 and 8.5e+03±6.8e+03 for the vital and non-vital region. Scatter plots revealed that combinations of the DCE parameters allow for segmentation between vital and non-vital regions.

Conclusion: The developed method offers the basis for an accurate spatial correlation of DCE-MRI derived parametric maps and histology. Non-rigid registration and an intermediate ex-vivo MRI are both essential in this process. Both pharmacokinetic and semi-quantitative DCE parameters can discriminate between vital and non-vital tumour regions. The combination of two well chosen DCE-MRI parameters will enable segmentation into vital and non-vital regions.

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CHANG-BASED ATTENUATION CORRECTION IN SMALL-ANIMAL SPECT/CT

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Introduction: In pinhole SPECT, attenuation of the photon flux on trajectories between the source and the pinhole apertures affects the quantitative accuracy of reconstructed images. This effect is smaller than in clinical SPECT, but not negligible. Therefore, attenuation correction is necessary in absolute quantitative small-animal SPECT studies. We propose a Chang-based [1] non-uniform attenuation correction (NUA) method for small-animal SPECT/CT with focusing pinhole collimation, and compare the quantitative accuracy with uniform Chang attenuation correction based on body contours (UA) [2].

Methods: SPECT/CT scans were performed with a small-animal NEMA image quality phantom and with different isotopes (i.e. ^{125}I, ^{99m}Tc and ^{111}In). SPECT images were reconstructed by using the pixel-based ordered subset expectation maximization method [3] combined with an energy-window-based scatter correction. CT images were reconstructed with a modified Feldkamp algorithm and registered to the SPECT images. CT numbers were converted to attenuation coefficients by means of a linear scaling. With the attenuation coefficients, a transmitted fraction map was created with the Chang algorithm for each scan and subsequently used for correcting attenuation in each SPECT image. Besides the phantom studies, measurements in rat cadavers with artificial sources were also conducted for evaluation in a more realistic condition.

Results: For ^{125}I, ^{99m}Tc and ^{111}In, the phantom experiments demonstrated that the average relative errors of quantification were 5.5%, 4.9% and 2.8%, respectively, with NUA. In contrast, these errors were 1.9%, 7.0% and 5.1% with UA, and 41.9%, 15.5% and 16.1% without attenuation correction. In the rat studies we obtained similar results as in the phantom studies. In general, the differences of accuracy on average between the results of NUA and UA were less than 3% except for the ^{125}I studies in which they were less than 6%.

Conclusions:We conclude that both NUA and UA are suitable for attenuation correction in small-animal SPECT, and result in high quantitative accuracy. NUA performs better than UA, except for ^{125}I in which other physical factors may have more impact on the quantitative accuracy than the selection of the attenuation correction method.

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COINCIDENCE BETA-PROBE VERSUS ENSEMBLE-LEARNING ICA FOR INPUT FUNCTION MEASUREMENT IN RODENTS

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Introduction PET kinetic modeling in mice is hampered by the fact that only a tiny volume of blood can be withdrawn to define the input function (time-activity curve in plasma, IF). In an FDG-study, we compared a non-invasive image-derived input function method based on ensemble-learning independent component analysis (EL-ICA) with real-time blood activity measurements with a coincidence beta-probe on a femoral arteriovenous shunt. We assessed whether EL-ICA could replace the shunt measurement in rats and mice and whether it could overcome problems related to bolus injection.

Methods Femoral artery and vein of rodents were catheterized to form an extra-corporeal shunt volume for blood activity measurement with a coincidence counter in parallel to the PET acquisition. Under continuous isoflurane anesthesia, animals were placed in a dedicated small animal PET-CT scanner. A dynamic PET acquisition was started along with the injection of about 10 (mice) or 20 (rats) MBq F18-FDG as bolus or infusion over 3 minutes. Blood activity was measured with a coincidence counter in parallel to the PET data. PET images were reconstructed with 3D-FBP/2D-OSEM and the cardiac region in PET images was used to derive IFs with an EL-ICA technique (1) implemented in MATLAB. These IFs were scaled with blood samples taken at the end of the scan. Invasively and non-invasively obtained IFs were compared and their fit with theoretical assumptions was assessed. They were also used for kinetic modeling of regional cerebral glucose metabolism to further establish their practical value.

Results EL-ICA extracted meaningful tissue and blood components from all data sets in both mice and rats. Both IF types could be closely fitted (r>0.97) with a function of type ca(t)=A1exp(-?1t)+A2exp(-?2t)A3exp(-?3t) with residuals being Gaussian distributed statistical noise. After the necessary corrections for whole blood to plasma values, delay of the coincidence counter data (using PMOD), and scaling of the tracer activity levels, blood concentration estimated from the image data correlated closely (r>0.95) with the coincidence counter data. IFs derived from images after bolus injection were prone to high frequency ripple artifacts and overestimation (the integral over the bolus EL-ICA IF-curve was about 10% higher than the integral over direct blood measurements) because of non-instantaneous mixing of blood and tracer in the first few seconds while IFs generated from slow infusion data sets were immune to these problems.

Discussion Invasive and non-invasive IF-determination yielded similar results. Our data suggest that despite low sensitivity during the early frames it may be favorable to use an infusion protocol instead of bolus injection. Overall, the use of EL-ICA is sufficiently robust to replace invasive methods for future studies in our lab.

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poster number: 051

PROTOCOL ASSESSMENT FOR QUANTITATIVE 19F MRI OF LOW CONCENTRATED COMPOUNDS ON A PRECLINICAL 7T MRI SCANNER: PRELIMINARY EVALUATIONS AND RESULTS

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Introduction: Fluorine magnetic resonance imaging (^{19}F-MRI) is a promising investigation tool for molecular imaging. The absence of a background signal allows to discriminate the accumulation sites of fluorinated molecules of interest and offers the possibility to perform quantitative studies. However ^{19}F-MRI is inherently a low sensitivity technique: a large number of fluorine atoms per voxel is required to obtain a detectable signal. Currently, ^{19}F-MRI studies are carried out on preclinical high field magnetic resonance scanners, mainly using perfluorocarbons for cell tacking applications (Srinivas et al., 2010). Efforts to reduce the detectability threshold are however still required, in order to reduce toxicity and acquisition time, mainly to enlarge the fan of usable fluorinated molecules and applications. At our laboratory, acquisition and processing protocols for quantitative images on phantoms containing low concentrated fluorinated compounds with a Bruker Biospec 70/30 USR 7T scanner were assessed for optimization. Preliminary evaluations and results are presented.

Methods and results: Phantoms of aqueous solutions of Potassium hexafluorophosphate (KPF_6, resonance frequency = 282MHz; T_1=3000msec; T_2=700msec, estimated with RAREVTR pulse sequence) at different concentrations (50, 10, 7, 5, 3, 1.5 mM) have been considered and the Rapid Acquisition with Relaxation Enhancement (RARE) sequence have been adopted. Four aspects concerning acquisition parameters were considered: 1) Set-up of the RF pulse. The automatic adjustment of the RF pulse amplitude fails if the concentration of ^{19}F atoms is too low. We verified the possibility of setting up the pulse by using a high concentration reference (if the molecule of interest is unavailable at high concentration, a molecule with a comparable Larmor frequency can be used). However, exact reproduction of calibration condition is required during subsequent scans. 2) RARE general set-up. Driven Equilibrium (Flip Back) permitted to reduce the acquisition time; filling the k-space starting from the centre improved SNR (true spin density weighting). Partial Fourier acquisition schemes were analyzed to reduce the total scan time (Partial-FT acceleration, Zero-Fill Acceleration). Zero-Fill Acceleration increased SNR up to 200%, while causing an acceptable blurring. 3) RARE molecule-specific set-up. Various combinations of the RARE parameters (T_R and RARE-factor, i.e. number of collected echoes) were explored, at fixed total acquisition time and voxel size. Improved SNR (up to 80%) was achieved, by calibrating T_R and RARE-factor on the magnetic properties of the specific molecule. 4) Possibility to perform quantification (linearity and reproducibility of the measurement). Crucial importance of correct shimming to reduce field inhomogeneity was verified. When multiple sites of fluorinate molecule are imaged, post processing correction procedures based on uniformity maps of B_0 (or even B_1) should be considered.

Conclusions: Preliminary protocol guidelines for ^{19}F-MRI imaging of low concentrated compounds with the RARE pulse sequence have been derived. The 1.5 mM sample of KPF_6 was detectable with vxsz=1.25mmx1.25mmx4mm, matrix 32x32, acquisition time =120min, T_R=1 sec, RARE-factor=16 and Zero-Fill=2.

References: Srinivas et al., '^{19}F MRI for quantitative in vivo cell tracking', Trends in Biotechnology 28 (7), 2010.

poster number: 052

NON-INVASIVE MULTISPECTRAL MOLECULAR IMAGING IN DISTINGUISHING FLUORESCENCE PROBES WITH SPECTRAL SIGNATURES IN CLOSE-PROXIMITY

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A wealth of information on various macromolecules and signaling pathways regulating diseases resulted from the scientific efforts during the Omics era. Since then, the quest to elucidate the role of the diverse players and pathways in vivo has been in the process. With optical imaging, especially fluorescence imaging emerging as a keen front runner in such discoveries of target molecules- investigation of multiple molecular events simultaneously is becoming common. Researchers utilizing the fluorescent molecular tools earlier used in the in vitro experiments, microscopy and cell culture, face some challenges attempting in vivo molecular imaging. Especially with molecular probes that fluoresce in the visible wavelengths. The key challenges include the attenuation of light by tissues, and light absorption by the endogenous macromolecules. Also, it is likely that some of the molecular probes available might have similar or overlapping spectral properties. Under these circumstances, we propose that automated analysis of such multiple probes can be undertaken using spectral unmixing process to overcome issues from multiple signal identification and the tissue autofluorescence. Here, we utilized a small animal in vivo imaging system to identify and quantify the individual components from multiple fluorescent signals, and bio-distribution patterns of fluorescent materials ranging from 10 to 100 nm in mice to substantiate the above. Further, we demonstrate the feasibility to reliably quantitate fluorescent probes with excitation optima as close as 20 nm.

poster number: 053

CONCEPT OF MULTI-DIMENSIONAL IMAGE ANALYSIS WITH FLEXIBLE OPERATORS AND CLUSTER-COMPUTING

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Introduction New developments in biological image acquisition enable more detailed and comprehensive views on the development of organisms. Our current use case focuses on the toxicology of distinct chemical substances on the early vertebrate development. State of the art microscopy generates three dimensional image data over time of fluorescent multicolor fish. These transgenic or transiently transgenic fish are coded with at least two different fluorescent tags, which corresponds to a fifth dimension of the data. Quantitative in vivo imaging results in 5D-data sets up to TBs. These data sets challenge not only storage, but also efficient image processing. Flexible and robust generation of a workflow for image analysis An automated and flexible processing tool was developed to evaluate and analyze huge multi-dimensional biological data sets by means of cluster computing. Different algorithms are included in pipelines as operators, and can be connected with each other within the chosen analysis chain. Operators from many libraries (Matlab, ITK, VTK, Boost, OpenCV, etc.) are so turned compatible within the tool, where more than 200 operators are already implemented. Furthermore, new operators can be implemented as plug-ins. Visualization of 2-4D image data is integrated to assists parametrizing. Once a preferred image analysis chain has been identified, the sequence of operators is saved in the sequence-XML file.

Workflow execution and parallel processing The subsequent processing of the large scientific data sets is performed on the Large Scale Data Facility's Hadoop-cluster, to profit from the increased throughput and flexibility compared to external processing. Within the automatically execution of the workflow the distributor combines any data set tagged for processing and the operators (sequence-XML) to one single workflow instance by using four input parameters: the original data folder, the output folder, the path to the sequence-XML file and a job name. The distributor manages all the data, distributes the corresponding folders among processing nodes and finally executes the required jobs in the cluster. Hadoop runs the project processor task in multiple instances by the MapReduce principle. The project processor then combines the partial results, evaluating the complete chain provided in the sequence-XML. All properties and results derived from this process are stored in new result-XML files which can be used for follow-up analysis. In this case the operators used can be of any kind - in the current project mainly filters and segmentation procedures.

Conclusion The workflow processing described above combines all the advantages of a scalable mass storage system with an automated operator processor on a powerful computer cluster. The processing as well as the workflow are designed as robust, flexible and user friendly as possible. Finally this system enables the automatic and problem-orientated analysis of huge image data in a short period by cluster computing.

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poster number: 054

THE NUCLEAR RECEPTOR PPARγ IS A REGULATOR IN SEBOCYTE DIFFERENTIATION, PROLIFERATION AND INFLAMMATORY RESPONSE

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Introduction: Peroxisome proliferator-activated receptor-gamma (PPARγ) has been implicated in lipid metabolism and inflammation. PPARγ is expressed in lipid rich human sebocytes, and it appears to have roles in their functions.

Methods: In our current study we show the role of PPAR??mediated signaling in human sebaceous gland (SG) biology, using immortalyzed human sebocyte cell culture model SZ95 and laser microdissected human SG. From both laser microdissected and cultured cells we isolate RNA and run RT-qPCR, which gives opportunity to detect and compare gene expression levels. To detect changes in neutral lipid production of the sebocytes we apply both Nile red staining with quantitative fluorimetry and oil Red-O staining. For more precise and detailed quantitative lipid analysis we use HPLC. With immunhistochemistry we detect nuclear receptors in various disorders of sebaceous glands and in SZ95 cells.

Results: Demonstrating that mRNA of PPARγ and its lipid metabolism-associated target genes, ADRP and PGAR are expressed in sebocytes upon their differentiational stage in laser microdissected sebaceous cells, we show that the transcriptional factor is more active in well-differentiated SG. Moreover, although PPARγ is present in normal and hyperplastic SG, immortalized SZ95 cell line, we show that PPARγ is barely expressed in undifferentiated SG adenoma and carcinoma cells. Furthermore, selective inhibitor of PPARγ reduces sebocyte proliferation rate and suppresses citokine production and we could proove that arachidonic acid regulates special phospholipid synthesis via PPARγ.

Conclusions: These data indicate the major role of PPARγ signaling in sebocyte biology, and suggest that PPARγ is a promising target in the clinical management of proliferative/ inflammatory SG disorders.

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poster number: 055

TARGET VALIDATION FOR HUNTINGTON´S DISEASE IN THE Q175 HD MOUSE MODEL BY AAV-MEDIATED GENE TRANSFER

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Huntington's disease (HD) is primarily caused by an elongation of trinucleotide repeats in exon 1 of the huntingtin gene. Animal models carrying the mutant huntingtin allele were shown to resemble the disease and constitute invaluable tools for the evaluation of target proteins as the basis for development of new therapies for HD. To investigate potential HD targets in vivo we have employed a recombinant AAV transduction system and the Q175 HD mouse model (Levine et al., 1999). Importantly, this animal model displays a late onset of neuroanatomical and behavioral abnormalities mimicking the slow progression of the disease. Intranuclear huntingtin inclusions become apparent at the age of 4 months and can reliably be detected by immunohistochemistry in about 24.7 ± 3.8 % of the striatal neurons. To quantify the extent of the nuclear huntingtin accumulation we have established automated image analysis and revealed robust differences between Q175 and wildtype mice. Since huntingtin itself is the prime candidate for pharmacological intervention or knock down therapy it was chosen for being the first target to be studied by shRNA-mediated knock down. Two independent shRNAs were confirmed to knock down huntingtin in primary neurons and selected for in vivo target validation. The corresponding AAVs have been pseudo-typed for serotype 1/2 and up to 109 particles were injected into the right lateral ventricle of neonatal mice to allow the infection of diverse neuronal populations within the brain. Efficient transduction of striatal and cortical neurons could be demonstrated at 15-45 days post injection without obvious morphological changes or inflammatory responses. The shRNA-mediated knock down within the transduced neurons is currently investigated by immunohistochemistry and automated image analysis (OperaTM, PerkinElmer). As previously reported for other HD models, RNAi-mediated knockdown of mutant huntingtin expression is expected to be neuroprotective and to ameliorate the cellular phenotype such as aggregation of huntingtin. The improvement of this predominant hallmark of HD would demonstrate the feasibility of target validation by AAV-mediated gene transfer and the value of this late onset HD mouse model for investigating potential targets in vivo. The automated imaging of the fluorescently labeled brain sections as well as the image analysis will be discussed in detail.

poster number: 056

QUANTIFYING DCE-MRI FOR RESPONSE PREDICTION OF CANCER TREATED BY ILP

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Clinical relevance/application: Isolated limb perfusion, as a treatment option for irresectable extremity soft tissue sarcoma, results in varying tumor response. The prediction of tumor response to ILP would aid in planning and thereby in personalization of treatment.

Purpose: The heterogeneity in DCE-MRI maps provides essential information for the assessment of tumor response to treatment. The aim of this work was to evaluate whether heterogeneity in DCE-MRI parametric maps can predict the tumor response to treatment.

Method and materials: 18 Patients (male 10, female 8 median age: 56.5y) with irresectable extremity soft tissue sarcoma were treated with Isolated Limb Perfusion (ILP). DCE-MR images were acquired before ILP with a 1.5 T MRI scanner (Vision, Siemens, Erlangen, Germany) using a bolus injection of 0.1 mmol/kg gadopentetate dimeglumine. Perfusion parametric maps were calculated using a two-compartment pharmacokinetic model and a model free method. Heterogeneity measures based on the co-occurrence method were calculated for the various parametric maps: Ktrans, Slope, Area Under the Curve (AUC) and Maximum Enhancement. The response category was based on histopathological finding and categorized in responders and non-responders. The Mann-Whitney U-test was used to assess differences in parametric map heterogeneity based on the pre-treatment DCE between the response categories. In addition the accuracy, sensitivity and specificity of the heterogeneity measures were determined based on a classification experiment using a support vector machine in a leave-one out approach.

Results: There is a good correlation between pharmacokinetic and model free parametric maps (Spearman's rank correlation coefficient > 0.75, $p = 0.01$). The heterogeneity, as computed from both type of maps, show significant differences for all tested parameters ($p<0.05$) between the two response groups. The accuracy ranged from 67% for the AUC to 83% for Ktrans, the sensitivity ranged from 82% for Slope to 91% for Ktrans and the specificity ranged from 43% for AUC to 71% for Ktrans.

Conclusion: Heterogeneity features, computed from both pharmacokinetic and heuristic parametric maps, show potential as a biomarker to predict tumor response in sarcomas.

poster number: 057

QUANTITATIVE MEASUREMENT OF PERIPHERAL ADIPOSE IN SMALL ANIMALS WITH A DUAL ENERGY X-RAY METHOD

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Introduction For years, transgenic mice have been utilized as a model to study obesity. A critical component of executing pre-clinical obesity research is to measure murine body composition; including fat content, lean content, and bone density under a number of genetic and biological conditions. These key metrics allow investigators to quantitatively assess the biological and environmental factors that contribute to obesity and subsequently measure treatment efficacy. X-ray imaging provides a safe, convenient, and non-invasive means for the direct visualization of anatomical features. A common technique for determining whole-body and regional bone, fat, and fat-free mineral-free soft tissue masses in both humans and mice utilizes a dual-energy X-ray absorptiometry (DXA) scan. Here we propose a simplified X-ray strategy for direct observation and enumeration of peripheral fat in small animals. One of the underlying merits of our method is that the prone placement of a rodent under its own weight extrudes the peripheral fat in the torso, revealing the fat to unobstructed imaging.

Methods Rats of approximately the same age were exposed to a high fat diet (n = 4) and a low fat diet (n = 3) ad libitum. Two X-ray images were taken on a Carestream In-Vivo MS FX PRO imaging system: one at a lower mean energy (keV = 10.371) and one at a higher mean energy (keV = 14.426). A ratiometric image was produced by dividing the high energy X-ray image from the low energy X-ray image. The ratiometric image was then false colored, contrasted to optimized ranges, and Regions of Interest were drawn around regions representing Lean + Bone tissues, and Fat + Lean + Bone tissues to find the area of each tissue grouping. Peripheral fat was then calculated through the following equation: PeriFat Area% = (Area[Fat+Lean+Bone] - Area[Lean+Bone]) x 100 Area[Fat+Lean+Bone]

Results Peripheral fat measurements differed significantly between the fat and control groups. PeriFat Area% for the fat group had a mean of 33.9 ±1.9 (32.9, 32.1, 36.4, 34.3) and the control group presented with less than half measured PeriFat Area% with a mean of 15.82 ± 1.34 (15.1, 15.0, 17.4).

Conclusion Here we present a facile method of measuring peripheral fat in rodents. The procedure presented as an effective method in a comparison between cohorts of fat and lean animals and should have value in longitudinal measurements of body fat changes under various experimental conditions of rodents in the same cohort.

poster number: 058

SIMULTANEOUS CHEMILUMINESCENT AND FLUORESCENT DETECTION: A NOVEL APPROACH FOR DOUBLE WESTERN BLOT ANALYSIS OF LOW COPY PROTEIN TARGETS

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Western blotting remains one of the most utilized applications for the identification of proteins. While labor intensive, it is cost effective and can performed with minimal investment. The detection of multiple proteins (a so called Multiplex Western blotting strategy), on a blot usually involves processes such as stripping which will remove protein in a non-uniform manner and ultimately degrade the reliability of the data. However, the recent proliferation in the availability of fluorescent labels makes designing Multiplexing strategies for Western blotting protocols easier, with the major caveat usually coming from the availability of unique 1° antibody species. While fluorescence in general demonstrates a wider linear dynamic range than enzyme driven photonic output, the detection sensitivity is not on par with chemiluminescent labels using current detection technologies. Therefore, we developed a technque presented in the following abstract that incorporates simultaneous hybridization of first primary, and then secondary antibodies. The secondary antibodies include both chemiluminescent labels as well as fluorescent labels which provides a means for obtaining both maximum sensitivity as well as multiplexed detection in a single protocol.

poster number: 059

PROBE DESIGN

THERMOGRAPHIC ASSESSMENT OF COLD STIMULATION ON AUTONOMIC VASCULAR REACTIONS

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Introduction: The objective of this study is to determine the potential usefulness of thermal imaging technology as a tool to evaluate autonomic vascular reactions, and to establish whether non invasive thermal imagery can be used to effectively detect pre-clinical stages of diabetes and identify other circulatory problems.

Methods: To test my hypothesis a double blind cold stimulation test was preformed on 60 participants using a radiometric thermal imager. The participants included diabetics, smokers, and a normal/control group. Participants submerged their left hand in 62ºF ± 2º water for 20 minutes while keeping the right hand on a wood surface. A thermal image was recorded of the right hand every 5 minutes starting with a preliminary test image.

Results: Three groups of people were clearly defined by the thermal imager. The diabetic group had no visible thermographic finger tip change while the overall average temperature did decline slightly by an average of 2°F. The smoker group had an extremely mottled and uneven thermographic appearance of all fingers and hand with a slight decline initially in temperature while rising 2°F and holding constant until the end of the 20 minute test period. The normal/control group's thermographic assessment of the circulatory system showed that the right hand was significantly affected, with a steady decline in surface temperature of 8°F± 2°.

Conclusion: Using a radiometric thermal imager test results were immediate and visual, clearly identifying differences in vascular reactions of the three groups. Two undiagnosed participants with family history of diabetes had thermal images identified as diabetic. My objective was met and significantly impressed the participants with a visual image to be more aware of their health. Using this thermal imaging technique may prove to be the most cost effective solution for third world countries, since only a low cost, mid-performance imager is needed. The data from the test supports my hypothesis in regards to thermal imaging being a useful tool for examining circulation. This research proves that thermal imaging can be used effectively to detect pre-clinical stages of circulatory problems and would be an excellent tool to use to quickly access compromised or damaged autonomic vascular reactions.

poster number: 060

RELIABLE PRODUCTION OF 18F-RADIOPHARMACEUTICALS IN AUTOMATED PLATFORM FOR PET IMAGING APPLICATIONS

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Introduction: PET modality is one the most rapidly growing areas of medical imaging thanks to the availability of innumerous clinical centers with their own biomedical cyclotrons. To cope with this rapid development flexible and reliable synthesizer platform (IBA Synthera) was designed. Well-known conventional chemistry steps were fully automated allowing the synthesis of multiple 18F-tracers beyond FDG.

Method: The platform consists of a synthesizer and a HPLC purification module. The synthesizer employs a disposable system (IFP=integrated fluidic processor) where the entire synthesis takes place. The IFPs are named after conventional synthesis steps they are designed to perform: nucleophilic, chromatography, alkylation, distillation, and reformulation. One IFP only is needed for a single synthesis, but they can be connected in series for multi-step processes.

Results: Several 18F-labeled tracers were successfully synthesized by nucleophilic fluorination: direct fluorination (SN2 and SNAr) and synthon chemistry. The former can be performed in one step (18F-FP-DTBZ, 18F-fallypride) or in two steps; fluorination followed by removal of protective groups (18F-FDG, 18F-FMISO, 18F-FAZA, 18F-FHBG, 18F-FA, 18F-F-A85380, 18F-ML-10, florbetaben). Synthons like18F-FBM (fluorobromomethane), 18F-SFB (succinimidylfluorobenzoate) was also synthesized and they may be coupled to adequate precursor via acylation, alkylation or amide formation. For instance, 18F-FCH[1] synthesis; 18F-FBM synthon produced in the first step N-alkylates the precursor resulting in 18F-FCH. This example shows the system is be able to carry out multistep process. In most cases, the crude synthesis product required HPLC purification (FLT, FAZA, ML-10, Florbetaben, FHBG, DTPZ, Fallypride, FBE, FA85380) while for the others cartridge purification (FDG, FCH, FMISO, SFB) was sufficient. In every synthesis parameters were optimized with respect to precursor amount, reaction time, temperature and concentration. In all cases synthesis time was < 60 min. even when HPLC purification was included. Good synthesis yields and high radiochemical and chemical purity (>95 %) were obtained.

Conclusions: The yields and the final product specifications obtained were superior when compared to manual synthesis (yields at least doubled). Other advantages in comparison to manual syntheses include less exposure, stable yields and shorter synthesis time. As a conclusion, the platform has proved to be a flexible for the routine production of wide range of radiopharmaceuticals in high yields, in a reliable and consistent way, suitable for human injection.

Acknowledgments: IBA thanks all the authors for their contribution for this paper.

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poster number: 061

ISOQUINOLINE-BASED LANTHANIDE COMPLEXES AS BIMODAL PROBES FOR MAGNETIC RESONANCE AND OPTICAL IMAGING

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Introduction: Among the state of the art bioimaging modalities, some are characterized by high resolution but low sensitivity (magnetic resonance imaging, MRI) and others by high sensitivity but low macroscopic resolution (optical imaging). Luminescent/MRI bimodal imaging offers the advantage of combining the high resolution of MRI with the high sensitivity of luminescence and the development of contrast agents active in both techniques is of prime importance. Despite it was long thought that the conditions required for both applications were non-compatible, we have previously reported a versatile pyridine-based scaffold for lanthanide complexation where MRI and near infrared (NIR) luminescence requirements are both satisfied using the same ligand.1 Aiming to improve the luminescent properties of this probe, isoquinoline-based complexes were designed to shift the excitation wavelength towards biologically compatible values.

Methods: The synthesis of the ligands will be described. Potentiometric titrations have been performed to assess the thermodynamic stability of the complexes and their selectivity for lanthanide cations. To characterize the MRI properties, the relaxation properties of the Gd3+ complexes were determined by temperature-dependent 17O NMR measurements and NMRD profiles. The NIR spectra of the corresponding Nd3+ and Yb3+ were recorded, as well the quantum yield of the complexes to quantify their luminescent properties. In vitro and in cellulo detection of the NIR lanthanide emission was realized with a macroscope.

Results: The bishydrated complexes are found to be thermodynamically stable and the ligands show a significant selectivity for Ln3+ over endogenous cations such as Zn2+, Ca2+ and Cu2+. High relaxivities for the Gd3+ complexes are observed thanks to the two water molecules and the good relaxation parameters. All the complexes give rise to NIR emission and the quantum yields are remarkable. They are in the same range as those of non-hydrated complexes optimized for fully protecting the NIR emitting Ln3+ for aqueous applications. The substitution of the pyridine with isoquinoline and the introduction of electron-donating moieties led to a red shift of the excitation wavelengths. The high efficiency of the isoquinoline antenna allows observing the NIR emission at the macroscope either in a capillary or in incubated cells.

Conclusions: The isoquinoline scaffold is a prime candidate for the development of bimodal NIR/MRI imaging probes, as the bishydrated Ln3+ complexes are thermodynamically stable and display high NIR quantum yields. The modification of the pyridine to an isoquinoline did not modify the thermodynamic properties of the complexes, but resulted in a shift of the excitation energy towards lower values preventing damages to biological samples and allowing deeper tissue penetration of the excitation photons. Moreover, these probes proved their efficiency in biological media as NIR emission was observed in cellulo.

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poster number: 062

IMAGING OF TYROSINE KINASE INHIBITORS WITH PET: RADIOLABELING AND PRECLINAL VALIDATION OF [11C] SORAFENIB

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Introduction: Receptor tyrosine kinases play a pivotal role in the signal transduction of vital processes of the cell, e.g. cell growth, survival and proliferation. Uncontrolled activation of these receptors is often related to tumor formation and therefore, tyrosine kinases have become important drug targets in the treatment of cancer.[1,2] Although several tyrosine kinase inhibitors have demonstrated clinical value, most TKIs developed are only effective in a small subset of patients (typically 10 - 30 %). In our center, PET imaging with radiolabeled targeted drugs (monoclonal antibodies and TKIs) is used as quantitative imaging strategy to speed up drug development and to facilitate patient selection. The aim of the present study was to develop radiolabeled [11C]sorafenib, to be used for the selection of patients who might benefit from treatment. Sorafenib was labeled at two positions to obtain [11C]methylamide sorafenib or [11C]urea sorafenib. Both labeled compounds are currently preclinically evaluated for there tumor targeting properties as well as their active metabolites.

Methods: [11C]methylamide sorafenib was synthesized by reacting desmethyl sorafenib with [11C]MeI in a solution of TBAOH in DMF at elevated temperature. For the synthesis of [11C]urea sorafenib, a Rhodium promoted reaction between the corresponding azide and amine was performed at elevated pressure and temperature in the presence of [11C]CO. The crude mixtures were purified by HPLC (C18 Alltima; Eluent: MeCN/H2O/TFA, 55:45:0.1, v/v/v) and the isolated product fraction was formulated by solid phase extraction in ethanol and 2.5% polysorbatum in 0.9% saline solution (1:9 v/v).

Results: [11C]methylamide sorafenib was synthesized in a decay corrected yield of 59% and [11C]urea sorafenib was synthesized in a decay corrected yield of 53%. Using analytical HPLC (C18 Alltima; Eluent: MeCN/H2O/TFA, 60:40:0.1, v/v/v), for both [11C]methylamide sorafenib and [11C]urea sorafenib, the radiochemical purity was determined to be higher than 99% and the identity of the product was confirmed by coinjection of the labeled product with reference sorafenib. A metabolite analysis in rats, revealed that sorafenib is slowly metabolised. The percentage of intact product in blood-plasma samples after 45 minutes are up to 90% for [11C]methylamide sorafenib, and 96% for [11C]urea sorafenib, respectively. Currently, both are tested for in vivo tumor targeting.

Conclusions: Reliable labeling procedures were developed for the synthesis of [11C]methylamide sorafenib and [11C] urea sorafenib. Both were obtained in a high yield and purity. Furthermore, both products have a high metabolic stability in rats. Finally, [11C]methylamide sorafenib and [11C] urea sorafenib will be biologically evaluated for their tumor targeting properties.

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poster number: 063

DESIGN AND SYNTHESIS OF A POLYFLUORINATED PROBE FOR TARGETED 19F-MAGNETIC RESONANCE IMAGING

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Introduction: The proton (^{1}H) and the ^{19}F-nucleus are the most sensitive particles for magnetic resonance (MR) technologies. While the protons of water are typically used in clinical examinations (with or without contrast enhancing agents), imaging of ^{19}F-nuclei has only recently become more popular and is not clinically established. The use of ^{19}F as a reporter nucleus has the great advantage of virtually no natural background signal in the body, easily facilitating identification of desired target structures. However, large amounts of the tracer are required for detection. While a number of strategies have been proposed to increase detection sensitivity, we tackle this problem by design of a probe containing a large number of equivalent fluorine nuclei contributing to the detectable signal.

Methods:Starting from tris(hydroxymethyl)aminomethane (TRIS), acrylic nitrile, a fluorinated aromatic and an amino acid a 36 magnetically and chemically equal fluorine atoms containing probe was synthesized. The MR properties of one compound were examined with a 9.4 T MRI system. Relaxation times of the compound and the detection limit were determined from phantom studies. To evaluate its in vivo properties, different amounts of the compound were suspended in biogel and injected subcutaneously into CD1 nude mice and ^{1}H and ^{19}F MR images were acquired.

Results:The synthesis of the probe was accomplished in 8 successive synthetic steps. TRIS was combined with three equivalents of acrylic nitrile, Boc-protected and hydrogenated. The fluorinated aromatic was then coupled to the resulting amine. After deprotection and amino acid addition, the probe was finally prepared as an active ester. T1 and T2 of one intermediate were determined from DMSO solutions and were 810 ms and 262 ms, respectively. Using a 10 mm diameter lab-built 19F solenoid coil, down to 25-30 nmol of the probe per voxel could be imaged (FID-FISP gradient echo sequence, acq time 15 min). In in-vivo ^{19}F MR imaging experiments the probe in the biogel pellet could clearly be visualized.

Conclusions: We successfully designed and synthesized ^{19}F-based probe that can easily be coupled to antibodies or proteins, yielding targeted tracers for combined ^{1}H/19F imaging. Together with high field MR scanners and newly developed ^{19}F coils, this approach offers the possibility of simultaneous ^{1}H/^{19}F MR imaging, yielding both, anatomical and molecular information on a preclinical platform. Optimization of the synthesis and the MR sequence will add towards establishing practicable targeted molecular imaging using ^{19}F-based tracers.

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SILICA NANOPARTICLES WITH MULTIPLE FUNCTIONALITIES FOR OPTICAL AND MAGNETIC RESONANCE IMAGING APPLICATIONS

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Introduction: Magnetic resonance imaging (MRI) with its high temporal and spatial resolution is currently one of the most powerful noninvasive medical diagnostic tools. Contrast agents (CAs) are applied in many examinations to increase the specificity and sensitivity of MR imaging. Importantly, the development of such agents is gaining increased attention lately because these agents require optimization to generate maximum contrast. One sound strategy to increase contrast is to anchor many Gadolinium (Gd^{3+}) complexes on nano sized systems like silica nanoparticles (SiO_2-NP) [1]. SiO_2-NP has a large surface area which can be differentially functionalized and modified so as to make them multifunctional devices.

Methods: Spherical, nonporous and monodisperse SiO_2-NP (average diameter of 100 nm), functionalized with carboxylic acid group at the terminus[2], were activated and reacted with Lys (Dde)-OH to make the exterior surface bifunctional namely with protected amine and carboxylic acid groups. The latter were reacted with Gd-DO3A-hexylamine serving as CA for MRI while the former were deprotected and a fluorophore (FITC or Cy 5.5) alone or octaarginine containing FITC for optical imaging and intracellular delivery were coupled. Two types of SiO_2-NP differing in the length of the alkyl chain attached on the surface were tested. Cellular uptake was estimated by fluorescence microscopy in 3T3 mouse fibroblasts. MRI experiments were performed with SiO_2-NP suspended in 1.5% agar in a 3T MRI scanner.

Results: A high surface concentration of Gd^{3+}-complexes was confirmed by ICP-AES and T_1-measurments. The relaxivity per gadolinium was slightly higher for Gd^{3+}-complexes on the SiO_2-NP compared to unbound molecules in solution. Contrast enhancement ability in vitro was also shown by imaging the SiO_2-NP in a 3T MRI scanner. Fluorescence microscopy demonstrated that all types of particles entered the cells in a concentration dependent manner although also larger agglomerates were formed which could not be taken up by the cells. There was no significant difference in cellular uptake between octaarginine coupled and non coupled particles. In an initial study Cy 5.5 functionalized NP were injected *i.v.* into mice, animals were sacrificed 10 min or 24 h later, and optical imaging of ex vivo derived organs was performed. A strong fluorescence signal was observed in the lung already after 10 min. In addition, fluorescence microscopy analysis of histological sections showed an accumulation in the kidneys and, to a much higher extent, in the liver. Only in the liver Si-NPs could be detected after 24 h.

Conclusions: A simple and straightforward method of modification as well as coupling of biomolecules on a bifunctional surface of spherical and nonporous silica support was developed. The applicability of such SiO_2-NP as bimodal probe for optical and MR imaging could be proven in initial biological studies.

Acknowledgements: Funding was provided by the Max Planck Society and the German Ministry for Education and Research (BMBF, FKZ 01EZ0813). The work was performed in the frame of COST action D38.

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GDDO3A-PIB DERIVATIVES, POTENTIAL MRI MARKERS FOR ALZHEIMER'S DISEASE

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Introduction**:** Alzheimer's disease (AD) is the most frequent form of intellectual deterioration in elderly individuals, characterized by the brain deposition of amyloid plaques and neurofibrillary tangles. Early detection of the beta-amyloid (Abeta) deposits in vivo is very difficult. Recently ^{11}C-radiolabeled small-molecules have been developed, capable of entering the brain and specifically targeting amyloid plaques for imaging with PET, such as several Thioflavin T derivatives [1-2]. In particular, the uncharged analogue 6-OH-BTA-1 (Pittsburgh compound B - PIB) is highly efficient both in crossing the BBB and in selective binding to AD amyloid aggregates. A major limitation of PET is the requirement for the markers to be labelled with short-lived isotopes. The use of Abeta marker linked to a MRI CA would constitute an attractive noninvasive in vivo imaging approach. Recently, Poduslo used CA aided MRI to image AD plaques with Gd(III)DTPA conjugated to a putrescine-modified human Abeta peptide able to cross the BBB and selectively target individual amyloid plaques in the brain of Alzheimer's disease transgenic mice. Nevertheless, due to its large size, several days (weeks) are necessary to obtain the labeling of amyloid plaques in transgenic mouse brain *in vivo*. In an attempt to label Aß plaques using small metal complexes for the diagnostics of Alzheimer disease, we have synthesized a series of new PIB-derivatives of DO3A.

Methods**:** DO3A-PIB derivatives were synthesized using a new, versatile strategy. The Gd^{3+} complexes have been characterized by relaxometric methods. Most of the compounds form micelles in aqueous solution and the critical micellar concentration has been measured. Immunostaining studies on human brain slices have been carried out to assess binding of the compound to the beta-amyloid deposits. Blood Brain Barrier studies to evaluate the permeation of the compounds were performed on a cellular BBB *in vitro* model [3].

Results**:** The ^1H NMRD profiles evidence aggregation of the GdDO3A-PIB complexes in aqueous solution, with cmc values varying form 1.0-1.5 mM. The Immunostaining experiments on human brain slices show good binding affinity of the LnDO3A-PIB derivatives towards the amyloid plaques. Permeation was detected in the BBB *in vitro* studies.

Conclusions**:** We synthesized and investigated a series of novel Gd^{3+} complexes with good binding properties to beta-amyloid deposits in human brain slices and we detected transfer in our *in vitro* BBB model studies.

Acknowledgement**:** We thank the support from the F.C.T. Portugal (financed grant SFRH / BD / 46370 / 2008) and COST D38.

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OPTIMIZING THE CELL LABELING STRATEGY WITH CATIONIC GD-LIPOSOMES: LONGITUDINAL *IN VIVO* FOLLOW UP OF GD-LABELED MSCS

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Purpose: To develop an optimized labeling protocol for mesenchymal stem cells with Gd-liposomes that allows for sensitive and longitudinal tracking of cell fate in vivo.

Materials and methods: Gd-DTPA was incorporated in the water phase of cationic liposomes containing cholesterol, DOTAP and DPPC at molar ratios 2.35:1.65:1.0. Rat mesenchymal stem cells (MSCs; Millipore, Billerica, MA, USA) were labeled with 125, 250, 500 or 1000 µM lipid, corresponding to 52±3, 104±6, 209±13 and 420±25 µM Gd respectively. Control cells were incubated with HEPES-containing liposomes. Labeling was performed for 1h, 4h or 24h. The cellular Gd load, cellular toxicity, cell proliferation rate and cell differentiation were determined as a function of labeling time and lipid dosage. Intracellular retention of Gd was assessed over time (20d). The intracellular fate of Gd-liposomes was studied with confocal microscopy, using fluorescent dyes in the water-core and bilayer. MRI visualization of Gd-labeled cells was performed on a 1.5T as well as a 3.0T clinical scanner. For in vivo experiments, cells were transplanted in skeletal muscle and imaged for at least 3 weeks. At several time-points animals were sacrificed and histology was performed to correlate MRI contrast to the presence of Gd-liposome labeled cells.

Results: Labeling for 4h with 125 µM lipid is the most preferred labeling strategy, combining time-efficiency with a sufficient cellular Gd uptake (30±2.5 pg Gd cell-1), without significant effects on cell viability, proliferation and cell differentiation. Gd-liposomes were retained well intracellularly, in an endosomal distribution pattern. MSCs labeled with Gd-liposomes were imaged with MRI at both 1.5T and 3.0T, resulting in excellent visualization both in vitro and in vivo. At least 10.000 Gd-MSCs could be detected. Histology showed the liposomes to be still present at the site of injection, inside the labeled cells. Prolonged in vivo imaging of 500,000 Gd-labeled cells was possible for at least two weeks (3.0T).

Conclusion: In conclusion, Gd-loaded cationic liposomes are an excellent candidate to label MSCs, without detrimental effects on cell viability, proliferation and differentiation allowing sensitive and longitudinal visualization by MRI.

Clinical relevance/application: Cell labeling with Gd-liposomes might prove valuable for cell tracking in vivo in a clinical setting, for example as a therapy evaluation of stem cell therapy.

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NOVEL [18F]FLUOROALKOXYINDOLEACETAMIDES FOR IMAGING THE TSPO 18KDA WITH PET

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Objectives: The large overexpression of the translocator protein 18 kDa (TSPO, a mitochondrial protein previously known as the peripheral benzodiazepine receptor (PBR)) in the brain during microglial cell activation in response to brain insults has attracted considerable interest for the development of PET-radioligands as imaging biomarkers of neuroinflammation. Several radioligands are currently being developed to replace the original [11C]PK11195, which is still the compound of reference [1]. This includes the indoleacetamide [11C]SSR180575 [2], the lead compound of a highly attractive new chemical class of structures. In the present work, two fluoroalkoxy derivatives of SSR180575 (FE-O-SSR180575 and FP-O-SSR180575) were synthesized, labelled with fluorine-18 and pharmacologically evaluated in a rat model of neuroinflammation by PET imaging.

Methods: The fluoroethoxy and fluoropropoxy derivatives, as reference compounds, as well as the corresponding tosyloxy derivatives, as precursors for labelling with fluorine-18, were all synthesized in two steps from the corresponding common para-methoxy-substituted key-intermediate. Fluorine-18 labelling of both compounds was performed using a TRACERLab FX-FN synthesizer (GEMS) and comprised (1) dissolving of the no-carrier-added, dried (activated) K[18F]F-Kryptofix®222 complex in dimethyl sulfoxide (700 µL) containing 1.5 to 2.0 mg of the adequate tosylate; (2) heating the reaction mixture at 160°C for 10 min; (3) dilution of the residue with twice 2 mL of the HPLC mobile phase; (4) pre-purification on a SepPak® Alumina N™ cartridge; (5) HPLC purification on a semi-preparative Symmetry-M® C-18 column and (6) SepPak® cartridge-based removal of the HPLC solvents and formulation for i.v. injection. Acute neuroinflammation was induced in Wistar rats (300 g, male) by stereotaxic injection of 7.5 nmol of AMPA in the right striatum for both ex-vivo autoradiography and in vivo PET-imaging (Focus P220 Concorde, control kinetics, displacement experiments with PK11195 and non-labelled SSR180575 derivatives (1 mg/kg)).

Results: The fluoro- and tosyloxy derivatives of SSR180575 were obtained in 59%, 54%, 42% and 61% overall yields respectively. [18F]FE-O-SSR180575 and [18F]FP-O-SSR180575 were obtained in 15-18% decay-corrected yields, based on starting [18F] fluoride. Typically, starting from a 30 GBq [18F]fluoride batch, 3.0 to 3.7 GBq of both fluorine-18-labelled derivatives, > 99% radiochemically pure and ready-to-inject, were obtained in 60 min and with specific radioactivities ranging from 50 to 90 GBq/µmol. In PET experiments, both derivatives showed a higher contrast between the lesioned area and the corresponding area in the intact contralateral hemisphere when compared to [11C]PK11195 (ratio ipsi/contra at 20 min and 60 min post-injection (n=4): [18F] FE-O-SSR180575: 1.8 and 2.4; [18F]FP-O-SSR180575: 2.1 and 2.6; [11C]PK11195: 1.7 and 1.6). Furthermore, both [18F]FE-O- and [18F]FP-O-SSR180575 were displaced by PK11195 or non-labelled 1a and 1b, respectively. Immunohistochemical analyses showed that the signals detected by PET-imaging were correlated with significant activation of microglia in and around the lesion. Conclusions: Two novel fluoroalkoxyindoleacetamides were successfully synthesized and labelled with fluorine-18 and dynamic µPET studies in rats demonstrated their potential to image neuroinflammation.

Acknowledgment: Supported by a Joint INCa / DAAD Translational Research Programme 08-006. References: [1] Chauveau et al. (2008), Eur. J. Nucl. Med. Mol. Imag., 35, 2304-2319 ; [2] Thominiaux et al. (2010), J. Label. Compds Radiopharm., 53, 767-773.

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IRON OXIDE NANOPARTICLE-CONTAINING MICROBUBBLE COMPOSITES AS MR-US DUAL-MODALILTY CONTRAST AGENTS

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Introduction: Magnetic resonance (MR) and ultrasound (US) imaging are widely used diagnostic modalities for various experimental and clinical applications. In this study, iron oxide nanoparticle-embedded polymeric microbubbles were designed as multimodal contrast agents for hybrid MR-US imaging.

Methods: These magnetic nano-in-micro imaging probes were prepared via a one-pot emulsion polymerization to form poly(butyl cyanoacrylate) microbubbles, along with the oil-in-water (O/W) encapsulation of iron oxide nanoparticles in the bubble shell. They showed a narrow size distribution of 2.2µm (microbubbles) and 10nm (embedded iron oxide nanoparticles) in diameter respectively, and the unique nano-in-micro embedding strategy was validated using NMR and electron microscopy.

Results: These hybrid imaging agents exhibited strong contrast in US and an increased transversal relaxation rate in MR. Moreover, a significant increase in longitudinal and transversal relaxivities was observed after US-induced bubble destruction, which demonstrated triggerable MR imaging properties. Proof-of-principle in vivo experiments confirmed that these nanoparticle-containing microbubble composites are suitable contrast agents for both MR and US imaging.

Conclusion: In summary, these magnetic nano-in-micro hybrid materials are highly interesting systems for bimodal MR-US imaging, and their enhanced relaxivities upon US-induced destruction recommend them as potential vehicles for MR-guided US-mediated drug and gene delivery.

poster number: 069

BODIPY DOTA DERIVATIVES: NEW MULTIMODAL RADIO-(PET/SPECT) AND FLUORESCENCE IMAGING AGENTS

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Introduction: Multimodal imaging agents can provide complementary information improving the accuracy of disease diagnosis and enhancing patient management.[1] Each imaging modality (SPECT, PET, Optical Imaging, MRI) has its own strengths and weaknesses, and thus, combining different and complementary systems can overcome inherent limitations associated with any one individual technique. In particular dual-modality Optical/Nuclear imaging may find important preclinical and clinical applications. One possible approach seeks to fuse the two imaging systems into one molecule (MonOmolecular Multimodality Imaging Agent [MOMIA])[2] in order to ensure the same biodistribution of the two probes.

Methods: In this work, we report the synthesis of the first bodipy-DOTA derivatives. The combination of these two versatile components within a single molecule should lead to promising bimodal imaging agents. Indeed, on the one hand the DOTA-like part may allow complexation of radiometals for nuclear imaging (SPECT or PET). [3] On the other hand the bodipy moiety is a valuable probe those fluorescent properties can be finely adjusted. [4]

Results: Interestingly, the attachment of the polyaminocarboxylate to the bodipy makes it soluble in water and complexation of different metal cations of interest in the macrocyclic cavity does not significantly alter the luminescence properties of the whole system. In addition, we showed that the use of an appropriate linker between bodipy and DOTA-like units, i.e. a 4-nitrophenylalanine derivative, could provide a bimodal tag for labeling antibodies or peptides. [5]

Conclusion: The complexe of bodipy-DOTA (In, Ga, Cu) are water soluble, thus solving one of the problem preventing the use of bodipy-agents for in vivo optical imaging. Modification of the second generation bodipy-DOTA containing the isothiocyanate function are undertaken in our laboratory, i.e. derivatization reaction to reach the near-IR range. Introduction of tacn derivative or cross bridged cyclam adapted for Ga and Cu respectively is also investigated.

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99MTC(I) TRICARBONYL COMPLEXES BEARING DIALKYLAMINOALKYL PHARMACOPHORES: SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION

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Introduction Searching for radioactive probes suitable to detect in vivo melanoma and its metastases, we have synthesized and evaluated fac-[M(k^3-L)$(CO)_3$] (M= ^{99m}Tc , Re) complexes (Tc1-Tc4/Re1-Re4) stabilized by a new (S,N,O)-donor bifunctional chelator. Tc1/Re1 correspond to model complexes without pharmacophore, while Tc2-Tc4/Re2-Re4 are complexes bearing, respectively, N-diethylethylamine, N,N-diethylpropylamine and ethylpyrrolidine as melanin-avid groups. Herein, we report on the synthesis, characterization and in vitro/in vivo biological evaluation of this new family of organometallic complexes.

Methods The new tricarbonyl complexes (Tc1-Tc4/Re1-Re4) were synthesized by reaction of fac-$[M(H_2O)_3(CO)_3]^+$ (M=Re, ^{99m}Tc) with the respective ligands (L1-L4). The rhenium compounds were fully characterized by the common techniques and used as surrogates in the identification of the ^{99m}Tc complexes. The in vitro evaluation of the ^{99m}Tc complexes comprised lipophilicity measurements, binding to synthetic melanin, and transchelation studies against cysteine and histidine in the case of Tc1. Cellular uptake studies were performed for Tc2-Tc4 in a melanotic murine B16F1 cell line versus the amelanotic A375 human cell line. Haloperidol blockade studies were performed to ascertain the influence of sigma receptors, another possible intracellular target of these complexes. Biodistribution studies in healthy (Tc1) and melanoma-bearing C57BL/6 female mice (Tc2-Tc3) were also done for the ^{99m}Tc complexes.

Results The high stability found for the model complex Tc1, both in vitro and in in vivo, prompted the synthesis of the congeners (Tc2-Tc4) functionalized with the melanin-binding pharmacophores. With the exception of Tc3, all the complexes have a moderate lipophilic character. The melanin binding affinity of Tc2-Tc4 is low to moderate, as well as their cell uptake. Complex Tc2 has shown the highest uptake in the melanotic murine B16F1 cell line but presented a negligible uptake in the amelanotic A375 human cell line. Consistently, Tc2 has also shown the highest tumor uptake in a B16F1 melanoma-bearing mouse with a fast clearance from non-target organs. Upon haloperidol blockade, significative reduction of the cell uptake of Tc2 was only observed for concentrations as high as 10^{-5} M.

Conclusions New Re/^{99m}Tc (I) tricarbonyl complexes bearing melanin-avid groups were synthesized and biologically evaluated. From these complexes, Tc2 presented the most promising biological profile for in vivo targeting of melanoma, with rather favorable target/non-target ratios. Apparently, no significant contribution from sigma receptors seems to be involved in the cell uptake of Tc2. Eventually, the results found for Tc2 may reflect its better ability to cross the cellular membrane, being retained inside melanotic tumor cells upon interaction with intracellular melanin despite its relatively low binding affinity towards this pigment.

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TARGETED MICROCAPSULES AS CONTRAST AGENTS FOR IMAGING OF COLORECTAL CANCER BY FLUORINE (19F) MRI

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Introduction: Early diagnosis of colorectal cancer (CRC) is a realistic approach to reduce its high mortality rates. Currently available methods for early detection of CRC do not distinguish the 5% of adenomas expected to progress into CRC (high-risk adenomas) from adenomas with low risk of progression. We aimed to develop targeted contrast agents for molecular imaging of colorectal tumors by fluorine (19F) MRI.

Methods: The contrast agents were prepared by a modified solvent emulsification-evaporation process in order to obtain polymeric microcapsules encapsulating the perfluorooctyl bromide (PFOB)1. Then the microcapsules were modified to attach covalently or non-covalently a monoclonal therapeutic antibody to epidermal growth factor receptor (anti-EGFR, Cetuximab). Microcapsules were first characterized physico-chemically and the encapsulated amount of PFOB was quantified by 19F NMR. In vivo 19F spectroscopic imaging was performed to verify the signal intensity of the non-targeted microcapsules. For this purpose, a newly developed sequence named 19F ultra-fast Turbo Spectroscopic Imaging (F-uTSI) was used 2,3. Then the influence of the antibody on microcapsule size and morphology was evaluated by laser diffraction and confocal microscopy and scanning electron microscopy. The amount of antibody associated to microcapsules was quantified by radiolabeling the antibody with Iodine 125 prior to coupling. The surface modification of the microcapsules was verified by X-ray photoelectron spectroscopy (XPS). In vitro targeting efficacy was tested with EGFR positive cancer cells, A-431 cells.

Results: Two types of microcapsules were obtained: non-targeted and targeted with a mean diameter around 2 µm with core-shell structure. 19F NMR allowed the quantification of PFOB at each step of the preparation process of the microcapsules. Fluorine images of a mouse colon were obtained with non-targeted microcapsules using the 3D-F-uTSI sequence after a rectal administration (50 µl 0.3% v/v). The 19F maps were overlaid onto proton anatomical images of the abdomen. The surface modification of the microcapsules with the antibody did not change the physico-chemical characteristics of the microcapsules. XPS and radioactivity results confirmed the presence of the antibody and the in vitro results showed the specific targeting properties of the microcapsules.

Conclusion: The targeted polymeric microcapsules developed are promising 19F-MRI contrast agents for the CRC imaging.

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LIFETIME IMAGING AND LIFETIME QUALITY ANALYSIS IN COMBINATION WITH PH-SENSITIVE NIR FLUORESCENCE PROBES TO IMPROVE TUMOR DETECTION *IN VIVO*

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Introduction Optical imaging via fluorescence provides a relatively simple and inexpensive method to noninvasively detect tumors in living mice. Despite imaging in the near-infrared (NIR) range, high background fluorescence is still a major problem hampering detection of specific signals. Time-domain measurements provide an advantage compared to simple intensity measurements, as they enable the identification of fluorescence lifetimes (LT) of the detected signals. Nevertheless, difficulties in the identification of specific signals arise in vivo as the probe's LT can be influenced by the environment. Herein we present an improved approach for separation of probe-derived fluorescence from autofluorescence based on evaluation of the quality of LT calculation. This is done in combination with pH-sensitive fluorescent probes which are activated only at the targeted tumor site thus circumventing background signals from unbound probe.

Methods We used a pH-sensitive NIR fluorescent dye, CypHer5E, coupled to the Trastuzumab antibody (pH-T), which is directed to the Her2/neu receptor, highly expressed on breast tumor cells. The pH-T was studied at a low (~1.5) and a high (~4.5) dye-to-protein ratio (DPR). An always-on fluorescent dye, Alexa Fluor 647, coupled to Trastuzumab at comparable DPRs was used as a control. Fluorescence intensities of all probes were analyzed spectroscopically in PBS as a function of pH. The LTs of the probes were determined in different matrices in vitro and in vivo. The functionality of the pH-Ts was confirmed by NIR fluorescence microscopy using Her2/neu-positive and -negative human breast tumor cells. The in vivo time-domain fluorescence imager, Optix MX2, was applied to image nude mice bearing orthotopic breast tumors after intravenous application of the fluorescent probes.

Results Spectroscopic analyses showed a remarkable increase in fluorescence intensity of the pH-Ts with decreasing pH values in contrast to the control probes. Slight changes of LT values of all probes were observed in vitro and in vivo. In vitro, when incubated with Her2/neu-positive cells, the pH-T probes showed fluorescence only after receptor-mediated internalization into the acidic lysosomes whereas no signals from the membrane-bound probes were observed. In contrast, the fluorescence intensity of the always-on probes was independent on internalization. In vivo, after application of the pH-Ts, fluorescence was detected only at the tumor site presumably as a result of the receptor-mediated internalization, and the acidic pH within tumors. Here, no increase in background fluorescence was observed. In contrast, the always-on probes showed strong fluorescence at the tumor site accompanied by high background fluorescence deriving from the circulating unbound probe. Therefore, only for the pH-T probe we could efficiently apply the quality parameter of the LT calculation in order to decouple fluorescence in the tumor from autofluorescence. This strategy for background elimination could not be accomplished using the always-on probes which generate strong probe-derived signals with lifetimes of similar quality in tumor and background.

Conclusions Here we present a novel method for separation of probe-derived fluorescence from autofluorescence by the combined use of quality-LT imaging and pH-sensitive tumor-specific probes. By this approach we could selectively visualize the targeted tumor site in living mice.

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SYNTHESIS, CHARACTERIZATION AND RELAXOMETRIC EVALUATION OF LN3+ CHELATES OF NEW DO3A-N-?-AMINO PROPIONATE AMIDE LIGAND DERIVATIVES AS MRI CONTRAST AGENTS

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Introduction: We have reported recently the design, synthesis and characterization of a novel metal chelator, DO3A-N-?-aminopropionate, bearing a propionate arm functionalized with an amine group for coupling to (bio)molecules, whose Gd3+ chelate displays exceptional stability and a fast water exchange rate, in the ideal range for attaining high relaxivity at intermediate magnetic fields. To take full advantage of the promising properties of this new chelator as MRI Contrast Agent, we report a robust synthetic methodology for preparing amide conjugates and characterize their Ln3+ complexes as MRI and optical imaging probes.

Methods: We evaluated the effect of amide formation on the water exchange rate of the complexes and the effect of simultaneous optimization of the water exchange rate and the rotational dynamics on the relaxivity using relaxometric 1H NMRD and 17O NMR. The micelle formation of an amphiphylic chelate was studied by DLS, relaxivity and fluorescence measurements. Energy transfer beween Ln3+ cations and the lgand antenna was studied by luminescence. The in vivo animal biodistribution in Wistar rats of 153Sm3+-labeled compounds was studied by gamma scintigraphy.

Results: The ligands discussed here are the DO3A-N-(a-benzylamido)propionate and DO3A-N-(a-pyrenebutanamido) propionate conjugates, and are compared with the parent chelator. The Gd3+ complexes are pH and Zn2+ transmetallation stable, as studied by relaxometry, and the complexes of the a-pyrenebutanamido derivative forms supramolecular assemblies (micelles), whose cmc was obtained by DLS, relaxivity and fluorescence measurements. The full relaxometric analysis of the two Gd3+ complexes was carried out below and above the cmc. Fast water exchange rates were obtained, close to the value reported for the parent complex (eg. the Gd(DO3A-N- (a-pyrenebutanamido)propionate) complex displays a kex298 =(6.2±0.5) x107 s-1 The DO3A-N- (a-pyrenebutanamido) propionate ligand has the ability to sensitize near-infrared emitting Nd3+ and Yb3+ cations in the corresponding complexes, making them promising candidates for near-infrared imaging in biological system applications. The 153Sm(DO3A-N- (a-pyrenebutanamido) propionate) complex displays a biodistribution profile at 1 hour pi similar to other micellar systems. After 24 hours pi there is a strong hepatic and renal uptake suggesting hepatic elimination.

Conclusion: The Ln3+ (Gd, Nd, Yb) complexes of DO3A-N-?-aminopropionate coupled as amides to benzylamide and pyrenebutanamide, as models for biomolecules, show optimal properties and potential MRI contrast agents (Gd) and NIR optical imaging probes. One of them forms micelles and displays in vivo (Wistar rat) hepatic uptake and elimination characteristic of micelle formation.

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LN-CONTAINING LIPOPARTICLES AS NOVEL BIMODAL IMAGING PROBES

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Introduction: Nanoparticle-based molecular imaging has emerged as an interdisciplinary area, showing promises to understand the components, processes, dynamics and therapies of a disease at a molecular level.1,2 Among the bioimaging modalities available, some are characterized by high resolution but low sensitivity (MRI), others by high sensitivity but low resolution (OI). Luminescent/MRI bimodal imaging offers the advantage of coupling the advantages of both techniques. Lanthanide complexes are well suited for the design of bimodal imaging probes: they combine optimized magnetic and optical properties, while the similar chemical reactivities allow facile substitution of one Ln3+ by another. Lanthanide cations provide complementary properties over organic fluorophores,3 nevertheless, the use of a chromophore, acting as an antenna transferring its energy to the Ln to become luminescent, is required. Herein we present the preparation and characterization of novel lipoprotein-like particles, including: (i) amphiphilic Ln3+ complexes3,4 (imaging probes); (ii) Apolipoprotein ApoE4, (target towards LDL receptors, located in the Blood-brain barrier or in tumour cancer cells); and (iii) chromophore (antenna). In vitro biological studies were performed to evaluate the applicability of the particles.

Methods: The particles were synthesised by the dry film methodology. Their characterisation was performed by Dynamic Light Scattering, Atomic Force Microscopy, Gel Electrophoresis and Fluorescence of Tryptophan residues. Their NMRD profile was recorded and their luminescence properties were studied, including by a macroscope adapted to NIR detection. In cellulo studies have been preformed.

Results: Novel synthetic lipoparticles containing a amphiphilic Ln3+ complex (Ln = Gd3+/Nd3+/Yb3+); ApoE4; and anthracene (incorporated in the lipidic core) were synthesized and characterized. The incorporation of ApoE4 into the liposomal moiety was confirmed by gel electrophoresis and fluorescence of the tryptophan residues. The anthracene allowed the sensitisation of both NdIII and YbIII ions, as shown by luminescence studies and capillary macroscope image. The NMRD profile corresponds to that of large particles, and their relaxivity is high. Studies in an in vitro BBB model showed the crossing of the particles.

Conclusions: New lipo-nanosystems were successfully prepared. The anthracene allowed the sensitisation of lanthanide ions as well as their detection under a macroscope. Their relaxivity is higher than the commercially available GdIII-contrast agents. The presence of ApoE4 leads to their targeting towards the BBB. These results seem promising for the use of such particles as bimodal MRI/OI agents.

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ALTERING THE TUMOUR ACCUMULATION OF LIPOSOMES BY CD13-TARGETING

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Introduction: Angiogenesis is required to supply malignant tumours with oxygen and nutrients. Targeting angiogenic vasculature has proven a successful technique for the visualisation and treatment of cancer. This research employs a CT image-based approach to compare the in vivo performance of several different liposome formulations targeted to the tumour neovasculature cell surface marker aminopeptidase N (APN/CD13) via surface conjugation of peptides containing the asparagine-glycine-arginine (NGR) amino acid sequence. Previous studies have demonstrated that NGR-targeting can significantly increase tumour accumulation and therapeutic efficacy of antineoplastic drugs [1].

Methods: A linear hexapeptide containing the NGR amino acid motif was conjugated to maleimide-functionalised PEG-DSPE. Liposomes encapsulating the low molecular weight CT agent iohexol were formed using DPPC, cholesterol (CH), and PEG2000-DSPE. NGR-PEG-DSPE was transferred into the bilayer of these liposomes such that four formulations were prepared: (a) 55:40:5 DPPC:CH:PEG2000-DSPE, (b) 55:40:4.36:0.64 DPPC:CH:PEG2000-DSPE:NGR-PEG2000-DSPE, (c) 55:40:2.44:2.56 DPPC:CH:PEG2000-DSPE:NGR-PEG2000-DSPE (d) 55:40:4.36:0.64 DPPC:CH:PEG2000-DSPE:NGR-PEG3400-DSPE. In vitro characterisation consisted of measuring size, zeta potential, and iodine loading. Pharmacokinetics and biodistribution were examined in female athymic mice bearing ~50 mm3 subcutaneous human non-small cell lung cancer tumours. Micro-CT scans were performed pre-injection and at 10 min, 8, 24, 48, 72, 96, and 144 hr post-injection. Iodine concentration in the tumour, blood (aorta), liver, spleen, kidneys, and muscle were quantified by comparing the density change in the given volume pre- and post- contrast administration.

Results: In vitro characterisation confirmed all formulations to be physically similar. All liposomes were ~ 85 nm in diameter, ~ -30 mV in electrostatic potential, and iodine loading was 45 ± 2 mg/mL. Flow cytometry confirmed binding of the targeted liposomes to cells expressing CD13. The half-life of all contrast agents in the blood pool was ~ 30 hr and accumulation in healthy organs did not differ between formulations, whereas there were statistically significant ($p < 0.05$) differences in tumour accumulation between formulations. Micro-CT images clearly identified heterogeneous liposome accumulation throughout the tumour. Maximum tumour accumulation for formulations (a), (b), and (c) occurred at 48 hr. Compared to the non-targeted formulation, inclusion of 0.64 mol% NGR-PEG2000-DSPE resulted in a 2-fold increase in maximum tumour accumulation (i.e. 1.2 ± 0.4 vs. 2.3 ± 0.7 mg I / mL tumour). Inclusion of 0.64 mol% NGR-PEG3400-DSPE shifted the maximum tumour accumulation to 72 hr and increased the tumour AUC 2-fold compared to the non-targeted formulation (i.e. 117 ± 44 vs. 243 ± 48 mg*hr/mL).

Conclusion: Seemingly small changes to probe formulation variables such as targeting ligand density and PEG spacer length can alter tumour accumulation kinetics by increasing maximum achievable concentration at the tumour site and significantly increasing tumour AUC. These are therefore important considerations in formulation design and can lead to the improved development of advanced theranostics for disease staging and intervention planning.

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poster number: 076

67GA- ESTRADIOL DERIVATIVE COMPLEX FOR BREAST CANCER IMAGING

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Introduction: Breast cancer is a major cause of death and the most malignant form of diagnosed cancer among women in the Western world. Breast tumors overexpressing estrogen receptors (ER) have a great impact in human health and welfare's regardless of the progress in its early detection and treatment. ER is an important tumor biomarker since malignant cells of breast carcinoma, express high ER concentrations when compared with normal cells. Moreover ER status can predict the tumor prognosis or response to hormonal therapy [1,2]. Hence, the search for new imaging agents based on ER-ligands to specifically target and visualize ER in tumors would improve the diagnosis and monitoring of individual therapeutic responsiveness' of tumors [3]. Herein, we report on the synthesis of a new 16a-DOTA-estradiol derivative and subsequent radiolabelling with ^{67}Ga to assess the feasibility of ^{67}Ga- estradiol for functional imaging of ER positive tumors.

Methods: The new 16a-DOTA-estradiol derivative was synthesized by reaction of TBS-16a-bromo-estrone with $DO_3{}^tBu_3$ followed by deprotection and reduction of the ketone group to give the ligand L1 which was fully characterized by the common techniques such ^{1}H and ^{13}C-NMR, ESI-QTMS, IR and HPLC. The cold gallium (GaL1) and ^{67}Ga- complexes (^{67}GaL1) were obtained after reaction of L1 with $Ga(NO_3)_3$ and $^{67}GaCl_3$, respectively. GaL1 was analytically characterized and used as surrogate in the identification of the ^{67}Ga complex. Radiolabelling yield and in vitro stability evaluation of the ^{67}Ga complex was assessed by reverse phase-HPLC and instant thin layer chromatography (ITLC). Lipophilicity was determined through octanol/PBS partition coefficient (Log $P_{o/w}$) obtained by the 'shake-flask' method. Cellular uptake kinetics of the ^{67}Ga-complex was studied in MCF-7 and MDA-MB-231 breast cancer cell lines.

Results: L1 was labeled with ^{67}Ga in high yield. HPLC and ITLC analysis show that ^{67}GaL1 complex is stable in physiological conditions at 37ºC up to 36h. A moderate cellular uptake was found in both cell lines which decrease when MCF-7 cells were previously treated with estradiol.

Conclusions**:** We successfully prepared a new ^{67}Ga-estradiol derivative in high radiochemical yield with high in vitro stability. The preliminary biological studies in MCF-7 cells suggest that uptake occurs via an ER-mediated process.

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WATER-SOLUBLE FLUORESCENT MOLECULAR PROBES FOR THE DETECTION OF ROS

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Introduction:Fluorescent probes, which are well-suited to detect biologically relevant species due to their high sensitivity and selectivity, can be employed for imaging purposes. Most fluorescent probes utilize modulated Photoinduced Electron Transfer (PET) quenching,[1] which implies that the fluorescence intensity changes dramatically upon interaction with a predefined analyte. PET probes have been constructed by a modular chromophore-spacer-receptor architecture. Recently we have introduced the highly fluorescent water-soluble 7-amino-1-methylquinolinium (AMQ) chromophore.[2] This chromophore can be substituted at multiple positions and we have constructed excellent pH probes based on it.[3] The sensitive detection of hydroperoxides and other reactive oxygen species (ROS) is important in a broad range of fields, and in-situ monitoring of ROS concentrations in live cells is highly desirably for understanding their role in biochemical processes.[4] Here we introduce a novel water-soluble fluorescent PET probe for the detection of ROS,[5] by combining the AMQ chromophore and a ROS-responsive thiomorpholine receptor unit.

Methods: The fluorescent probe 1S and its oxidation products were synthesized and characterized. Photophysical properties were determined by spectroscopic techniques like UV-Vis absorption spectroscopy, steady state fluorescence, and fluorescence lifetime measurements. Changes in absorption and emission wavelength, fluorescence quantum yield upon oxidation were determined. Finally the probe oxidation was monitored in a fluorescence spectrophotometer and by fluorescence microscopy.

Results: The fluorescent probe, 1-methyl-7-thiomorpholino-quinolin-1-ium tertafluoroborate (1S), was synthesized along with the corresponding oxidation products, the sulfoxide 1SO, and the sulfone 1SO2. Upon exposure to hydrogen peroxide and other ROS in water, 1S is readily oxidized to 1SO, but not to 1SO2. This process is accompanied by moderate 25 nm blue-shifts in absorption and emission and a 14 fold increase in fluorescence quantum yield, from 0.03 to 0.45. Using a 405 nm excitation source a 17.5-fold increase in emission can be accomplished, and this value can be increased by additional filtering.

Conclusions: We have synthesized and characterized a water-soluble fluorescent molecular probe for the detection of hydrogen peroxide and other ROS. In the probe design we have used the AMQ chromophore, which can be excited at 405 nm and a thiomorpholine receptor unit. The probe readily reacts with hydrogen peroxide at room temperature and for the 520 nm emission a 17.5-fold enhancement has been achieved.

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THE IMAGING PROBE DEVELOPMENT CENTER AT THE NATIONAL INSTITUTES OF HEALTH: PRODUCTION OF A WIDE RANGE OF MOLECULAR IMAGING PROBES

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The Imaging Probe Development Center (IPDC) was established as part of the National Institutes of Health (NIH) Roadmap for Medical Research Initiative (http://nihroadmap.nih.gov/), under the 'Molecular Libraries and Imaging' section of the 'New Pathways to Discovery Program'. IPDC was set up with the recognition that molecular imaging, and the probe chemistry that underlies it, constitute key technologies in 21st century science. The IPDC is dedicated to the production and provision of known and novel imaging probes including optical, radionuclide, ultrasound and magnetic resonance agents and may produce any type of imaging contrast agent. IPDC has already worked collaboratively with over fifty principal investigators from within the NIH intramural research community and beyond. As a trans-NIH effort IPDC core synthetic chemistry serves each of these Institutes and Centers and so its influence can be expected to impact widely different subject matter spanning biological research. Examples of probes already prepared or under preparation will be described in order to illustrate the breadth of the chemistry undertaken by IPDC staff along with a brief outline of the diverse biological applications for which various probes are intended. The spectrum of probes synthesized by IPDC spans from small organic compounds to complex nanoparticles, i.e. fluorogenic enzyme substrates, fluorescent dyes and analogs, radiolabeled low MW compounds, caged derivatives which become fluorescent upon uncaging, radio- and fluorescent-labeled antibodies and other proteins, liposomes, dendrimers etc. Going forward it is planned that IPDC may offer its synthetic, radiochemistry and bioconjugate chemistry services to the broader imaging community, particularly to those imaging scientists who may be limited by their lack of chemistry support.

To learn more readers are encouraged to visit the IPDC website at http://www.ipdc.nih.gov

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NEW POTENTIAL BIMODAL IMAGING CONSTRAST AGENTS BASED ON DOTA-LIKE AND PORPHYRIN MACROCYCLES

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Non-invasive imaging techniques are perfect tools for diagnostic of many diseases, like cancers. The most routinely used analyses at the clinical stage are MRI (Magnetic Resonance Imaging), SPECT (Single Photon Emission Computed Tomography), PET (Positron Emission Tomography)[1]. Some others imaging techniques, less known, are developed at the pre-clinical stage and based on optical imaging, e.g. FRI (fluorescence reflectance imaging), FMT (fluorescence-mediated tomography) or MPM (multiphotons microscopy)[2]. Our researches recently focused on the synthesis of new bimodal contrast agents for medical imaging. Conveniently, the use of bimodal contrast agents could allow the study of the same biological structures at different depths and resolutions, to obtain the most relevant diagnostic.

We have prepared a bifunctional chelator featuring (DO3A-AM)-Porphyrin units[3]. The high-field relaxivity of the Gd complex is similar to those of currently available contrast agents clearly showing that the first coordination sphere remains identical to the parent molecule [Gd(DO3A-AM)(H2O)], with one coordinated water molecule. In this project various covalent bonds are envisaged for the linker, for example a triazolic cycle or a peptidic bond[4].

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poster number: 120

RAPID VISUALIZATION OF HUMAN TUMOR XENOGRAFTS THROUGH OPTICAL IMAGING WITH A NEAR-INFRARED FLUORESCENT ANTI-EGFR NANOBODY

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Introduction: The epidermal growth factor receptor (EGFR) is known to be overexpressed in many types of human epithelial cancers[1]; therefore, non-invasive molecular imaging of EGFR is of great interest. A number of studies have employed monoclonal antibodies (mAbs) as probes; however, their characteristic long half-life in the bloodstream, which delays imaging, has encouraged the development of smaller probes[2]. Nanobodies[3] are the smallest functional fragments of naturally occurring heavy-chain only antibodies (HcAb), and although smaller than mAbs (15 kDa, instead of 150 kDa), nanobodies can bind very specifically and with high affinities to their targets. In this study, an anti-EGFR nanobody-based probe is developed and tested in comparison with the anti-EGFR mAb cetuximab for application in optical molecular imaging.

Methods: The anti-EGFR nanobody 7D12 and the control nanobody R2 were produced as described in[4]. The near-infrared fluorophore IRDye800CW-NHS (IR, LI-COR Biosciences) was conjugated to the nanobodies and to cetuximab. Binding studies were performed on living cells at 4°C. Nude mice bearing A431 tumor xenografts at the hind legs were injected via the tail vein and imaged at several time points post injection (p.i.) with an IVIS Lumina (Caliper Life Sciences). Organs were collected at 2 h and 24 h p.i. and their probe content was quantified in percentage of injected dose per gram of tissue (% ID/g). The distribution of the probes on tumor sections was assessed with an Odyssey scanner (LI-COR Biosciences).

Results: The IR-conjugation had no detrimental effect on the binding properties of 7D12, which specifically bound human EGFR with an affinity of 2 nM. A431 tumors were visible 30 min after the injection of 7D12-IR (with best images obtained 2 h p.i.), whereas with cetuximab IR the best images were obtained 24 h p.i. The quantification of the IR-conjugated proteins in the tumors revealed ' 17% ID/g tumor uptake 2 h after injection of 7D12-IR; while 24 h after injection of cetuximab-IR ' 10% ID/g tumor was obtained. Tumor sections presented a homogeneous distribution of 7D12-IR, whereas cetuximab-IR was irregularly distributed throughout tumors.

Conclusions: 7D12-IR allows the visualization of tumors at earlier time points than cetuximab-IR. Furthermore, higher tumor uptake is obtained with 7D12-IR compared to cetuximab-IR, which is suggested to be related to the more homogenous distribution of this smaller probe throughout the tumors.This study stimulates further research on the development of nanobody-based probes for rapid preclinical optical molecular imaging, which holds promise as a complementary diagnostic tool in humans.

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NANOPARTICLE-BASED RATIOMETRIC APPROACH FOR OXYGEN SENSING IN TUMORS

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Introduction: Hypoxia is one of the major attributes of solid tumors, caused by a combination of relatively high oxygen consumption and insufficient oxygen supply of usually fast growing tumors due to e.g. poor vascularization. Accordingly, there is a high demand for sensitive and cost effective tools for the determination of tumor oxygenation *in vivo* and in real time. So far, oxygen sensors based on oxygen-induced quenching of the photoluminescence are restricted mainly to compounds emitting in UV-vis and to in vitro purposes. Here, we present the development of near-infrared fluorescent (NIRF) nanoparticle-based ratiometric optical probe with high potential for measurements of the tumor oxygenation status *in vivo*.

Methods: For the preparation of the ratiometric NIRF oxygen nanosensor (Ox-NP), 100 nm sized polystyrene nanoparticles were stained with two spectroscopically distinguishable fluorophores, an oxygen-sensitive NIR-emissive metal ligand complex (DYE_{Ox}) and an oxygen-insensitive reference dye (DYE_{Ref}), both excitable at 635 nm. For the calibration of Ox-NP, ratios between the fluorescence intensity of DYE_{Ox} and DYE_{Ref} (DYE_{Ox} / DYE_{Ref}) were measured in the presence of different concentrations of the oxygen scavenger sodium sulfite (Na_2SO_3) and plotted against the effective oxygen concentration determined with a PreSens OxoPlate®. MH-S murine aveolar macrophages and human breast tumor cells were used for NIRF microscopy in order to assess probe uptake and to measure the response to hypoxia. The applicability of the Ox-NPs for *in vivo* imaging was evaluated with the small animal imager, Optix MX2 using a 635 nm laser for excitation. Nude mice bearing subcutaneous human tumor xenografts were used for *in vivo* imaging.

Results: We successfully doped 100 nm polystyrene nanoparticles with the two fluorophores DYE_{Ox} and DYE_{Ref} without alteration of their size or shape. Spectroscopic studies revealed that the emission spectra of both dyes can be easily separated after excitation at 635 nm. The phosphorescence of DYE_{Ox} is completely quenched in oxygen-saturated solution, while the reference signal is not affected by oxygen. A linear relation between the oxygen concentration and the ratiometric response of Ox-NP was measured. It showed a ~4-fold increase in signal intensity for oxygen-free compared to oxygen-saturated conditions. A comparable response with an intensity increase by a factor of ~4.6 upon deoxygenation was determined with Optix MX2. NIRF microscopy revealed that Ox-NP was efficiently taken up by MH-S macrophages cultivated under different oxygenation levels (1 % O_2 up to 20 % O_2). Moreover, the DYE_{Ox} / DYE_{Ref} fluorescence was strongly increased in cells incubated with Ox-NP under hypoxic conditions (1% O_2) in comparison to cells incubated with Ox-NP under normoxia (20% O_2). The hypoxic status of the cells was confirmed by the accumulation of the hypoxia marker HIF-1a, analyzed by Western blot. Furthermore, first results in tumor-bearing mice showed an increase in the ratiometric response between high and low oxygenation conditions in tumor tissue. Conclusion: We developed a novel NIR fluorescent ratiometric system consisting of dye-loaded polystyrene nanoparticles that can be applied not only for intracellular oxygen sensing, but also for the *in vivo* assessment of the oxygenation status in tumor tissue.

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CELL LABELING EFFICACY AND BIOCOMPATIBILITY OF DIFFERENTLY FORMULATED LIPID-COATED NANOCLUSTERS OF IRON OXIDE

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Introduction In recent years, various studies have shown that cell labeling with magnetic material enables in vivo monitoring of cellular migration with MRI[1]. With the increasing availability of multimodal imaging settings, which allows for more extensive characterization of the fate of labeled cells, there is a growing interest in multimodal contrast agents. Therefore, in this study we propose fluorescent lipid-coated iron oxide formulations for simultaneous magnetic and fluorescent cell labeling. Murine macrophages were incubated with these contrast agents to assess labeling efficiency, biocompatibility, as well as multimodal detectability of labeled cells.

Materials and methods Nanoparticles: Three different fluorescently labeled lipid-coated iron oxide nanoclusters were prepared using a solvent evaporation technique[2]. Each nanoparticle consisted of a PEG2000-DSPE/DSPC/Rhodamine-PE lipid monolayer, encapsulating multiple oleic acid-coated iron oxide crystals only (SPION) or dispersed throughout soybean oil (SPION-Soy) or medium-chain triglycerides (SPION-MCT). Nanoparticle size and morphology were studied with dynamic light scattering and transmission electron microscopy (TEM), while MR relaxivities were acquired at 9.4T (Varian Inc.). Cell labeling: Murine macrophages (RAW 264.7) were incubated with nanoparticles for 3 hours (200 µg Fe/ml). Intracellular iron content was determined with 1,10-phenantroline and fluorescence was quantified with flow cytometry. Nanoparticle localization was visualized using TEM, fluorescence- and light microscopy, and T_2*-weighted MRI of homogeneously distributed labeled cells was performed at 9.4T. Cell viability was assessed with an MTT assay and 7-AAD staining.

Results The solvent evaporation procedure resulted in lipidic nanoparticle aggregates of 62-79 nm. Each of these nanoparticles showed a distinct distribution of iron oxide crystals within the lipid monolayer core, giving rise to differences in relaxivities r_2 ranging from 267 $mM^{-1}s^{-1}$ for dispersed iron oxide throughout the oil phase (SPION-Soy and SPION/MCT), to 402 $mM^{-1}s^{-1}$ for densely packed iron oxide (SPION). TEM of labeled cells showed internalized nanoparticles within endolysosomal compartments, which agreed with compartmentalized iron staining (Prussian Blue) and fluorescence, as observed with light and fluorescence microscopy. Macrophage labeling efficiency ranged from 1.0 pg Fe/cell (SPION-Soy) to 5.3 pg Fe/cell (SPION). T_2*-weighted contrast increased accordingly, and allowed MRI of single cells in case of labeling with SPION-MCT and SPION. Cell viability was largely maintained after labeling with SPION, whereas incubations with SPION-MCT and SPION-Soy reduced cell viability. Furthermore, SPION-Soy caused a time- and oxygen-dependent reduction in cell viability, which was not observed for SPION and SPION-MCT.

Conclusions and discussion Lipid-coated nanoclusters of iron oxide represent an attractive, potent and flexible platform that allows the detection of single cells with MRI and optical (imaging) techniques. Nanoparticles that did not contain an oil phase exhibited superior properties in terms of relaxivity, labeling efficiency, biocompatibility and shelf-life. Biocompatibility of SPION-Soy decreased with nanoparticle storage time, which was most probably caused by iron-catalyzed fatty acid peroxidation of the oil phase. These results emphasize that nanoparticles, even though composed of biocompatible compounds such as phospholipids, iron oxide and vegetable oils, should be carefully designed, as the combination of biocompatible building blocks may seriously affect their biocompatibility.

poster number: 123

BIOLOGICAL CHARACTERIZATION OF SEVERAL FUNCTIONALIZED USPIO DERIVATIVES DESIGNED FOR THE MOLECULAR MRI OF ALZHEIMER'S DISEASE

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Introduction : Alzheimer's disease is a neurodegenerative, progressive and incurable pathology, which is one of the leading causes of death in the developed countries. Its diagnosis is done nowadays by the evaluation of cognitive symptoms and is often probable. Amyloid plaques represent the main molecular hallmark of Alzheimer's disease and the ideal target for the development of molecular imaging probes and treatment strategies. Two cyclic heptapeptides (PHO and PHI) were previously identified by screening a randomized library of phage display (1). Aiming to evaluate their aptitude to cross the blood-brain barrier (BBB), independently or associated with putrescine (Put), the peptides were conjugated in the present work to USPIO, and their biodistribution was evaluated in mice. The affinity for amyloid-beta peptide (ABP) of the functionalized USPIO derivatives was evaluated by ELISA, and their cell toxicity was assessed by using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) method, being compared to that of the non-conjugated peptides. The ability of peptides to inhibit the amyloid fiber formation was evaluated by thioflavin T (ThT) aggregation assay.

Methods : PHI and PHO were conjugated to the carboxyl groups exposed by the bisphosphonate coating of USPIO through their amino-terminal groups. Put followed by PEG-NH2 were then conjugated to the free carboxyl groups of USPIO. NMRI mice were injected with 100 µmol Fe/kg b.w. of one of the following USPIO derivatives: USPIO-PHO, USPIO-PHO-Put, USPIO-PHI, USPIO-PHI-Put, USPIO-Put. Ninety minutes later, the mice were sacrificed and the blood and several organs (brain, liver, spleen, kidneys, heart, and lungs) were collected. The transverse relaxation rate of water protons (R2) was then measured at 60 MHz on a Bruker Minispec mq60. For affinity measurements by ELISA, the USPIO derivatives bound to ABP were detected with an anti-PEG antibody, which was then identified by a biotinylated secondary antibody, followed by streptavidin and peroxidase-biotin. Cytotoxic effects were evaluated on mouse neuroblastoma N18TG2 cell line.

Results : The hydrophobic character of PHO appears to play a significant role in the access of USPIO across the BBB. The hydrophilic peptides like PHI restrain the USPIO transfer into the brain, while Put enhances their liver sequestration. When combined with either of the peptides, Put contributes to the fast blood clearance of the functionalized USPIO nanoparticles. The ThT assay has shown that our peptides are able to limit the ABP aggregation, and their binding affinity is preserved even after conjugation to USPIO. The peptides are not toxic for neurons, but USPIO derivatives conjugated to Put produce a significant decrease of the cell viability.

Conclusions : The peptides have a high potential for molecular targeting of amyloid plaques, either as carriers of molecular imaging compounds or as amyloid fiber disrupting agents. USPIO-PHO seems to be the better candidate because it is not captured by the liver, its plasma clearance is slow, and it probably crosses the BBB in the absence of a permissive molecule like putrescine.

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poster number: 124

CFO-DPA-714, A NOVEL FLUORINE-18 LABELLED ANALOGUE OF DPA-714 DEVOID OF THE METABOLICALLY UNSTABLE FLUOROALKOXY MOIETY

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Objectives: Nearly all brain diseases reveal pronounced changes in the functional states of glial cells and most prominent is the presence of activated microglia in areas of progressive disease or tissue destruction. Microglia activation is characterized by the over-expression of the translocator protein 18 kDa (TSPO) on the outer mitochondrial membranes supporting, for over two decades, considerable efforts in the design of radioligands for the in vivo imaging of this pharmacological target by Positron Emission Tomography [1]. A particular attractive and today well-recognized chemical family of selective TSPO radioligands are the pyrazolo[1,5-a]pyrimidineacetamides, a series that includes the fluoroethoxy derivative [18F]DPA-714. However, recent studies clearly demonstrated that this compound is rapidly and extensively in vivo metabolized in both rodents (rats) and non-human primates (baboons), with notably formation of fluorine-18-labelled metabolites resulting from O-dealkylation [2]. Based on these observations, a novel series of pyrazolo[1,5-a]pyrimidineacetamides were designed by replacing the oxygen atom bridging the phenyl ring and the fluoroalkyl chain by a methylene group.

Methods: CfO-DPA-714 as reference compound as well as its tosylated derivative as precursor for labelling with fluorine-18 were synthesized in nine steps from commercially available methyl 4-hydroxybenzoate. The key-intermediate was the corresponding propylalcohol derivative, which was reacted at the last stage, in dichloromethane, either with DeoxoFluor to afford CfO-DPA-714 or with TsCl and triethylamine to afford the tosylate derivative. Fluorine-18 labelling of CfO-DPA-714 was performed using a TRACERLab FX-FN synthesizer (GEMS) and comprises (1) dissolution of the no-carrier-added, dried (activated) K[18F]F-Kryptofix®222 complex in dimethyl sulfoxide (700 µL) containing 4.0 to 4.5 mg of the tosylated derivative (7.2 to 9.8 µmol); (2) heating the reaction mixture at 160°C for 5 minutes; (3) dilution of the reaction mixture with twice 2 mL of the HPLC mobile phase; (4) pre-purification on a SepPak® Alumina N™ cartridge; (5) HPLC purification on a semi-preparative X-TerraTM RP18 column (eluent : aq. NH4OAc 0.1 M pH10 / MeCN : 60/40 (v:v)) and (6) SepPak® cartridge-based removal of the HPLC solvents and formulation for i.v. injection.

Results: CfO-DPA-714 and its tosylated analogue were obtained in 52% and 85% yields respectively from the common propylalcohol intermediate. [18F]CfO-DPA-714 was obtained in 25-30% non decay-corrected yields, based on starting [18F] fluoride. Typically, starting from a 30 GBq cyclotron-produced [18F]fluoride batch, 7.5 to 9.0 GBq of [18F]CfO-DPA-714, > 99% radiochemically pure and ready-to-inject, were obtained in 55-60 min (including HPLC-purification, Rt : 11 min) and with specific radioactivities ranging from 50 to 90 GBq/µmol.

Conclusions: CfO-DPA-714, a first candidate of a novel series of pyrazolo[1,5-a]pyrimidineacetamides, putatively less prone to metabolism and in particular more stable with regard to radiodefluorination, was successfully synthesized and labelled with fluorine-18. Comparative PET-imaging (Focus 220 Concorde) is currently underway in a rat model of neuroinflammation (unilaterally, AMPA-induced, striatum-lesioned rats) to demonstrate its potential to image the TSPO.

Acknowledgment: Supported by Joint INCa/DAAD Translational Research Programme 08-006. References: [1] Dollé et al. (2009), Curr. Med. Chem., 16, 2899-2923 ; [2] Peyronneau et al. (2009), J. Label. Compds Radiopharm., 52 (suppl 1), S385.

poster number: 125

TUMOR BRACKETING AND SAFETY MARGIN ESTIMATION USING MULTIMODAL MARKER SEEDS

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Introduction: One of the main reasons for locoregional treatment failure in breast cancer is the inability to fully excise the primary tumor during surgery. An approach that optimizes the surgical precision can have a positive influence on the clinical outcome. One approach to improve surgical guidance is the preoperative placement of markers around the tumor. The challenge here is to generate marker seeds that: 1) can be accurately placed at different positions surrounding the tumor, 2) can be preoperatively detected with non-invasive imaging techniques, 3) can be relocated during surgical procedures and 4) will also provide post operative guidance for pathologists.

Methods: To achieve all this, we have generated multimodal marker seeds that are based on a 'cocktail' of a dual emissive inorganic dye, lipids, and pertechnetate and evaluated their potential using a phantom wherein a surrogate tumor was placed.

Results: Due to their contents these markers can be easily placed under ultrasound guidance and detected afterwards with X-ray imaging, single photon emission tomography, magnetic resonance imaging, and fluorescence imaging. Placement of several markers around the surrogate tumor enable bracketing of the entire lesion. Furthermore, the dual emission by the dual emissive inorganic dye (combined exciton and defect emission), gives the unique opportunity to also determine the depth of the seeds via multispectral imaging; exciton emission (520 nm) <5mm penetration and defect emission (open filters) <12 mm penetration.

Conclusion: The difference in tissue penetration between the two emissions may aid surgeons in the resection of a marker bracketed tumor using a > 5 mm safety margin. Furthermore, when using particles with different (fluorescent) colors, the original geographic orientation of the excised tissue can be determined.

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EARLY DETECTION OF PRIMARY AND DISSEMINATED MELANOMA WITH [18F]ICF01006: PRECLINICAL STUDY

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Malignant melanoma represents a public health problem with a dramatic increase in incidence and mortality due to a high metastatic potential and resistance to conventional cytotoxic agents. Surgery is the most efficient therapy and early diagnosis and staging of melanoma remain crucial. Positron emission tomography (PET) using [18F]FDG has been widely used for melanoma imaging, but lacks specificity. Consequently, more specific radiotracers are still needed to allow identification, characterization, monitoring and guiding therapeutics for disseminated melanoma. Our lab developed for many years arylcarboxamides as melanoma tracers due to melanin binding . From this series, [18F]-N-[2-(diethylamino)ethyl]-6-fluoro-pyridine-3-carboxamide ([18F]ICF01006), demonstrated promising results for the early detection and monitoring of primary and disseminated in experimental B16BL6 melanoma in C57BL/6J mice.

For primary melanoma model, melanoma cells (1.5x105cells/mouse) were injected subcutaneously in the shoulder of mice. Pulmonary metastatic model was induced by intravenous injection of melanoma cells (1.5x105cells/mouse) in lateral tail vein of mice. In the two experimental melanoma models, mice were imaged by PET at different time points of tumoral development. In the primary model, B16BL6 melanoma tumours were detected at Day-5 after inoculation with [18F]ICF01006 uptake of 3.4±1.5% ID/g of tumour as early as 1 h post administration. Tumoral uptake of [18F]ICF01006 increased as pathology progressed and reached a value of 11.5±2.7 %ID/g at Day-14. B16BL6 tumours were all clearly visible with a high contrast to background ratio (i.e: tumour-to-muscle ratio=5.5±2.6 and 7.6±0.6 at Days-5 and Day-14, respectively). Significant uptake was also observed in other melanotic tissue as eyes and spleen. In contrast, activity in nontarget tissues displayed fast washout at 1 h p.i.

In pulmonary metastatic model, [18F]ICF01006 allowed detection of melanoma lesions at Day-9 after tumour cell inoculation with a value of 1.2±0.51% ID/g, while lung radiotracer accumulation increased with melanoma invasion. At Day-21, [18F]ICF01006 uptake reached a value of 5.7±1.4% ID/g in lungs versus 0.4±0.1% ID/g in controls. At autopsy, macroscopic observation showed that the degree of lung tumour burden could be related with [18F]ICF01006 accumulation observed in PET images. Comparison with [18F]FDG PET imaging at stage day-14 showed that both radiotracers were able to detect pulmonary lesions but [18F]ICF01006 was superior in terms of contrast and specificity. [18F]ICF01006 exhibited a highly contrasted tumour imaging as early as 1 h post injection for primary model permitting thus an early diagnosis of subcutaneous tumours before they were palpable. The high specificity of [18F]ICF01006 for melanin and its rapid elimination allow an early detection of pulmonary lesions suggesting its ability to detect small lesions in different sites of metastases. Our promising results suggest an excellent potential of [18F]ICF01006 PET imaging for early diagnosis and follow-up of disseminated melanoma.

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poster number: 127

A CELL-PERMEABLE B-GALACTOSIDASE TARGETED CONTRAST AGENT FOR OPTICAL AND MR IMAGING

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Introduction: Noninvasive monitoring of intracellular targets like cytosolic enzymes by means of magnetic resonance imaging (MRI) is rapidly gaining relevance in various research fields. It inevitably requires the development of suitable cell-permeable imaging probes that can specifically interact with the target characterizing the cellular or molecular process of interest. We developed a dual-labeled probe, Gd-DOTA-k(FR)-Gal-CPP, to report the presence of intracellular ß-galactosidase (ß-gal) by MRI. The probe consists of a galactose core, serving as cleavable spacer, inserted between a cell penetrating peptide (CPP, D-Tat_{49-57}) and two reporter moieties (Gd-DOTA, fluorescein (FR)). Cellular retention of these imaging reporters is expected in the presence of ß-gal due to the cleavage of CPP following internalization of the probe into cells.

Methods:Gd-DOTA-k(FR)-Gal-CPP was synthesized by utilizing a facile building block strategy, which involved the preparation of required building blocks, their subsequent assembly on the solid phase using Fmoc chemistry and complexation of the obtained ligand with $GdCl_3$. The longitudinal relaxivity, r_1, was measured in aqueous solutions at 3T and room temperature. Cellular uptake of Gd-DOTA-k(FR)-Gal-CPP in ß-gal expressing C6/LacZ (ß-gal$^+$) and enzyme-deficient C6 (ß-gal$^-$) rat glioma cells was confirmed by fluorescence spectroscopy, MR imaging and ICP-AES measurements. Cellular distribution was evaluated by fluorescence microscopy.

Results:Gd-DOTA-k(FR)-Gal-CPP showed a considerably higher relaxivity r_1 (16.8±0.6 $mM^{-1}s^{-1}$) than the commercial Gd-DOTA (4.0±0.12 $mM^{-1}s^{-1}$). The observed enhancement of r_1 can be attributed to the additive effects of a slower global tumbling rate and restricted intrinsic local motion. Reduction of steric hindrance nearby the Gd-chelate in a second derivative, Gd-DOTA-Gal-k(FR)-CPP (changed positions of k(FR)), with the same molecular weight, resulted in significant lowering of r_1 (10.0 ± 0.8 $mM^{-1}s^{-1}$). Gd-DOTA-k(FR)-Gal-CPP was efficiently internalized into both cell lines and a higher accumulation of imaging reporters was observed in ß-gal$^+$ compared to ß-gal$^-$ cells by all methods used. Fluorescence spectroscopy revealed an almost twofold higher content of imaging probe in ß-gal$^+$ cells. Although the observed slightly increased cellular relaxation rate in ß-gal$^+$ vs. ß-gal$^-$ cells loaded with Gd-DOTA-Gal-k(FR)-CPP was not statistically significant, subsequent ICP-AES studies on cell lysates revealed a significant about fourfold higher Gd-content in ß-gal$^+$ cells. The relatively high nonspecific background signal detected in ß-gal$^-$ cells probably originated from vesicularly entrapped and unconverted Gd-DOTA-k(FR)-Gal-CPP as observed by fluorescence microscopy. This unfavorable cellular distribution allowed only a restricted interaction of the probe with the cytosolic ß-gal enzyme in ß-gal$^+$ cells and was responsible for the observed nonspecific background signal in the ß-gal$^-$ cells. Conclusions:A novel, cell-permeable and dual-mode imaging probe targeting an intracellular enzyme was developed. Increased specific retention of our probe in ß-gal positive cells was observed although further improvements are needed to circumvent its predominantly vesicular entrapment inside the cells. However, our probe was successfully applied for cell imaging using MRI and optical modalities.

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A THIOL-RESPONSIVE GD(III) BASED MRI CONTRAST AGENT FOR THE EVALUATION OF THE TUMOR REDOX MICROENVIRONMENT

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Introduction. Mammalian cells display a large number of reactive protein thiols on the extracellular side of the plasma membrane (Exofacial Protein Thiols, EPTs). These EPTs are quite reactive and can be chemically labeled by suitably designed MRI contrast agents. We have recently shown that compound Gd-DO3A-PDP, containing the 2-pyridinedithio chemical function for the recognition of EPTs, can label cells ex vivo through the formation of disulfide bridges with the cell's EPTs. [1,2] The Gd(III) chelate thus anchored on the cell surface is transported into the cytoplasm, and by this route up to 1.2xE10 Gd atoms per single cell can be internalized. The amount of internalized Gd is dependent upon the availability of EPTs, whose levels are influenced by the extracellular redox microenvironment. In solid tumors, cells proliferate under a hypoxic (thus reducing) microenvironment, therefore they are expected to display high levels of EPTs. The extent of labeling of these cells in vivo by Gd-DO3A-PDP would therefore report about the tumor extracellular redox, readily seen as DCE in MRI images.

Methods. To assess whether Gd-DO3A-PDP could be used to label tumors in vivo, murine melanoma B16 cancer cells have first been assayed in vitro for their ability to take up Gd-DO3A-PDP as a function of the EPTs levels (modified by chemical thiol blockers / reductants). Then, Gd-DO3A-PDP has been delivered to tumor areas in mice grafted with a B16 melanoma derived tumor and the signal enhancement monitored over time by MRI at 1 T.

Results. The signal enhancement in tumors treated with Gd-DO3A-PDP has been shown to decay very slowly with time (it is still clearly detectable at 48 hours post injection), especially if compared with that of tumors treated with control Gd-DO3A. The long lasting signal enhancement of Gd-DO3A-PDP in tumors is in line with the compound being internalized into cells through the EPTs route.

Conclusion. We think that the results herein presented constitute a good proof-of-concept about the possibility to obtain molecular images of EPTs in vivo, that in turn can give information about the tumor extracellular redox.

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poster number: 129

SYNTHESIS AND EVALUATION OF A DUAL LABELED PEPTIDE FOR CXCR4 IMAGING

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Introduction: The chemokine receptor 4 (CXCR4) is over-expressed in 23 types of cancer were it plays a role in, among others, the metastatic spread. For this reason it is a potential biomarker for the field of diagnostic oncology. Our aim was the development of a bimodal CXCR4 peptide imaging probe, using a multifunctional single-attachment-point (MSAP) reagent.[1] This MSAP reagent consisted of a DTPA chelate, a Cy5.5-like dye and an activated ester for conjugation. This design allowed coupling of the label to one amine functionality distant from the pharmacophore of the Ac-TZ14011 peptide.

Methods: The MSAP reagent has been prepared using solid phase and solution phase synthesis and it was conjugated to the antagonistic CXCR4 peptide Ac-TZ14011. In vitro binding studies using flow cytometry and confocal microscopy were conducted. SPECT/CT, fluorescence imaging and biodistribution studies were performed to evaluate the probe in mammary tumor models.

Results: Flow cytometry and confocal microscopy demonstrated that the imaging peptide had a high CXCR4 receptor affinity and a good selectivity. Initial in vivo experiments revealed good tumor specificity.

Conclusions: Our results demonstrate the potential of a bimodal peptide-based CXCR4 antagonist. A potential application for such a multimodal probe could be an integrated use of preoperative diagnostics and surgical planning (radioactivity-based), with intraoperative surgical image guidance (fluorescence-based) to the predefined lesion location.[2-3]

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MULTIMODAL MOLECULAR IMAGING OF TUMOR MARGINS AND DISTANT METASTASES GUIDED BY AVB3 INTEGRIN EXPRESSION

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Introduction: Multimodal imaging agents create the possibility to unite the advantages of two imaging modalities into a single imaging approach. Combining SPECT/CT and fluorescence imaging makes it possible to plan a surgical intervention towards the primary tumor and (distant) metastases. Intraoperatively, the surgeon can use fluorescence imaging to approach the tumor margins and excise the metastases. This approach has shown its added value in complex clinical sentinel lymph node procedure.1 The goal is to apply this same approach with a targeted multimodal imaging agent. avß3 Integrin, which is involved in (tumor induced) angiogenesis, is a promising candidate for the tumor specific visualization of both primary tumors and their distant metastases. The necessity of tumors to induce angiogenesis to grow larger than 2 mm makes avß3 integrin targeting compounds into possible general tumor markers. The cyclic pentapeptide c[RGDfK] is well validated and has the possibility to be labeled via the lysine without affecting the receptor binding.2

Methods: A hybrid imaging agent targeted to the avß3 integrin, 111In-MSAP-RGD, was synthesized by combining solid phase peptide chemistry and a multifunctional single-attachment-point (MSAP) reagent,3,4 which contains a chelate (DTPA) and a cyanine dye (CyAL-5.5). Tumor bearing mice were injected with 111In-MSAP-RGD and subjected to SPECT/CT, FMT-XCT, fluorescence imaging/ microscopy.

Results: Tumor specific 111In-MSAP-RGD uptake could be visualized in 3D by SPECT/CT and FMT-XCT imaging. In comparison to 111In-DTPA-RGD, the multimodal variant showed a prolonged retention in vivo and an increased accumulation in the primary tumor. Next to the primary tumor, also distant metastases were clearly visible in the SPECT/CT images and could be accurately excised guided by the fluorescent signal.

Conclusions: Our bifunctional RGD-derivative shows great potential in preoperative planning and fluorescence based surgical intervention. Both imaging modalities complement each other in the process of locating the lesions preoperatively and finding and excising the lesions intraoperatively. The general requirement of tumors to induce angiogenesis and the fast and complete biodistribution of this small peptidic avß3 integrin marker makes this a good and very useful candidate for clinical translation.

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IN VIVO FLUORESCENCE IMAGING OF T CELLS: COMPARISON OF NANOBODIES AND CONVENTIONAL MONOCLONAL ANTIBODIES

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Introduction: The toxin-related ecto-ADP-ribosyltransferase 2 (ART2) on murine T cells can apoptotically delete various T-cell subsets (1). This process can be blocked by ART2-specific nanobodies in vitro and in vivo (1,2). The objective of our study was to compare the pharmacokinetic, biodistribution and efficacy of fluorochrome-conjugated ART2 inhibitory nanobodies with conventional monoclonal antibodies and to determine their in vivo fluorescence imaging properties.

Methods: Three different types of ART2-specific antibody molecules were used in our experiments: A recombinant llama nanobody (s+16, 14 kD), a fusion protein of s+16 with the Fc domain of murine IgG1 (s+16-mFc, 75 kD) and a conventional rat mAb (Nika102, 150 kD), labeled with Alexa-Fluor dyes. In vitro biodistribution and enzyme-inhibition analyses were performed with cells prepared from ART2-deficient, wildtype, and ART2-overexpressing transgenic mice that were sacrificed at different time points after intravenous antibody injection. Circulating and excreted Abs were monitored in plasma and urine samples. Inhibition of ART2 mediated cell surface ADP-ribosylation was monitored by a FACS assay. Semiquantitative in vivo fluorescence imaging was carried out with an IVIS-200 optical imaging system and compared to results from in vitro analyses.

Results: FACS analyses of plasma revealed a rapid decline of circulating nanobody, with a corresponding appearance in urine, whereas Fc-fusion protein and conventional mAb showed a slow decline of plasma levels and were barely detectable in urine. Blood derived T cells revealed rapid and equal staining by all three antibody constructs. Lymph node derived T cells where stained much faster with nanobody and Fc-fusion protein (within 10 minutes) than with conventional mAb (2 hours). In vivo fluorescence imaging provided further visual evidence for rapid renal clearance of nanobodies (bladder signal) and labeling of lymph node T cells in ART2-expressing wildtype and ART2-transgenic mice, but not in ART2-deficient mice.

Conclusion: Our results provide a proof of principle for the utility of fluorochrome-conjugated nanobodies for both, the inhibition of a T cell surface enzyme, as well as for the noninvasive imaging of T cells. Single domain nanobodies seem particularly suited for reversible, short-term uses, e.g. tumor imaging or therapy of acute inflammation. Reformatted nanobody-Fc fusion proteins, in contrast, appear particularly suited for long-term uses, e.g. therapy of tumors and chronic inflammation.

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NEAR INFRARED OPTICAL IMAGING OF CCK2R EXPRESSING TUMOURS USING A NEW MINIGASTRIN ANALOG

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Introduction: The cholecystokinin-2-receptor (CCK2R), also called gastrin receptor has recently been evaluated as promising target for early detection of a wide range of tumours. In this study, we aimed at developing a new CCK2R targeted probe which is detectable via optical imaging. The probe was designed as a near-infrared peptide probe, which allows for rapid, non-invasive imaging of CCK2R positive tumours.

Methods: The optical peptide probe (dQ-MG-754) was synthesized by coupling a newly constructed 13 aa minigastrin peptide to the hemicyanine fluorescent dye DY-754. Probe properties are defined to be highly hydrophilic and to show a near-infrared fluorescence to enable fast, high-contrast imaging. To determine receptor binding and probe specificity in vitro, receptor positive cells (A431/CCK2R cells stably transfected with CCK2R) and receptor negative cells (A431/WT wildtype cells) were incubated with probe, probe together with a 10-fold excess of unlabeled minigastrin, free dye or medium, respectively. Laser scanning microscopy was used to visualize probe binding and internalization into cells. Subcutaneous xenografts in athymic nude mice were induced by implantation of A431/CCK2R on the right shoulder and A431/WT cells on the left shoulder to allow for simultaneous imaging of both tumours. Probe accumulation and kinetics were observed via fluorescence reflectance imaging (FRI) over 72 h (n=5) after systemic application. In vivo specificity was tested by coinjection of probe and a 10-fold excess of unlabeled minigastrin (n=2). Ex vivo probe biodistribution was determined 4h, 8h, 24h and 72h p.i. (n=5/group). Vascularization was compared using CD31 immunohistochemistry on cryosections of A431/CCK2R and A431/WT xenografts.

Results: A strong receptor binding was revealed by a 16-fold higher fluorescence in A431/CCK2R cells compared to A431/WT cells after probe incubation. Blocking of A431/CCK2R cells with a 10-fold excess of unlabeled minigastrin led to a signal reduction by 58% (SD ± 3.6%), indicating probe specificity. LSM imaging showed internalization of the probe into A431/CCK2R cells and a cytoplasmatic localization. In vivo A431/WT xenografts showed a 63% lower (SD ± 7.9%) fluorescence than A431/CCK2R xenografts 8 h p.i.. In vivo coinjection of probe and unlabeled minigastrin reduced fluorescence in A431/CCK2R xenografts by 54% (SD ± 20.8%), supporting specific probe uptake. Tumour to normal tissue ratio (TNR) 8 h p.i. was 3.9 (SD ± 0.76) in A431/CCK2R xenografts, 1.4 (SD ± 0.28) in A431/WT xenografts and 2.03 (SD ± 0.23) in blocked A431/CCK2R tumours. Biodistribution of the probe showed a high kidney and moderate liver uptake. In contrast, nearly no probe was detected in the brain, heart, lungs, spleen and muscle. CD31 immunohistochemistry showed the same vascularization pattern in both tumour types, indicating that comparable probe accessibility.

Conclusion: In conclusion, our probe dQ-MG-754 selectively targeted CCK2R expressing xenografts. A 4-5 fold increase in contrast between A431/CCK2R tumours and normal tissue or A431/WT tumours demonstrated preferential uptake into A431/CCK2R tumours. Based on the occurrence of CCK2R expression in the development of many neuroendocrine and gastrointestinal malignancies, such as medullary thyroid cancer and colon carcinoma, our probe could be a promising candidate for early detection of laparoscopically or endoscopically detectable tumours.

poster number: 133

EVALUATION OF [18F] FEBSA AS A PET BLOOD POOL TRACER FOR LVEF QUANTIFICATION AND ITS COMPARISON TO GOLD STANDARD GATED CARDIAC MAGNETIC RESONANCE IMAGING

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Objectives: PET tracers for blood pool imaging may provide an attractive alternative to non-invasively assess left ventricular (LV) cardiac function [1]. In this study a novel PET tracer that allows in vivo labeling of RBCs 4-[18F]fluoroethoxybenzene sulfonamide, [18F] FEBSA was evaluated in normal Wistar rats. The left ventricular ejection fraction (LVEF) was measured in rest and stress (dobutamine) with gated PET and the results were compared with cardiac MRI as the reference method.

Methods: The [18F] FEBSA was synthesized with good radiochemical yield and purity as described in [2]. About 48.1 MBq/0.7 mL of the tracer was administered to the Wistar rats (n=2) via a tail vein, followed by electrocardiogram (ECG) triggered-respiratory-gated micro-PET acquisition at rest (0.5 h) and stress (0.5 h) by infusion of dobutamine 10 µg//Kg/min. The data was reconstructed into a series of 12 ECG gated images and were filtered with a Gaussian with FWHM of 1 mm in 3D. Along the time axis, the images were filtered in the FFT-domain by keeping the DC-value and the first four harmonics. CineMRI was perfomed with a 9.4T horizontal magnet (Bruker Biospec) and a retrospectively gated FLASH sequence (TR/TE=7.6/1.8ms, flip angle=17deg, matrix=256x256, FOV=6x6cm, 10 to 12 1mm thick short axis slices covering the LV, 15 frames; using INTRAGATE®, Bruker) under isofluorane anesthesia in the same animals and with a similar dobutamine stress test as for the micro-PET.

Results: The LVEF values with micro-PET for rat 1 and rat 2 was found to be 75% and 61% at rest and during the dobutamine challenge 84% and 85% respectively. Using cardiac MRI the LVEF was found to be 53% and 73% at rest and under stress 81% and 82% respectively.

Conclusions: We have shown that our novel PET tracer [18F] FEBSA can be used to assess LVEF and temporal changes in response to pharmacologic stress. PET was in good agreement and comparable with MRI as a reference technique.

Acknowledgement**:** In vivo molecular imaging research (IMIR), Katholieke Universiteit Leuven, Leuven, Belgium.

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poster number: 134

MEO-SSR180575, A NOVEL INDOLEACETAMIDE-BASED CANDIDATE LABELED WITH CARBON-11 FOR PET IMAGING OF THE TSPO 18 KDA

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Objectives: The 3-isoquinolinecarboxamide [11C]PK11195, despite its low brain uptake and high level of non-specific binding, is still the most widely used PET-radioligand for the in vivo imaging of the peripheral benzodiazepine receptor (PBR or TSPO 18 kDa). Several new PBR radioligands are currently developed to replace [11C]PK11195 [1], e.g the pyrazolo[1,5-a]pyrimidineacetamides [11C]DPA-713 and [18F]DPA-714, the imidazo[1,2-a]pyridineacetamides [11C]CLINME and [18F]PBR111 and the N-benzyl-N-(2-phenoxyaryl)-acetamides [11C]PBR28 and [18F]FEDAA1106. Another attractive newly identified chemical class of structures are the indoleacetamides and notably compounds derived from the lead compound SSR180575 [2]. Herein are reported the synthesis and the labelling with the positron-emitter carbon-11 (half-life : 20.38 min) of a novel derivative of SSR180575, bearing a para methoxy function on its phenyl ring, as well as a preliminary pharmacologically evaluation of this radioligand in a rat model of neuroinflammation by PET imaging.
Methods: p-MeO-SSR180575 was synthesized from commercially available 4-chloro-2-nitrotoluene in 10 steps. O-demethylation, performed with a boron tribromide solution in dichloromethane at low temperature, afforded the free phenol derivative as precursor for labelling with carbon-11 (p-HO-SSR180575). Carbon-11 labelling of p-MeO-SSR180575 was performed using a TRACERLab FX-C Pro synthesizer (GEMS) and comprised (1) trapping of [11C]MeOTf at -10°C in acetone (0.3 mL) containing p-HO-SSR180575 (0.6-0.9 mg) and aq. 3N NaOH (8 µL) ; (2) heating at 110°C for 2 min ; (3) concentration to dryness and taking up the residue in 1.0 mL of the HPLC mobile phase ; (4) purification using semi-preparative reversed-phase HPLC and (5) SepPak®Plus C-18-based formulation for i.v. injection. Acute neuroinflammation was induced in Wistar rats (300 g, male) by stereotaxic injection of 7.5 nmol of AMPA in the right striatum for both ex-vivo autoradiography and in vivo PET-imaging (Focus P220 Concorde, control kinetics, displacement experiments with PK11195 (1 mg/kg)).
Results: p-MeO-SSR180575 was obtained in 10% overall yield. The tricky and low-yielding step in our approach was the pyridazine ring formation reaction that proceeded partially: the maximum conversion of the intermediate diester-indole reacting with 4-methoxyphenyl hydrazine was only about 35%. p-HO-SSR180575 was obtained in 93% yield from p-MeO-SSR180575. p-[11C]MeO-SSR180575 was obtained in 18-22% decay-corrected yields, based on starting [11C]carbon dioxide. Typically, starting from a 74 GBq cyclotron-produced [11C]carbon dioxide batch, 3.7 to 4.5 GBq of p-[11C]MeO-SSR180575, > 99% radiochemically pure and ready-to-inject, were obtained in 38 min (including HPLC-purification, Rt : 7.5-8.0 min). Specific radioactivities ranged from 75 to 150 GBq/µmol. In PET experiments, p-[11C]MeO-SSR180575 showed a higher contrast between the lesioned area and the corresponding area in the intact contralateral hemisphere when compared to [11C]PK11195 (ratio ipsi/contra at 20 min and 60 min post-injection (n=4): p-[11C]MeO-SSR180575: 2.2 and 3.0 ; [11C]PK11195: 1.7 and 1.6). and was also displaced by PK11195.
Conclusions: p-MeO-SSR180575 was successfully synthesized and labelled with carbon-11. Preliminary dynamic µPET studies in rats demonstrated its potential to image neuroinflammation.

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poster number: 135

DESIGN AND APPLICATIONS OF NEW FLUORESCENT BISPHOSPHONATE IMAGING PROBES

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Introduction: Nitrogen-containing bisphosphonates (N-BPs) are well-accepted therapeutic agents for treatment of bone disorders such as osteoporosis and Paget's disease, and are finding a general role in cancer therapy. To elucidate details of the skeletal distribution, cellular uptake and mechanisms of these drugs, fluorescent imaging probes are required that mimic their structures and pharmacological properties.

Methods: Recently, we described the first synthesis [1] of novel fluorescent conjugates of the heterocyclic N-BP drug risedronate and related analogues. The synthesis is based on attachment of a functionalized linker to the drug under exceptionally mild reaction conditions (aqueous conditions at near neutral pH; 40 °C). The drug-linker derivative is then facilely conjugated to a suitable activated form of an imaging agent of choice, yielding a fluorescent BP imaging probe.

Results: Our 'magic linker' synthesis methodology has now been extended to all three of the major heterocyclic N-BPs (risedronate, zoledronate, and minodronate) and several related analogues. A series of fluorescent dyes, including near infrared (NIR) fluorescing dyes, Alexa Fluor 647 [2] and IRDye 800CW, were incorporated into the fluorescent BP probes. The probes can be prepared in good yields (50-80%) and high purity (> 95%), and are fully characterized by HPLC, UV-VIS and fluorescence emission spectroscopy, ^{1}H and ^{31}P NMR and high-resolution MS. The fluorescent probes generally retain significant affinity for bone mineral, reflecting the varying affinities of their parent drug components. Labeling of drugs having different bone affinities by dyes with distinguishable fluorescent emission spectra generates a fluorescent probe 'toolkit', allowing simultaneous detection of individual low and high affinity BPs and PCs in bone, bone tissues, and cells [3]. We have now obtained evidence that such conjugates have anti-osteoporosis effects in vivo, suggesting that they retain key pharmacological characteristics of the parent drug in vivo.

Conclusions: The development of N-BP drugs including zoledronate and minodronate linked to diverse VIS and NIR fluorescent labels provides an expanded basis for imaging studies of this clinically important group of compounds.

Acknowledgement: Financial support was provided by NIH, USC and PUF (Partner University Fund).

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SYNTHESIS, CHARACTERISATION AND EXAMINATION OF GD(III)CHELATE-FUNCTIONALISED SILICA NANOPARTICLES AS CONTRAST AGENTS FOR MRI

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Introduction: The development of magnetic resonance imaging (MRI) towards one of the most powerful techniques in clinical diagnosis is accompanied by progress in the design of paramagnetic contrast agents (CAs) to enhance imaging sensitivity. Most of the currently applied CAs for enhanced T1-contrast are based on gadolinium(III) chelate complexes and are mainly extracellular agents which only distribute non-specifically throughout the circulatory system and interstitial spaces. Since those agents are excreted easily and quite fast from the body, they are not suitable for targeting or long-term tracking applications. Therefore, nano-sized materials were developed and they are gaining increasing importance in medical diagnosis and treatments. Silica nanoparticles (SiO2NPs) can serve as a matrix not only for Gd(III) chelate complexes, but also for vector- and sensor-biomolecules for targeting applications [1].

Methods: Spherical, non porous and monodisperse SiO2NPs with diameters of 50-100 nm were synthesised by means of the Stöber process [2]. The surface of the bare SiO2NPs was functionalised with carboxylic acid groups and amino groups, respectively, thus allowing to build peptide bonds with Gd(III) chelate complex systems and/or biomolecules [3,4]. Lysine was used to introduce a bifunctional linker. The materials were fully characterised after each synthetic step by dynamic light scattering (DLS), scanning electron microscopy (SEM), diffuse reflectance infrared fourier transform (DRIFT) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy and thermogravimetry (TG). Zetapotential measurements showed that the materials are able to form stable suspensions in phosphate buffered saline (PBS) and serum-containing cell culture medium. ICP-AES measurements confirm Gd(III)-concentrations as determined by T1-measurements. Furthermore, MR-images of Gd-containing materials embedded in 1.5% agar were recorded with a 3T MRI human whole body scanner.

Results: The agglomeration of the SiO2NPs can be controlled by the length of the coupled spacer. High surface concentrations of Gd(III) complexes of up to 83 µmolg-1 were determined. The relaxivity per gadolinium is higher for the surface bound complexes in the hybrid materials than for the corresponding single molecules dissolved in water. T1-weighted images show a clear concentration-dependent contrast enhancement in the agar layer. Next to Gd(III) complexes, fluorophores, peptides and antibodies were coupled to the silicasurface which allows multimodal and targeting imaging.

Conclusions: We successfully synthesised, modified and characterised SiO2NPs to obtain novel functional materials for various imaging applications, such as MRI and optical imaging. It was possible to covalently modify the surface of the SiO2NPs with diverse functional groups, thus providing the opportunity to couple Gd(III) complexes, fluorophores and biomolecules such as peptides and antibodies. SiO2NPs are therefore promising candidates to not only circumvent sensitivity-issues of MRI applications but also to allow multimodal and targeting approaches.

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PHARMACOKINETICS AND BIODISTRIBUTION IN FVB MICE OF RADIOACTIVE AND FLUORESCENT TRIPLY-LABELED LIPID NANOPARTICLES

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Introduction: Nanovectorization is a promising strategy to improve tumor targeting and easily design multimodality imaging probes. Lipidots, dye-loaded lipid nanoparticles [1], have previously been described as promising tumour-targeted nanocargos for fluorescence imaging [2]. To quantitatively assess their fate in healthy or breast cancer tumour bearing mice, triply-labeled-particles, incorporating two radiotracers and a fluorophore, have been synthesized. Correlation between the three tracers biodistribution, indicative of the lipidots integrity and metabolization in vivo, is investigated.

Methods: Lipidots are lipid-core nanoparticles stabilized in aqueous phase by phospholipids and PEGylated surfactants. Cholesteryl-hexadecyl-ether-3H, cholesteryl-oleate-14C and DiD dye are incorporated into the oily phase (lipids and phospholipids), which is mixed with the aqueous phase (PEG surfactants and glycerol) before sonication is performed at room temperature for a whole 10 minutes period. Encapsulation integrity of the three tracers, particle size and size distribution are evaluated by respectively steric exclusion chromatography (SEC) and dynamic light scattering (DLS). The in vivo pharmacokinetic and biodistribution of lipidots are investigated thanks to 3H and 14C radioactivity counting and DiD fluorescence (Fluobeam® 700) in FVB female mice. Organ and cellular localization of nanoparticles is follow using DiD fluorescence by microscopy on histological sample from the mice. Passive tumor accumulation of the nanoparticles is also investigated in FVB female mice injected in fat pad with PyMT tumors breast cancer cells.

Results: More than 98% of the radiotracers and 520 molecules of DiD are encapsulated in the lipidots. Loading does not modify nanoparticle physicochemical properties: 55 ± 1 nm diameter with a polydispersity index of 0.19 ± 0.01, and long-term stability (> 5 months in PBS). The injection of the free radioisotopes in FVB mice leads to their accumulation mainly in lungs, and less extendingly in spleen and liver and for free DiD, uptake in spleen and liver is observed. The injection at the same dose of the triply-labeled lipidots leads to a different biodistribution, evidencing a nanovectorization effect. The particle integrity seems to be preserved in blood and the nanoparticles are rapidly captured by the liver (t1/2 around 30 minutes). All the 3 tracers are taken up in liver, adrenals and ovaries with similar kinetics. Using fluorescent microscopy, particular localization of the fluorescent dye is observed in ovaries and adrenals. In the PyMT model, lipidot accumulation is rapidly observed in the tumor site (around 5 h), certainly due to the Enhanced Permeability and Retention (EPR) effect of the nanoparticles.

Conclusion: Lipidots modify the biodistribution of the tracers they encapsulate. Both radioactivity and fluorescence tracers have the same biodistribution at least up to 5 hours post-injection, indicating the nanoparticle integrity for this time lapse. A specific localization is observed in steroid organs probably due to an affinity between these organs and the nanoparticle nature. Lipidots, thanks to their nanometric size, take advantage of the EPR effect and passively accumulate at tumour sites.

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IMPROVEMENT OF PROTEASE-SPECIFIC IRON OXIDE PARTICLES FOR MAGNETIC RESONANCE IMAGING

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Introduction: *In vivo* imaging of enzyme activity is currently dominated by activatable fluorescent probes. Recently we introduced protease-specific iron oxide nanoparticles (PSOP) as a novel platform for imaging protease activity by MRI. We focused on the matrix metalloproteinase family (MMP), which is up-regulated in important pathologies like atherosclerosis and cancer. PSOP are based on electrostatically stabilized superparamagnetic very small iron oxide particles (VSOP), coated with peptide-methoxypoly(ethylene glycol) (mPEG) copolymers. The peptide contains a cleavage site for MMP-2/-9. As a result of specific cleavage by MMPs, the nanoparticles rapidly lose their sterically stabilizing PEG-shell and switch from a stable low-relaxivity 'stealth' state to become adhesive, aggregating high-relaxivity particles. The purpose of this study was to improve the coating of the copolymers on the VSOP surface to extend the stability of PSOP for *in vivo* applications.

Methods: To synthesize variants of PSOP, mPEG5000 chains were covalently attached to different fluorescently labeled peptides resulting in peptide-PEG-copolymers (MMP-PEG). The copolymers were characterized by RP-HPLC and MALDI-TOF-MS. For one type of PSOP, arginine-rich peptides were used to attach the copolymers electrostatically to the citrate-coated VSOP. For the other type of PSOP, lysine-rich peptides were employed to covalently link the lysine residues to the NHS-activated citrate surface of VSOP. The extent and stability of the coating in different salt concentrations and in cell culture medium was analyzed via fluorescence quenching and hydrodynamic size measurements. To demonstrate the specific activation of MMP-9-PSOP by MMP-9, a time course of fluorescence and hydrodynamic size was measured before and after the addition of MMP-9.

Results: RP-HPLC and MALDI-TOF-MS showed that the different copolymers were successfully synthesized and purified. Incubation of PSOP with increasing salt concentrations leads to partial dequenching for PSOP coated electrostatically with MMP(Arg)-PEG, whereas the dequenching is strongly reduced for PSOP coated with MMP(Lys)-PEG via covalent linkage of the lysine residues to the citrate surface. MMP-9 specifically activated both types of PSOP resulting in aggregation and increasing fluorescence.

Conclusions: MMP-9-specific activation and inhibition confirmed the principal function of PSOP. Electrostatically coated PSOP were less stable at higher salt concentrations, indicating partial dissociation of MMP(Arg)-PEG copolymers and VSOP. The particle stability was improved for PSOP coated with the copolymer MMP(Lys)-PEG, which show a higher stability at physiological salt concentrations and in cell culture medium based on the covalent binding between the lysine residues and the citrate surface of VSOP. These promising results need to be affirmed in further experiments and *in vivo*.

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poster number: 139

67GA- AND 99MTC-LABELED SHORT NEUROPEPTIDE Y ANALOG FOR BREAST CANCER IMAGING

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Introduction: The overexpression of many peptide receptors on human tumor makes such peptide-ligands attractive agents for diagnostic and therapy of cancers. Breast carcinomas have shown extremely high incidence and density of Neuropeptide Y receptor subtype 1 (NPY Y1) whereas normal breast tissue mainly expresses Y2-receptors [1]. Thus, Y1-selective targeting became a challenging topic for the required early and selective breast cancer diagnosis. A potent and specific full-length (36 amino acids) Y1-NPY analog, labelled with ^{99m}Tc allowed clear and specific tumor images in patients with breast cancer [2]. However, smaller peptides are preferred because they usually present better labelling efficiencies and several efforts have been made to find small and specific Y1 agonists [3].

Methods: In this study, the truncated Y1-receptor selective agonist [Pro^{30},Nle^{31},Bpa^{32},Leu^{34}]NPY(28-36) (NPY1) [3] was conjugated to DOTA and to a pyrazolyl-diamine containing chelator (pz), labeled with ^{67}Ga and ^{99m}Tc, respectively. Their Y1-receptor targeting properties were also evaluated. NPY1 peptide and pz- and DOTA-NPY1 conjugates were prepared by Fmoc-based Solid Phase Peptide Synthesis, purified by HPLC and characterized by ESI-MS. Whereas pz-NPY1 conjugate was labelled using the *fac*-[^{99m}Tc(CO)$_3$]+ moiety, the ^{67}Ga was incorporated into DOTA-NPY1 conjugate using ^{67}GaCl$_3$. Both radiopeptides were controlled, purified by HPLC and characterized by HPLC comparison with the corresponding complexes prepared at the macroscopic level. Cellular uptake studies were performed using Y1-expressing MCF-7 and MDA-MB-231 breast cancer cell lines. Pharmacokinetic properties were determined using healthy mice and the evaluation of tumor uptake in MCF-7 xenografts is underway.

Results: Peptide and peptide-chelator conjugates presented a purity >95%. Both radiopeptides were obtained in a high labelling yield and fully characterized. The radiocomplex ^{67}Ga-DOTA-NPY1 was hydrophilic, whereas ^{99m}Tc-pz-NPY1 was lypophilic (Log D o/w: -1.86±0.14 versus 0.67±0.03). Both radiocomplexes were stable in vitro and bound very quickly to the receptor presenting moderate to high cellular uptake. Biodistribution data revealed that ^{99m}Tc-pz-NPY1 was excreted slowly by the hepatobiliar route while ^{67}Ga-DOTA-NPY1 a faster excretion and clearance from non-target tissues.

Conclusions: NPY Y1 receptors are a promising target for tumor imaging. This work presents the first radiolabeled short linear NPY analog prepared for tumoral Y1-targeting and warrants further study.

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IMAGING CARDIOVASCULAR DISEASES

poster number: 158

CONTRAST-ENHANCED CARDIAC MRI OF VASCULAR REMODELING AFTER MYOCARDIAL INFARCTION USING LIPID-BASED NANOPARTICLES

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Introduction: Vascular adaptations of the coronary circulation following myocardial infarction may have a negative impact on global cardiac remodeling. Therefore, *in vivo* characterization of the condition of the cardiac vasculature can yield useful insights in the progression of cardiac remodeling. In this study, the ability of paramagnetic, long-circulating nanoparticles (micelles and liposomes) to visualize the vascular remodeling process following myocardial infarction was explored. Nanoparticles are initially restricted to the intravascular compartment, but can extravasate when vascular integrity is compromised. Therefore, it is hypothesized that in acute (one day) and chronic (one week) phases of myocardial infarction, nanoparticles can report on vascular integrity as well as revascularization.

Materials and methods: *Contrast agents* - Paramagnetic, fluorescent micelles (0.05mmol Gd/kg, diameter d=20nm, r_1=6.3mM^{-1}Gd•s^{-1} at 9.4T) and liposomes (0.05mmol Gd/kg, d=125nm, r_1=3.4mM^{-1}Gd•s^{-1}) were prepared. Gd-DTPA (0.3mmol Gd/kg, r_1=3.9mM^{-1}Gd•s^{-1}) was used as a control. Blood circulation half-lives were determined in mice (n=9) by evaluating the blood longitudinal relaxation rates (at 9.4T) up to 48h after intravenous administration. *Experimental setup* - Myocardial infarction was induced in mice by permanent coronary artery ligation (n=39). In acute myocardial infarction, contrast agents were injected intravenously (i) directly after surgery, followed by MRI after 24h of circulation or (ii) one day after myocardial infarction, with MR-image acquisition up to 1.5h after administration. In chronic myocardial infarction (iii), contrast agents were administered one week after surgery and MRI was performed up to 48h post administration. *In vivo MRI* - T_1w short-axis multi-slice FLASH images were acquired at 9.4T to evaluate contrast agent distribution. Global cardiac function was determined from long- and short-axis CINE FLASH images. Infarct composition was assessed with HE and MTC stainings. Nanoparticulate fluorescence was visualized with confocal laser scanning microscopy (CLSM) and was correlated with leukocytes (CD18/CD68) and blood vessels (CD31).

Results: Circulation half-lives of micelles (3.90±0.44h) and liposomes (2.31±0.40h) were prolonged compared to Gd-DTPA (0.30±0.05h). In acute myocardial infarction, (i) administration of nanoparticles immediately after coronary artery ligation caused MR-signal enhancement after 24h of circulation. CLSM showed high affinity of micelles for necrotic myocardium, whereas liposomes were often colocalizing with blood vessels, but not with leukocytes. (ii) When contrast agents were injected 24h after myocardial infarction, micelles induced minor MR-signal enhancement within 1.5h, whereas liposome accumulation was not observed. In chronic myocardial infarction (iii), remarkable MR-contrast differences were observed between reperfused and necrotic/fibrotic myocardium immediately after administration of nanoparticles. In time, accumulation of micelles in the necrotic myocardium was detected with MRI, whereas liposomes induced only minor signal enhancement. CLSM confirmed *in vivo* MRI findings.Administration of Gd-DTPA in acute and chronic myocardial infarction led to immediate, short-duration hyperenhancement of infarcted myocardium.

Conclusion: Paramagnetic lipid-based nanoparticles have potential to provide detailed insights in the vascular remodeling process following myocardial infarction using *in vivo* MRI. In the acute phase of myocardial infarction, loss of vascular integrity led to size-dependent nanoparticle extravasation. In chronically infarcted myocardium, circulating nanoparticles illustrated the revascularization process to restore local perfusion.

poster number: 159

SYNTHESIS AND SCREENING OF IRON OXIDE NANOPARTICLES FOR ENRICHMENT IN ATHERSCLEROTIC PLAQUES

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Introduction The noninvasive diagnosis of vulnerable atherosclerotic plaques, which are responsible for most acute vascular events like stroke and myocardial infarction, is still a challenging goal in clinical practice. MRI of vulnerable plaques is possible using sterically stabilized iron-oxide nanoparticles, but not less than 24 hours after administration. Novel low molecular weight contrast-agents based on Gadolinium generate good contrast 1 hour after application, but considering their specific accumulation in potentially vulnerable atherosclerotic plaques their safety remains to be demonstrated.

Aim This project aims to make and compare different Iron oxide nanoparticles by surface-coating of iron oxide cores with different small molecules (organic acids, proteins, carboxydextran), resulting in nanoparticles with different surface properties. These nanoparticles were screened for their accumulation in atherosclerotic plaques. Due to the different coatings, there are multiple potential targets within inflamed plaques, including modified glucosaminoglycan content and migrated macrophages or other immune cells.

Methods: To synthesize the iron oxide nanoparticle library, first magnetite cores were generated by co-precipitation of iron(II)-chloride and iron(III)-chloride at a ratio of 1:2 in an alkaline environment. Next, particles were coated by adding the respective substance, each under individually adapted coating conditions. Nanoparticles were characterized by measurements of dynamic light scattering (size) and relaxivity. Nanoparticle accumulation in plaques of ApoE-KO-Mice was analyzed for 8, 12, and 16 weeks of western-type diet (0,21% cholesterol, 21% lard). Mice received a particle dose of 300 µmol Fe/kg via tail vein under isoflurane-anesthesia. After 3 hours the animals were sacrificed and perfused with ice-cold PBS followed by 4 % PFA for fixation. The aorta and descending vessels were dissected, collected, and embedded in paraffin. Histologic examinations of paraffin-sections were performed, using prussian blue staining to examine iron-oxide-nanoparticle enrichment in plaques in the aortic arch and brachiocephalic trunk, and using immunohistochemistry for various immune markers.

Results: We successfully synthesized and characterized four particles, stabilized by BSA, DMSA, malate and carboxydextran, in addition to established citrate-coated very small iron oxide particles (VSOP). We then examined plaque enrichment of these particles in ApoE-KO-mouse after different periods of feedings with the western-type diet. VSOP were present 3 hours after injection, at levels depending on the duration of western-type diet, and their accumulation correlated with several components of the plaques. Furthermore, after 12 weeks feeding with western-type diet, malate-coated iron-oxide nanoparticles accumulated specifically in plaques in the aortic arch and brachiocephalic trunk, whereas no accumulation was detected for DMSA-coated nanoparticles.

Conclusions: VSOP is the first iron oxide contrast with rapid accumulation in atherosclerotic plaques of ApoE-KO-mice. The enrichment varies with structural and inflammatory changes within the plaque. VSOP accumulates in different regions of the intima, as well as in the media depending on the feeding-period of western-type diet.

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MOLECULAR MR IMAGING OF ATHEROSCLEROTIC PLAQUES BY A SCAVENGER RECEPTOR A1 TARGETED CONTRAST AGENT

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Introduction: Several MRI studies have shown atherosclerosis can be detected both in humans and animal models. Uptake of non-targeted contrast agents (CA) in the leaky endothelium of atherosclerotic plaque gives good plaque enhancement yet without discrimination of the different plaque components. In search of targeted molecular imaging modalities for discriminative detection of inflammatory high risk plaques, we investigated the imaging potential of scavenger receptor-AI (SR-AI) which is highly expressed by lesional macrophages and linked to plaque vulnerability.

Methods: A 15-mer binding peptide for SR-A1 was identified by pComb3 phage displayed peptide library screening. USPIO coated with dextran and conjugated with our synthetic peptide or a scrambled variant were produced. The in vivo imaging potential of SR-A1 targeted USPIOs was investigated both in aged ApoE-/- mice and LDLr-/- bone marrow chimeras with leukocyte SR-AI deficiency or expression of human SR-AI. MRI of the aortic arch was performed on a 9.4 T vertical Bruker system, 24 hours after intravenous injection of USPIO using a cine MRI FLASH sequence.

Results: We successfully identified a highly specific peptide with nanomolar affinity for human. SR-AI. High resolution MRI performed 24 hours after i.v. injection of USPIO in ApoE-/- mice with advanced plaques showed enhanced uptake of the P03 SR-A1 USPIO compared to the passive uptake of the scrambled variant. LDLr-/- mice were transplanted either with SR-AI deficient bone marrow or bone marrow with expression of human SR-AI in lysM1 macrophages. These latter mice showed a significant improvement in contrast-to-noise ratio for the plaque in aortic arches whereas the chimeras transplanted with SR-A1 deficient bone marrow only showed only moderate uptake, comparable to passive uptake of the scrambled USPIO in ApoE-/- mice.

Conclusions: Our data indicate that SR-AI targeted molecular imaging based on phage display derived binding peptides holds great promise for diagnosis of inflammatory plaques in manifest atherosclerosis.

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MINOCYCLINE INHIBITS CELL DEATH IN VIVO IN MURINE MODELS OF MYOCARDIAL ISCHEMIA AND REPERFUSION: EVALUATION WITH ANNEXIN A5 IMAGING

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Introduction:In the treatment of acute myocardial infarction (AMI), reperfusion therapies strongly reduce myocardial damage. However, they also cause ischemia/reperfusion (I/R) injury(1). Apoptosis, a form of programmed cell death, is central in I/R injury and therefore forms a target for therapeutic reduction of I/R injury(1). Minocycline (Mc) is a widely used, safe antibiotic with anti-inflammatory and anti-apoptotic properties(2). In cerebral I/R, Mc treatment at reperfusion reduces apoptosis(2). In-vivo imaging with radio-labeled or fluorescently-labeled Annexin A5 (AA5) can be used to measure myocardial apoptosis(3;4). The aim of this study was to determine whether treatment with Mc upon reperfusion reduces myocardial apoptosis after ischemia I/R injury-induced apoptosis. In-vivo and ex-vivo AA5 imaging were used to measure apoptosis.

Methods:Myocardial I/R was induced in mice (N=24) and rabbits (N=6) infarct models for 30/60 minutes and for 40/210 minutes, respectively. Mc was administered to subgroups of animals upon reperfusion. 99mTc-labeled AA5 was administered in the rabbits for ex-vivo SPECT imaging. In mice, Alexa568-labeled AA5 or 99mTc-AA5 was administered upon reperfusion and in-vivo fluorescence or SPECT imaging was performed subsequently. In a subset of mice, histopathologic characterization of the myocardial injury was subsequently undertaken.

Results: In Mc-treated mice (N=3), Alexa568-AA5 was approximately 60% lower at 60 minutes when compared with untreated mice (N=3). In a separate series of mice, absolute quantification by SPECT imaging showed a 40% lower 99mTc-AA5 uptake in treated mice (N=6, 1.31±0.32%ID/g) as compared to the control mice (N=6, 2.15±0.52%ID/g). In myocardial tissue sections of these mice, TUNEL staining revealed a 70% reduction of the percentage of apoptotic cells (0.16 ± 0.10% vs 0.54 ± 0.32% $P < 0.05$). In rabbits, there was a striking reduction in the radiotracer uptake in the treated animals (N=3) compared to the control animals (N=3) in-vivo SPECT imaging and the gamma counting showed 42% lower 99mTc-AA5 uptake in the myocardial area at risk in the treated (0.38±0.07%ID/g) vs untreated rabbits (0.65±0.1%ID/g).

Conclusions**:** Mc treatment at reperfusion reduces myocardial I/R-induced apoptosis. We recommend initiation of clinical trials in patients with myocardial infarction to determine whether addition of minocycline to standard reperfusion therapy improves outcomes.

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NON-INVASIVE MONITORING OF ANGIOGENESIS AFTER STROKE WITH MRI

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Introduction: Angiogenesis in the brain is up-regulated in response to stroke (1) and a few studies have attempted to image this process with magnetic resonance imaging (MRI). For example, using steady-state contrast enhanced MRI (SSCE-MRI), changes in microvessel density following permanent middle cerebral artery occlusion (MCAO) were observed in rats (2). The primary aim of the present project is to characterize a time course of this response in a transient stroke mouse model. An additional strategy to look at angiogenesis uses transgenic mice that over-express a hemagglutinin (HA) epitope tagged ferritin (Ferr) MRI reporter in response to activation of the vascular endothelial cadherin (VECad) promoter (3).

Methods: 13 male adult VECad-HaFerr mice and 17 age-matched wild type FVB mice received 30min of MCAO using the intraluminal filament technique. MRI was performed 7 days before, and 14 and 21 days after MCAO. The SSCE-MRI protocol included a combination of T2 and T2* sequences before and after injection of contrast agent (Sinerem, 30 mg/kg i.v.), as well as a diffusion-weighted MRI scan. Regions of interest (ROIs) were drawn on theT2 maps (infarct core and peri-infarct zone (PIZ)) and copied into the corresponding relaxivity (R2) and vessel density (Q) maps (4). Animals were sacrificed at day 21 and brain tissue was processed for immunohistochemistry.

Results: Prior to stroke R2 was significantly higher in the hippocampus, but not in the cortex or striatum, of VECad-HAFerr mice ($t(28) = -2.812$, $p=0.009$). MCAO produced extensive lesions, therefore final group sizes were reduced (n=5 wild type, n=2 VECad-HAFerr). There were no significant changes in microvessel density (quantity Q) over time in either region. However, R2 decreased significantly after stroke in both the infarct core ($F(2,10) = 36.934$, $p<0.0001$) and PIZ ($F(2,10) = 21.312$, $p<0.0001$). Interestingly, R2 was slightly higher in the PIZ of the VECad-HAFerr mice. Immunohistological analysis showed dilated microvessels in the ischemic hemisphere compared to the contralateral side but actual microvessel density counts are pending.

Conclusions: SSCE-MRI derived measurements of microvessel density (Q) did not show changes in the infarct core or PIZ after MCAO in mice. This could partially be due to the inherent image noise in the Q maps, particularly since changes in R2 were observed in the infarct core. It appears that higher R2 values are present in VECad-HAFerr mice, but more animals are needed to conclude this.

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poster number: 163

SYNTHESIS AND FUNCTIONALIZATION OF CONTRAST PARTICLES FOR MOLECULAR IMAGING OF ATHEROSCLEROSIS

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Introduction Atherothrombosis results from the aggregation of activated platelets following plaque rupture[1]. This occurs mostly without any warning signs, leading to myocardial infarction and stroke, a leading cause of worldwide mortality and morbidity. Despite considerable progress in biomarker and imaging technologies, definitive detection of vulnerable, rupture-prone plaques has not been achieved yet. Targeting of contrast agents to vulnerable atherosclerotic plaques offers the potential to identify such plaques before rupture, allowing suitable interventions and thus avoiding myocardial infarction and death. Currently, targeting can be achieved via chemical conjugation to specific antibodies, which typically results in the loss of antibody functionality[2]. We have designed and functionalized contrast imaging particles using a unique biotechnology approach which retained the antigen binding activity of the single-chain antibody and resulted in the successful targeting of contrast agents to atherosclerosis.

Methods 30-nm gold nanoparticles (AuNPs) were prepared by reducing HAuCl4 with sodium citrate. Different AuNPs were developed via chemically coating AuNPs with (1) modified heparin; (2) modified bovine serum albumin, or (3) modified dextran. Near infrared dyes were also incorporated on the coating to facilitate the binding studies. Iron-oxide microparticles (IOMP) were purchased from Invitrogen. GGG peptides were introduced to AuNPs and IOMPs using different chemical techniques. Anti-GPIIb/IIIa single-chain antibodies (scFv) containing LPETG motifs were site-specifically conjugated to AuNPs and IOMPs by condensation reaction between NH2-GGG- and -LPETG-COOH via Staphylococcus aureus sortase A enzyme. The scFv specifically binds to activated platelets which play a pivotal role in atherosclerosis, thrombosis and inflammation[3,4]. Targeting functionality of AuNPs and IOMPs was evaluated in both in-vitro and in-vivo studies.

Results The scFv was successfully conjugated to AuNPs and IOMPs (contrast agents for computed tomography and magnetic resonance imaging). The conjugation efficiency was between 50-70% and the bioactivity of the scFv after coupling was preserved. The targeting of scFv-coupled contrast particles to activated platelets was strong and specific as demonstrated in in-vitro static adhesion assays, in a flow chamber system under shear stress, in in-vitro human thrombus binding study and in mouse intravital microscopy.

Conclusions We have successfully synthesized and labelled Imaging contrast particles with scFv in a site-specific manner using a unique chemo-enzymatic approach, a versatile and broadly applicable tool for procuring targeted molecular imaging in cardiovascular, inflammatory diseases and beyond.

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poster number: 164

CHEMICAL SHIFT IMAGING OF CARDIAC METABOLISM WITH HYPERPOLARIZED [1-13C]PYRUVATE IN MIDDLE SIZE ANIMAL MODEL

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Introduction: Hyperpolarization of nuclear spins and their use in injectable solutions is a novel techniques in NMR spectroscopy and imaging. MRI with hyperpolarised 13C represents a promising modality for in-vivo spectroscopy and provides a unique opportunity for the non-invasive assessment of regional cardiac metabolism. In this work a 3D-IDEAL spiral CSI and hyperpolarized [1-13C]pyruvate were used to obtain spatially and spectrally resolved information of cardiac basal metabolism in middle size animal models at 3T.

Methods: Imaging experiments were performed on a 3T GE Signa HDx scanner (GE Healthcare) using a custom-made 13C quadrature birdcage coil (Rapid Biomedical). Three healthy male farm pigs (38±2 kg) were studied in basal condition, after injection of [1-13C]pyruvate, polarized with an HyperSense DNP polariser (Oxford Instrument).The preparation of a large dose of [1-13C]pyruvic acid was optimized for bolus injection in pigs following a three-step process: 1) Hyperpolarization 2) Fast Dissolution 3) neutralization and final formulation (final volume=21 mL): the final injectable solution contained 230 mM sodium [1-13C]pyruvate, 100 mM TRIS buffer, 0.11 mM Na2EDTA and 20 uM Dotarem). The injected [1-13C]pyruvate dose was 0.13 mmol/kg body weight. Anatomical reference images were acquired in the axial plane using a standard steady-state free precession (SSFP) sequence. Metabolic information covering the whole heart was obtained using 3D IDEAL spiral CSI prescribed on the same region imaged by the anatomical reference scan (FOV= 30x30 cm2, slab thickness=100mm), starting 20 s after the beginning of the injection. The IDEAL spiral CSI was prescribed with 11 echo times, a constant relative echo time spacing of 0.9ms, and constant FA = 7°. 3D image encoding was performed in form of a stack of planar, single-shot spirals (FOV= 30x30 cm2, matrix resolution=38, BW=62.5kHz) with standard phase-encoding along the z-direction. Data were reconstructed using spectrally-preconditioned CS inversion followed by gridding reconstruction implemented in Matlab. Image reslicing along cardiac short axis (SA) views and image fusion of 13C metabolite maps and anatomical 1H reference images were performed by PMOD software.

Results and discussion: Representatives map of bicarbonate, lactate and pyruvate in SA orientation through the heart have been produced. Pyruvate uptake and metabolic products bicarbonate and lactate are differentially localized in the heart: strong pyruvate uptake is localized in the chambers while lactate and bicarbonate are converted in the myocardium. The most intense myocardial uptake has been selected designing a ROI, and the spectroscopic mean intensity signal of bicarbonate and lactate normalized by the mean intensity signal of pyruvate in the same region have been assessed.

Conclusions: This ongoing study demonstrates the feasibility of whole-heart 13C cardiac metabolic imaging in pigs using 3D IDEAL spiral CSI at 3T. The proposed method could be applicable for the development and characterization of new pathophysiological models to investigate the mechanisms underlying cardiac disease and for the assessment of the pharmacokinetic properties of suitable 13C-enriched tracers for MRS.

poster number: 165

ENDOTHELIAL GAP JUNCTIONS PROVIDE A MEANS FOR THE EXPANSION OF LOCAL OXIDATIVE STRESS AND APOPTOSIS: A MODEL STUDY BASED ON CULTURED ENDOTHELIAL CELLS

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Background: Reactive oxygen species (ROS) have very short lifetimes and cannot propagate more than a few microns in the biological milieu(1-3). Yet, propagation of localized oxidative insults to distant sites characterizes important pathophysiological activities in plants and animals(2-7). It was recently proposed that ROS produced by one plant cell acquire auto-propagating ability to distal sites by stimulating ROS production in a bystander cell through extracellular H_2O_2 receptors(8-9). Here we explore the possibility that ROS mediated oxidative insult in animal pathophysiological activities, can auto-propagate to distal sites via gap junction intercellular communication (GJIC) among endothelial cells (ECs).

Methodology: A transient burst of $O2^{\bullet-}$ and •OH, was photogenerated in a geometrically confined (300X300?um) population of cultured ECs (bEnd.3 and H5V lines) pre-incubated with the photosensitizer WST11(10). Subsequent generation of ROS, reactive nitrogen species (RNS), Ca^{+2} level elevation, membrane integrity and apoptosis in the illuminated and non-illuminated regions were monitored at sub-cellular resolution by time-lapse fluorescence microscopy in wild type and genetically modified cells during 24h post illumination.

Results: A few minutes post illumination the illuminated cells underwent rapid necrosis. Slightly later, waves of calcium elevation, de-novo generation of ROS and RNS, accompanied by activation and nuclear translocation of c-Jun N-terminal kinase and massive apoptosis, started in bystander, non-illuminated cells. These secondary processes auto-propagated in a radial fashion to 700-800 um from the primary oxidative insult in the following 24 hours. The propagation was inhibited by chemical or genetic blockers of GJIC (7, 11-12) but not by extracellular ROS scavengers such as catalase.

Conclusions/Significance: Our study shows that cytosolic signal transduction through intercellular GJ among ECs provide a means for auto-propagation of ROS, RNS, and cell apoptosis. Since GJs in-vivo also connect ECs to myocytes, our observations suggest new approaches for exploring the etiology of major vascular diseases involving oxidative insult expansion and related therapeutics.

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poster number: 166

ACCURACY AND REPRODUCIBILITY OF NONINVASIVE MEASUREMENT OF INFARCT SIZE IN MICE WITH HIGH-RESOLUTION PET/CT

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Introduction: Positron emission tomography (PET) with ^{18}F fluorodeoxyglucose (FDG) is increasingly used for noninvasive evaluation of myocardial pathophysiology in small animals[1-2-3]. Accurate and reproducible noninvasive measurement of infarct size in mice with PET would benefit studies designed at the exploration of biochemical and functional changes associated with acute myocardial infarction (MI). We assessed the accuracy and reproducibility of ^{18}F-FDG for noninvasive measurement of infarct size in mice using a high-resolution animal PET/CT system.

Methods: C57BL/6 outbred wild-type mice were studied by ^{18}F-FDG PET/CT one week after induction of MI (n = 17) or sham procedure (n = 20)[4]. Imaging was performed 45 min after intravenous administration of ^{18}F-FDG (7.5 MBq) using a high-resolution dedicated PET/CT (eXplore Vista, GE Healthcare) with a PET spatial resolution of 1.6 mm FWHM and a CT spatial resolution of 0,2 mm. PET data were reconstructed using a 2D-OSEM including random and scatter corrections, and attenuation correction using CT data. Volumetric analysis was used to measure infarct size expressed as area/cm^2 and % area map. A subset of 20 mice (16 of MI group and 4 of sham group) was sacrificed immediately after PET/CT scan to perform histological analysis.

Results: The interobserver and intraobserver variability for infarct size measurement did not show any significant difference between examiners for both PET/CT and histological analysis. The correlation between the measurements (Spearman's coefficient of correlation) and their reliability (intraclass coefficient of correlation) were excellent (>0.9) for all the measurements. A high correlation between PET/CT and histology was found for measurement of infarct size ($R = 0.98$, $P < 0.001$), with linear regression curves close to unity (y = 1.06x 0.37). At Bland-Altman analysis[5], the mean difference between PET/CT and histology was 0.19 (95% confidence interval 0.09-0.28) and the lower and upper limits of agreement were -0.21 and 0.59, respectively.

Conclusion: Noninvasive measurement of infarct size in mice with high-resolution PET/CT has high accuracy and excellent intraobserver and interobserver reproducibility. This noninvasive methodology might allow longitudinal studies of cardiac biochemical parameters and facilitate studies to assess the effect of intervention after acute MI.

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FOLATE RECEPTOR IMAGING AS A MEASURE OF INFLAMMATION TO ESTIMATE VULNERABILITY WITHIN THE ATHEROSCLEROTIC CAROTID PLAQUE

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Background: Plaque inflammation is an important process in atherosclerotic plaque development. Active inflammation includes the accumulation of macrophages at sites of plaque rupture. The folate receptor ß is present on activated macrophages but not on quiescent macrophages or other immune cells. By labeling the folate receptor ß with an optical contrast fluid, activated macrophages can be visualized by optical imaging. The aim of this study is to distinguish vulnerable sites from more stable regions within the plaque by optical imaging.

Methods: Carotid specimens were taken from a total of 20 patients. Ex-vivo near-infrared fluorescence imaging was performed to determine the exact location where the folate/FITc uptake had taken place. Biopsies with high uptake (hot spots) were compared with biopsies with low uptake (cold spots) through immunohistochemistry and real time quantitative reverse transcription PCR.

Results: Hot spots showed a significantly higher folate/FITc uptake than cold spots. Significantly more macrophages and areas of hypoxia were found in hot spots compared with cold spots. In atherosclerotic plaques we found a positive correlation between mRNA levels of CD68 (marker for macrophages) on the one hand, and folate receptor ß and HIF-1a expression on the other hand.

Conclusion: In areas of high folate/FITc uptake, more macrophages and higher levels of hypoxia were present compared to areas with low uptake. It is therefore anticipated that NIRF molecular imaging might be a good indicator for plaque vulnerability.

poster number: 168

DETECTION OF ACTIVATED PLATELETS IN CAROTID ARTERY THROMBOSIS IN MICE WITH RADIOLABELED SINGLE CHAIN ANTIBODIES FOR PET

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Activated platelets are key players in thrombosis and inflammation and can be found in intravascular thrombosis. We previously generated a single-chain (ScFv) antibody that specifically binds to the highly abundant platelet glycoprotein integrin receptor IIb/IIIa in its activated, ligand-bound form (anti-LIBS)1. The antibody is specific for activated platelets and the small size of this recombinant fragment makes it particularly suitable for molecular imaging.

Here we describe the construction of a novel PET radiotracer targeting the LIBS-epitope on activated platelets. We hypothesize that this will allow selective and sensitive detection of platelet activation in thrombosis and inflammation as activated platelets play a major role in the development of vulnerable plaques, both in the early stages of plaque development and the formation of thrombi2.

The LIBS scFv was conjugated to N-succinimidyl 4-[18F]fluorobenzoate (S[18F]FB), an NHS ester PET prosthetic group. LIBS scFv was reacted with 6.25 mCi of SFB and separated from free SFB by filter centrifugation. The radiolabeled antibody was injected into mice with an in vivo formed thrombus formed in the carotid artery by topical application of a 5% ferric chloride solution. Biodistribution studies conducted on the mice show fast uptake of the radiotracer in the target thrombus and also fast clearance from the circulatory system. This novel radiotracer might be suitable for detecting activated platelets which typically line the surface of unstable, vulnerable, rupture-prone plaques. This radiotracer can potentially provide information about the morphology of the vessel wall. The potential to determine if or where patients have vulnerable plaques would allow implantation of prophylactic stents to stabilize fragile plaques and the initiation of aggressive medical treatment for primary/secondary prevention.

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poster number: 169

IN VIVO R2 MAPPING OF SPIO-LABELED MSC VIABILITY IN THE RAT HEART WITH BIOLUMINESCENCE VALIDATION

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Purpose: To assess in vivo the proliferation and viability of Superparamagnetic Iron Oxides (SPIO) labeled adult rat mesenchymal stem cells (rMSC) implanted in the rat heart during open heart surgery. To generate R2 MRI maps of SPIO-labeled rMSCs, allowing a correlation of (de)compartmentalization effects with cell viability, validated by bioluminescence. To show the feasibility of R2 mapping of the rat heart at 7T at a single cardiac phase, overcoming the challenges due to the very short cardiac resting phase.

Material and methods: rMSC transduced to constitutively express Firefly luciferase (Fluc) were labeled with SPIO (215µg Fe/ml) for 24h. 5x105 viable or non viable (from the same labeling stock, obtained by repeated freeze-thawing) rMSC were injected in the heart of Wistar rats during open heart surgery. BLI was performed after administration of D-Luciferin (100mg/kg). MRI images were acquired by using FSE black blood sequences.

Results: Animals underwent BLI and MRI imaging at days 2, 5, 7, 10 after cell implantation. BLI showed proliferation of implanted rMSC up to 7 days (15-fold increase) while corresponding R2 maps reported decreasing R2 values (0.02 ?0.004 ms-1). Reduced viability of cells, probably due to immune response, was observed at BLI on day 10 after implantation and it was associated with increased R2 values (0.04?0.004 ms-1). R2 values of implanted dead cells on day 2 corresponded to 0.06?0.003 ms-1.

Conclusion: R2 mapping of SPIO-MSCs in the rat heart at 7T is a feasible and robust technique. Changes in R2 values can be associated either to proliferation, upon dilution of SPIO and cell viability. Clinical relevance/application: Quantification of R2 of SPIO-labeled MSCs may prove a valuable tool to evaluate the cell fate of intramyocardial cell therapy non-invasively.

poster number: 170

PARAMAGNETIC PHOSPHATIDYLSERINE-CONTAINING LIPOSOMES FOR MR IMAGING OF MACROPHAGES IN MOUSE CARDIAC ISCHEMIA/REPERFUSION INJURY

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Introduction: After myocardial infarction, inflammatory cells infiltrate the injured myocardium. The aim of this study was to visualize with MRI the presence of inflammatory cells after cardiac ischemia/reperfusion injury in the mouse. For this purpose, paramagnetic and fluorescently-labeled liposomes were developed, in which the negatively charged phospholipid phosphatidylserine (PS) was incorporated to promote phagocytosis by inflammatory cells.

Methods: *Contrast agents* - Liposomal formulations containing 0, 6, 12 or 37 mol% of PS (named PC-L, PS-6-L, PS-12-L and PS-37-L respectively) were prepared. Hydrodynamic diameters were assessed by dynamic light scattering. Relaxation properties were measured at 9.4T. *In vitro* - Murine macrophages (RAW264.7) were incubated with the liposomal formulations (0.25mM Gd, 2h) and uptake was studied with MRI, inductively coupled plasma mass spectrometry (ICP-MS), confocal laser scanning microscopy (CLSM) and fluorescence activated cell sorting (FACS). *Blood circulation half-lives* - Blood samples were taken from Swiss mice (n=3/group) before and up to 24h after injection of PS-6-L or PC-L (0.05mmol Gd/kg). To calculate blood circulation half-lives, changes in blood R_1 with time, measured at 9.4T, were fitted with a bi-exponential decay function. *In vivo MRI* - Cardiac ischemia/reperfusion was induced in Swiss mice (n=4/group) by transient ligation (30min) of the left coronary artery. After three days, cardiac T_1-w short-axis multi-slice FLASH images were acquired at 9.4T, before and up to 24h after injection of PS-6-L or PC-L (0.05mmol Gd/kg). Regions of interest were drawn and the signal change in the infarct area normalized to remote tissue (NSC) was calculated. After MRI, mice were sacrificed and hearts were excised for CLSM.

Results: *Contrast agents* - Relaxivity r_1 values of all liposomal formulations were similar ($3.2\pm0.6 mM^{-1}s^{-1}$). With increasing molar percentages of PS, the diameter of liposomes decreased from 162nm to 112nm. *In vitro* - ICP-MS of macrophages revealed that PS-6-L resulted in the highest Gd^{3+} uptake. MRI, FACS and CLSM confirmed highest uptake for PS-6-L as well. Therefore, PS-6-L was used for subsequent in vivo studies. *In vivo* - Similar short blood circulation half-lives were found for PS-6-L and PC-L (0.4±0.2h and 0.5±0.2h respectively; p=NS). However, long blood circulation half-lives were significantly shorter for PS-6-L (2.0±0.9h versus 4.0±1.1h; $p<0.05$). At 3 days after ischemia/reperfusion, different NSC versus time curves were found for individual mice during the first 2.5h after injection of PS-6-L or PC-L. Preliminary CLSM results revealed specific accumulation of PS-6-L in macrophages 2.5h after administration, while for PC-L this was not seen. When MRI was performed 24h after injection, signal enhancement and positive NSC values (22% and 27% respectively) were observed for both PC-L and PS-6-L. At 24h CLSM revealed aspecific accumulation in infarcted myocardium and some co-localization with macrophages.

Conclusion: In vitro, PS-6-L displayed the highest uptake by macrophages. In mice with cardiac ischemia/reperfusion, variable MRI results were found for PS-6-L and PC-L during the first 2.5h after administration. Importantly, CLSM confirmed specific association of PS-6-L with macrophages present in the infarcted myocardium. Currently, the correlation between MRI signal and the number of macrophages in the infarct area is under investigation.

poster number: 171

ATHEROSCLEROTIC PLAQUE INVESTIGATION IN APOLIPOPROTEIN E-NULL (APOE-/-) MOUSE USING MRI AND HISTOLOGY FOLLOWING ADMINISTRATION OF B22956/1

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Introduction**:** The brachiocephalic artery is a site where ruptured plaques have been reported to occur in the apolipoprotein E -/- (apoE-/-) mouse. In the field of atherosclerosis this observation is of major significance, since acute events such as myocardial infarction or stroke result from plaque rupture. Aim of the study was the identification by MRI of plaques occurring in the brachiocephalic artery at different time points of high fat diet. In particular, the efficacy of the blood pool contrast agent (CA) B22956/1, a gadolinium complex containing a deoxycholic acid moiety as albumin-binding promoter, in enhancing the MRI sensitivity for plaque detection was investigated.

Methods**:** ApoE-/- mice were maintained for 8 or 12 weeks on a high fat diet (21% fat, 0.15% cholesterol). At the end of the dietary treatment, MR images were acquired before and after i.v. administration of 0.1 mmol/kg B22956/1. Animals were then sacrificed and brachiocephalic arteries were removed. Serial paraffin sections were stained with hematoxylin and eosin and Masson's Trichrome. Immunohistochemical staining specific for smooth muscle cells and macrophages were used to fully characterize the cell composition of the atherosclerotic plaques. The MRI signal intensity of the plaques was calculated using the post versus pre contrast injection signal enhancement of the plaque (*Signal Enhancement %*) in a defined region of interest (ROI) and the percent of contrast between the plaque and muscle ROIs (*Plaque to Muscle Contrast %)*. All the images were also scored by experts voters, required to determine if the plaque was visible in the image or not (*Consensus Vote*).

Results**:** Plaque volumes in apoE-/- mice sacrificed after 8 weeks of diet were similar to that detected in mice sacrificed after 12 weeks. Concerning the relation between the experts semi-quantitative characterization of the plaque and the quantitative MRI signal parameters, the best correlation was observed between *Plaque to Muscle Contrast %* and *Consensus Vote*. Moreover, starting from 30 minutes after CA injection, *Plaque to Muscle Contrast %* showed significantly higher values in animals with histologically confirmed plaques with respect to the non-histologically confirmed ones. Anyway, the unequivocal identification of the animals which developed atherosclerotic plaque by visual inspection of the MRI images was not possible. Thus, the distribution of the histological parameters in detectable and undetectable plaques was compared in order to identify if the MRI identification could be related to a specific interaction between the plaque and the contrast agent. Detected plaques showed significantly lower *% Extracellular Matrix Area* with respect to the undetected plaques (p-value < 0.05, Student's t-test). Moreover, a statistically significant correlation between *Plaque to Muscle Contrast %* and the *% Stenosis* at 60 minutes after CA injection was found (corr = 0.667, p-value < 0.05, Pearson Correlation).

Conclusions**:** The results showed that in our experimental conditions, B22956/1 is able to enhance the MRI sensitivity in detecting atherosclerotic plaques characterized by a low content of extracellular matrix, i.e. plaques at elevated risk of rupture.

poster number: 172

SMALL DIFFERENCES IN LEFT VENTRICULAR FUNCTION IN MICE: SENSITIVITY OF CONVENTIONAL IMAGING TECHNIQUES COMPARED

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Introduction: Many strategies for cancer therapy, like radiotherapy, are associated with an increased risk for the development of cardiovascular diseases. Early detection of small changes in left ventricular (LV) function is vital, for the earlier an abnormality can be detected, the sooner treatment can be adapted to minimise the risk of cardiac failure. Non-invasive imaging strategies for the assessment of LV function in mice have seen a vast increase in application over the past few decades. Our aim was to compare the parallel outcomes of three cardiac functional imaging techniques by looking at the similarity of LV functional parameters.

Methods: Mice received local irradiation to the heart with a dose of 16 Gy or 0 Gy (controls), and were imaged 20 weeks after treatment. Imaging was performed with classic 2D-Echocardiography, and two conventional methods of LV function measurement with SPECT: cardiac perfusion gated SPECT (99mTc-tetrofosmin) and cardiac blood pool gated SPECT (99mTc-HSA). B-mode short- and long-axis recordings were made with 2D-Echo and were manually analysed. The four-headed, dedicated small-animal SPECT/CT system from Bioscan, Inc. was used for gated SPECT acquisition and data were analysed using automated algorithms.

Results: With each of the three techniques we were able to demonstrate a significant decrease in LV end diastolic and systolic (LVED, LVES) volume in the irradiated animals, compared to the controls. However, the differences in LVED and LVES measured by 99mTc-HSA were less pronounced, therefore only 2D-Echo and 99mTc-tetrofosmin indicated significant increases in LV ejection fraction (LVEF) in irradiated mice. It was not possible to measure wall thickening with 99mTc-HSA, due to the characteristics of this tracer, but when measured with 2D-Echo and 99mTc-tetrofosmin, wall thickening in both the anterior and posterior wall of the LV was markedly increased after irradiation, to a very comparable degree.

Conclusion: 2D-echocardiography, 99mTc-tetrofosmin gated SPECT and 99mTc-HSA gated SPECT can reliably be used to detect small differences in LV function, however, 2D-Echo and 99mTc-tetrofosmin showed greater sensitivity than 99mTc-HSA. A general advantage of gated SPECT is that data is analysed in an operator-independent way, as opposed to the manual analysis of 2D-Echo data. Of all three modalities, 99mTc-tetrofosmin has the additional advantage of providing the most functional information in a single scan. For future strategies of investigating cardiotoxicity in early stages in genetic mouse models of cardiac dysfunction and/or after anti-cancer treatment, 99mTc-tetrofosmin is a promising and sensitive tool for use in screening methods of therapy-induced cardiotoxic effects in small animals.

poster number: 173

EFFECT OF INTRACORONARY INJECTED BONE MARROW STEM CELLS TO THE LEFT VENTRICULAR EJECTION FRACTION

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Objectives: Bone marrow stem cells (BMSC) may potentially be used for regeneration of damaged myocardium. We present our initial experience with the change of left ventricular function in patients post BMSC implantation.

Methods: We studied 4 patients with coronary artery disease, 3 men (50, 57 and 67 y/o) and 1 woman (50 y/o). Two pts were post acute MI (3 and 9 days, both men) and 2 with remote MI. 20% of injected BMSC were labeled with 20 mCi of 99mTc HMPAO and mixed with the remaining cells. They were injected in the total volume of 25 to 42 ml (2.5 x 108 mononuclear cells on average) into the left anterior descending coronary artery via angioplastic catheter during PTCA. The amount of cells remaining at the site of infarction at 2 and 20 hours post injection was calculated in percent of injected activity with appropriate corrections. Ejection fraction was measured by echocardiography before and 4 to 5 months after procedure.

Results: In the two men post AMI, there were 4.5% and 3.7% of injected BMSC at 2 hours, respectively declining to 1.34% and 1.9% at 20 hours post injection. Their LV ejection fraction at 4 months improved from 35% and 45% to 50% and 60%, respectively. In the man with remote MI, there was 1.6% BMSC at the site of infarction at 2 hours with decline to 0.4% at 20 hours. His LV EF at 5 months post implantation improved from 28% to 45%. In the woman with remote IM, there was 0.4% of BMSC at 2 hours and 0.17% at 20 hours post injection and her LVEF at 5 months post procedure remained unchanged (35%).

Conclusion: Based on our preliminary data on 4 pts, we can speculate that homing of sufficient amount of BMSC at the site of infarction can improve subsequent LV function. On the other hand, if the amount of BMSC at the site of infarction is low, no improvement could be expected.

poster number: 174

99MTC-MIBI-SPECT/CT USED FOR IMAGING MYOCARDIAL PERFUSION DEFECTS AFTER RADIOTHERAPY FOR LEFT-SIDED BREAST CANCER

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Purpose: To evaluate radiation-induced defects of myocardial perfusion after radiation therapy for left-sided breast cancer using 99mTc-MIBI-SPECT/CT (single photon emission computerized tomography - computed tomography).

Material/Methods: Two to four years after radiation therapy for left-sided breast cancer 12 patients received an myocardial rest/stress perfusion 99mTc-MIBI-SPECT/CT because of cardiovascular risk factors (diabetes mellitus, high blood pressure, elevated serum cholesterol, cigarette smoking, adiposity, positive family disposition). The findings are evaluated for clinical consequences and radiation-induced defects in myocardial perfusion. The 99mTc-MIBI-SPECT/CT images were matched with the computed tomography (CT) images performed for 3D-treatment planning.

Results: The patients have received a 3-dimensional (3-D) planned LINAC radiation therapy to a total dose of generally 50 Gy whole breast irradiation, in a single fraction of 2 Gy, 5x/week, plus 10 Gy electron boost (5 x 2 Gy/week). In one third of the patients (4/13) myocardial perfusion defects were observed. All abnormalities were confined to the cardiac apex. In this area the heart achieved the maximum dose. In the studied patients these findings did not correlate with clinical symptoms of coronary artery disease.

Conclusion: Myocardial perfusion abnormalities in patients treated for left sided breast cancer occur. We are currently evaluating the frequency of these abnormalities their dose dependence, influence of cardiovascular risk factors and the clinical significance of these abnormalities.

ANIMAL MODELS

poster number: 101

DPA-713 A PERIPHERAL BENZODIAZEPINE RECEPTOR LIGAND REVEALS THE PRESENCE OF FOCAL NEUROINFLAMMATION ASSOCIATED WITH MS-LIKE LESIONS AND METASTASIS IN THE BRAIN

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Introduction: The reliable detection and quantification of Peripheral Benzodiazepine Receptor (PBR) induction in microglia associated with MS lesions is recognised as an important goal, but we have recently discovered that microglial activation is also an early event associated with brain metastasis. Thus PBR binding may provide a means for the early detection of brain metastasis. [11C]-DPA-713 exhibits a higher signal-to-noise ratio than [11C]-PK11195, which is the archetypal PBR ligand, and is an important candidate for clinical studies as it is likely to provide more accurate quantification of PBR induction. DPA-713 also lends itself to the development of a more versatile multiplatform ligand as [124I]-DPA-713 can be used for PET and [123I]-DPA-713 for SPECT in clinical studies.

Objectives: The aim of the present study was to determine whether [125I]-DPA-713 would detect microglial activation in the focal (pattern I) Th1-type DTH model of MS and whether we could detect focal and disseminated brain metastases.

Methods: Animals were injected stereotaxically into the left striatum with heat-killed BCG or with 5,000 4T1 breast cancer cells. Intracardiac injections of 10,000 4T1 cells were used to generate disseminated brain metastases. Four weeks after the intrastriatal injection of BCG, a focal DTH lesion was initiated by an intradermal injection of BCG in CFA. PBR induction was assessed by the binding of the TSPO ligand [125I]-DPA-713 on ex vivo 10µm-thick cryosections.

Results: [125I]-DPA-713 binding revealed the presence of DTH lesions at day 12 and day 19 after the initiation of the lesion. The binding was unilateral in the striatum. However, strong binding was observed in association with the ependymal layer in both hemispheres in naïve animals and animals with lesions. Five days after the injection of 4T1 cells into the brain the presence of a focal tumour (area in plane ca. 0.008 mm2) could be easily resolved with the [125I]-DPA-713 ligand. As tumour associated microglial activation is more widespread than the tumour itself, the volume of DPA-713 ligand binding is considerably larger. We were also able to detect disseminated metastases at day 10 after intracardiac injection of 4T1 cells.

Conclusions: Our results show that DPA-713 detects the presence of immune mediated neuroinflammatory disease as expected. However, this ligand also provided the means to detect the early presence of brain metastasis as a consequence of the associated microglial activation.

poster number: 102

JUVENILE DEVELOPMENT AND AGEING MEDIATED CHANGES IN CORTICAL STRUCTURE AND VOLUME IN THE RAT BRAIN

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Introduction. Animal models can provide mechanistic insight into disease pathology and evolution, as well as a platform to test potential therapies. However, most pre-clinical research employs juvenile, 3month old (300g) animals, which is not necessarily representative of the natural disease state. We are conducting a lifespan study on rats, imaging the juvenile development as well as ageing processes of the brain with non-invasive techniques including functional and anatomical MRI and different PET-tracers, respectively. An explorative study like this benefits from an automated evaluation technique that is based on individual structural changes rather than manual ROI analysis. To overcome this limitation, we used elastic coregistration and individual deformation fields to address changes in the frontal cortex from MR images

Material and Methods. From postnatal day 21 two groups of four male Wistar rats were housed pairwise. Food restriction (80% of ad libitum consumption) started at months 3 in order to minimize obesity, a risk factor for age-related diseases (1). Animals were employed in MR experiments on a bimonthly basis. Group 1 was followed from the age of 3weeks up to 14months, Group 2 from 10 until 20months. MR experiments were conducted on an 11.7T Bruker BioSpec horizontal bore scanner. Animals were anaesthetized using 2% Isoflurane in 70:30 N2O:O2 and vital functions were monitored continuously. T2 maps were chosen for their anatomical detail and quantitative reproducibility. Maps were calculated from an MSME (multi slice multi echo) sequence (10echoes) with TE=10ms, TR=5000ms, FOV=28x28mm and a resolution of 0.146x0.146mm in plane and 0.5mm slice thickness (without gaps). For every individual all T2 maps from the different ages were coregistered non-rigidly using a Bspline transform (2), and the corresponding deformation fields calculated. Deformation maps indicated volumetric changes of brain regions. At specific time points (3weeks and 3months) a subset of rats was sacrificed for histological evaluation (cresyl violet).

Results. The deformation maps revealed a decrease in cortical thickness during juvenile development (3weeks to 3months). In parallel, quantitative evaluation of the frontal cortex showed a reduction of T2 relaxation time. A further T2 reduction was observed after the age of 6months, however, without significant changes in volume. Preliminary histological evaluation revealed a higher cortical cell density at 3months when compared to 3weeks of age.

Conclusion. Elastic coregistration is a useful tool for lifespan studies, providing unbiased information on volume changes on an individual basis. The deformation fields allow the creation of physiologically meaningful ROIs for quantitative analysis of imaging parameters. A combination of reduced T2 and decreased cortical volume is implying an increasing tissue density (myelination and cell number) during development, lowering the relative amount of free water, and thus reducing the cortical volume. This was confirmed by histological evaluation.

Acknowledgements. This work was financially supported by BMBF (0314104) and ENCITE EU-FP7 (HEALTH-F5-2008-201842) programme.

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poster number: 103

A UNIQUE NEW HUMANIZED MOUSE MODEL FOR MULTIPLE MYELOMA: OPPORTUNITIES FOR STUDYING MM IN ITS NATURAL ENVIRONMENT AND PRECLINICAL TESTING

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Introduction Multiple myeloma (MM), one of the most common hematological malignancies in adults, is a neoplasm of terminally differentiated B cells, i.e. plasma cells. The transition of a plasma cell to a fully transformed, aggressive myeloma is a multistep process, which requires the acquisition of mutations in multiple genes. Most of this evolution takes place in the bone marrow (BM). Studying the pathogenesis of MM is seriously hampered by the lack of appropriate conditions for the engraftment of patient-derived MM cells (pMM) which, unlike MM cell lines, strongly depend on a human microenvironment to engraft, survive and expand, indicating that the interaction of MM cells with the cellular and extracellular components of the human BM microenvironment plays a crucial role in the growth behavior of MM cells.

Methods Here we report the development of a unique mouse model to study the pathobiology of MM by implementing a technology for creating a natural human bone environment in the immune deficient RAG2$^{-/-}\gamma_c^{-/-}$ mouse. To this end we combined a procedure to culture-expand human BM-derived mesenchymal stromal cells (MSC) that were seeded on biphasic calcium phosphate (BCP) particles and subsequently implanted subcutaneously in RAG2$^{-/-}\gamma_c^{-/-}$ mice. Within 6 weeks this leads to the formation of so-called ossicles that contain substantial amounts of human bone, while the remaining open spaces are filled with mouse hematopoietic cells and blood vessels, creating an environment that strongly resembles the human bone marrow.

Results A striking finding was that this humanized environment in the mouse as a 3-D natural 'niche' for pMM cells. Intrascaffold injection of pMM cells resulted in engraftment and outgrowth of tumor cells in close contact with the human bone layer in the ossicles. In addition, intracardial injection, revealed that these primary tumor cells were also capable of homing to the implanted artificial BM-niches, while no tumor cells were detected in the mouse BM. The outgrowth of pMM in this model is accompanied by an increase in osteoclast number on the bone surface, indicating the presence of bone resorption, one of the most important clinical sequelae of MM. Interestingly, by gene-marking pMM cells with luciferase and using bioluminescent imaging, we were able to follow myeloma outgrowth in time, and visualize the effect of treatment.

Conclusion Hence, this novel humanized mouse model provides the first opportunity to investigate patient-derived MM plasma cells in a more natural environment, which may lead to better insights in the pathogenesis of this disease. Furthermore, it could serve as a model for preclinical testing of new therapeutic approaches for the treatment of MM patients.

poster number: 104

ESTABLISHMENT OF A NEW ORTHOTOPIC TUMOR MODEL FOR FLUORESCENCE GUIDED COLONOSCOPY IN MICE

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Introduction: Colorectal cancer (CRC) is one of most malignant human tumors, occurring in about 7 % of the population. Orthotopic mouse models of colorectal cancer, which feature cancer cells growing in their natural location, replicate human disease with high fidelity. The identification of small polyps and neoplasms with nonpolypoid morphology is a serious problem. During colonoscopy, early metaplastic and dysplastic changes of the mucosa are still missed in 25-30% of patients. To improve visualization of surface features of the intestine detection options have to be optimized. Near infrared contrast agents combined with fluorescence guided endoscopy are promising approaches to make these precancerous lesions visible.

Material and Methods: For the orthotopic implantation 2x106 tumor cells (HT29, HCT116 and Colo205) were injected into the wall of the descending colon. Tumor growth was monitored by bioluminescence imaging and endoscopy started on day 1 after surgery and was repeated once a week. Tumor development was scored by the total number and by the size of tumors visible in the lumen. In addition, fluorescence imaging with a fiber probe (MaunaKea) was established and validated by using optical contrast agents.

Results: All three cell lines could be implanted successfully into the colon wall of nude mice. Tumor growth could be monitored by bioluminescence imaging and by endoscopy with a rigid endoscope. The mean take rate for all cell lines was 88%. Molecular imaging using the fiber probe could be established. Using a contrast agent for integrin a5b3 we could clearly distinguish normal mucosa structures from tumor tissue. Furthermore, we could measure a significantly higher fluorescent signal on tumor areas compared to healthy mucosa tissue in vivo. The fold changes were as follows: 4.6 for HCT116, 4.6 for Colo205 and 2.8 for HT29.

Conclusion: We were able to establish a new orthotopic tumor model for colorectal cancer in nude mice for at least three colorectal cell lines. Tumor growth could be monitored by non-invasive bioluminescence imaging as well as by endoscopy. In addition the tumors showed a high contrast and thus we could clearly distinguish between normal and tumor tissues in vivo. Our newly established orthotopic CRC tumor model now provides the basis for further pre-clinical studies to validate new contrast agents and new therapy targets to obtain clinically relevant data.

poster number: 105

QUANTITATIVE MAGNETIC RESONANCE MONITORING OF MUSCLE DAMAGE/HEALING PROCESS THROUGH DIFFUSION TENSOR IMAGING AND T2-MAPS ASSESSMENT

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Introduction The possibility of a dynamic in-vivo assessment of structural changes occurring during the skeletal muscle repairing process represents an essential need in any preclinical and clinical project aimed to investigate the role of immune system in the muscle healing. Aim was to set up and validate a non-invasive Magnetic Resonance (MRI) based follow-up of acute muscle damage/healing process, able to describe the muscle changes with a quantitative readout.

Methods Acute muscle damage was obtained through cardiotoxin (CTX) injection into tibialis anterior and quadriceps muscles of 24 C57BL/6N mice. MRI was performed on a 7T magnet (Bruker, Biospec 70/30) before CTX and at 7 time points after damage (1, 3, 5, 7, 10, 15, 30 days post CTX), including T2w-MSME sequences and diffusion tensor images (EPI-DTI sequences) for quantification of T2 relaxation time (T2-rt) and fractional anisotropy (FA), respectively. At each time point 3 mice were sacrificed and histological results (hematoxylin/eosin) were correlated with MRI. Moreover, the number of infiltrating leukocytes and the percentage of regenerated muscle fibers were quantified on histological samples.

Results In healthy muscles T2-rt was 16,1±0,56ms and FA 0,31±0,063. After damage induction T2-rt strongly increased getting the maximum value at day 3 with a subsequent slow decrease until basal value at day 30 (day1:30,1; day3:35,9; day5:32,6; day7:30,2; day10:24,6; day15:26,6; day30:16,1 ms). T2-rt changes were well related with inflammatory changes detectable at histology; moreover T2-rt was strongly correlated to histological quantification of infiltrating leukocytes ($R=0.94$; $p<0.005$). FA showed a marked decrease at day 1 after damage, with a complete recover of normal values at day 5, in agreement with fibers destruction (FA decrease) and regeneration (FA increase) at histology (day1:0.08; day3:0.18; day5:0.47; day7:0,42; day10:0,39; day15:0.36; day30:0.32). Strong correlation was found between FA and percentage of regenerating fibers at histology ($R=0.89$; $p<0.005$).

Conclusions T2-rt and FA modifications seem sensitive and quantitative markers of two different phenomena occurring in the acute muscle damage, tissue edema with leukocyte infiltration and muscular architecture disruption. MR imaging allows quantitative and non-invasive monitoring of muscle healing process with edema reabsorption and fibers integrity recover. MRI can be used for quantitative monitoring of muscle healing process.

poster number: 106

CHRONIC MILD STRESS AFFECTS THE MICROSTRUCTURE OF REGIONS RELATED TO MAJOR DEPRESSIVE DISORDER

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Introduction: Major depressive disorder (MDD) is the leading psychiatric disease in the western world. A vast number of studies reported that microstructural changes in several areas, such as the prefrontal cortex, striatum and hippocampus, are implicated in the pathophysiology of MDD. However, little non invasive studies have been conducted on the topic. Previously, we found microstructural changes in the hippocampus of the chronic mild stress (CMS) rat model, using diffusion kurtosis imaging (DKI). [1] DKI is a more extensive method to characterize diffusion in the brain than the conventional diffusion tensor imaging (DTI). Whereas DTI assumes Gaussian diffusion, DKI has been developed to quantify the deviation of the water diffusion displacement profile from the Gaussian distribution of unrestricted diffusion. Now, we have used DKI to detect possible microstructural changes in other potential target areas.

Methods: We used a total of 21 rats, including 7 control and 14 CMS exposed rats, which had been split up into 7 stress sensitive and 7 stress resilient rats, based on the sucrose consumption test. [2] All rats were anaesthetized using isoflurane (1.5-2%) and were monitored carefully to maintain constant physiological parameters during measurements. The experiments were conducted on a 9.4T Bruker Biospec (Ettlingen, Germany). The imaging protocol included DKI scans which used 30 gradient directions and 7 b-values (0-2800s/mm²). Images were collected with a multi-slice spin echo 2-shot EPI sequence using following parameters: TR/TE=3000/25ms, d=5ms, ?=12ms, acquisition matrix=128*64, FOV=35*17.5mm², slice thickness=1mm, NEX=4. After realignment of the DW images with SPM5 diffusion II toolbox, diffusion kurtosis tensor and diffusion tensor derived parametric maps were computed. Anatomy-based region of interest analysis of the prefrontal cortex, caudate putamen and amygdala was performed using AMIRA (Mercury Computer systems, San Diego, USA). Statistical analysis testing for differences of diffusion parameters between control and CMS exposed rats, and stress sensitive and stress resilient rats, was performed.

Results: The analysis showed that the caudate putamen and the amygdala have different diffusion properties after CMS exposure, i.e. axial diffusion and radial diffusion, respectively, were significantly increased in CMS exposed animals. In addition, mean kurtosis was significantly decreased in the caudate putamen of stress sensitive rats as compared with stress resilient rats.

Conclusions: Our findings confirm that the caudate putamen and the amygdala are affected by continuous exposure to stress which can lead to MDD. The diffusion changes might reflect the dendritical remodeling found in these brain structures. [3,4] Interestingly, mean kurtosis in the caudate putamen discriminates stress sensitive and stress resilient animals. As stress sensitive animals are a model for MDD, decreased mean kurtosis in the caudate putamen is a potential non invasive marker for MDD.

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poster number: 107

INCREASED ARTERIAL STIFFNESS LEADS TO BRAIN INFARCTIONS AND SUDDEN DEATH IN APOLIPOPROTEIN E- DEFICIENT MICE

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Introduction: Arterial stiffness has been associated with increased cardiovascular risk. We previously showed that arterial stiffness promotes both the progression of atherosclerosis and a more unstable plaque phenotype in apolipoprotein E (ApoE)- deficient mice with a mutation in the fibrillin-1 gene [1]. In this study, we aimed to investigate whether these mice are suitable as an animal model for plaque rupture.

Methods: Mice with a mutation (C1039G+/-) in the fibrillin-1 gene, leading to fragmentation of the elastic fibres, were crossbred with ApoE-deficient mice. At six weeks of age, female ApoE-/- Fibrillin-1C1039G+/- (n=16) and ApoE-/- control mice (n=16) were fed a western type diet (21% fat, 0.2% cholesterol) for up to 35 weeks. Excised brains of perfused mice were examined by high resolution 3D SE-MRI (7T, TR 4000ms, TE 12ms, 256*256*96 matrix with an in plane resolution of 78µm2) for the presence of brain infarcts. Histological confirmation of infarcts was made on hematoxylin-eosin sections. In addition, detailed characterization was performed using oil-red O for neutral lipid staining, a- smooth muscle actin for smooth muscle cells and Mac-3 for macrophage detection.

Results: Between 13 and 35 weeks (average 20 weeks) of a western type diet 80 % (13 out of 16) of ApoE-/- Fibrillin-1C1039G+/- mice died suddenly, which was mostly preceded by head tilt and/or motor problems (loss of orientation and balance). In contrast, mortality was not seen in the control group (0 out of 16 mice at 35 weeks). MRI revealed one or more brain infarcts in all 16 ApoE-/- Fibrillin-1C1039G+/- mice as dark spots in the brain parenchyma, mostly in brain regions associated with motor nuclei and/or the vestibular system. Control mice displayed no brain infarcts ($p < 0.001$). Histologically, the brain lesions contained foam cells and cholesterol clefts, were positive for neutral lipids, smooth muscle cells and macrophages.

Conclusion: ApoE-/- Fibrillin-1C1039G+/- mice are a novel model for plaque rupture and stroke and open new perspectives for pharmacological interventions. The model can be characterized by ex-vivo MRI and histological sections are indicative for cerebral embolization of plaque material after rupture. In vivo MRI will be performed in future experiments for early detection of infarcts at various time points.

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poster number: 108

INTRODUCTION OF MACROPHAGE-ABLATION ANIMAL MODELS INTO BIO-MEDICAL IMAGING RESEARCH

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Introduction: Macrophages as an integral component of the reticulo-endothelial system engulf foreign particles such as larger imaging probes. This is pivotal for bio-medical imaging in two ways. First their phagocytic ability is utilized to enhance macrophage-rich tissue such as the reticulo-endothelial system. Liver tumors and metastases loose their phagocytic capacity, and therefore do not enrich imaging probes allowing intrahepatic tumors to be distinguished from healthy tissue. Secondly, unspecific phagocytosis reduces the circulation time of imaging probes, especially if the imaging probes are above a certain size range. This is a significant limitation in molecular imaging, because a short circulation time reduces the chance to reach sufficient local enhancement in the case of targeted imaging concepts. Macrophage ablation animal models have existed for a long time (1,2), and they may be a valuable research tool to investigate, manipulate or prevent unspecific probe uptake. The aim of this study was to introduce this tool into bio-medical imaging by showing its power to manipulate the kinetics of larger imaging probes.

Methods: The kinetics of the commercially available CT contrast media Exitron nano 6000 was compared in macrophage ablative mice and normal mice. An established macrophage ablation model was used (1,2). Therefore, 220 µg liposomes, loaded with clodronate at a concentration of approximately 7 mg/ml, were injected into the peritoneum of 3 female C57BL/6 mice, respectively. At the third day after the clodronate application, 200 µl of the particulate agent ExiTron nano 6000 were injected into three macrophage-ablative mice as well as three control mice. CT scans were acquired before and 3 minutes, 1, 6, 24 hours after the ExiTron application. Subsequent to the last scan, the animals were sacrificed; spleens and livers were removed. Relative CT values (CTV) were measured and analyzed in different organs of the respective animals.

Results: Liver and spleen enhancement of treated as well as of controls was increasing over time. However, the mean peak values were different with 218 CTV (min 184/max 252) for ablative and 622 CTV (min 480/max 805) for control mice in the liver and 443 CTV (min 350/max 508) for ablative and 1017 CTV (min 523/max 1553) in control mice in the spleen. Kidney enhancement showed little difference with 74 CTV in ablative and 71 CTV in control mice in peak enhancement at one hour. Muscle showed no enhancement.

Conclusion: Macrophage-ablation leads to a significant decrease of enhancement in organs containing high numbers of macrophages as liver and spleen, but only marginal changes in macrophage-poor organs such as kidneys. Therefore, we conclude that macrophage ablation can significantly influence the phagocytotic activity and thus open new potential to investigate and manipulate uptake of imaging probes.

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PRECLINICAL *IN VIVO* PET CHARACTERIZATION OF TUMOUR GRAFT MODEL OF LUNG CANCER

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Introduction: Lung cancer is the main cause of death for cancer in the world, with about 10% estimated survival at 5 years for non-small-cell lung cancers (NSCLCs). Low therapies efficacy is manly due to high tumoural heterogeneity. In recent studies, authors have observed the presence of cancer stem cells (CSCs) CD133 positive (1-2). These cells can be intrinsically resistant to chemo and radiant therapies and thus responsible for tumour relapse and metastasis. For these reasons, it is important to develop preclinical models that allow to test new therapies and that reproduce the heterogeneous aspect of human pathology. Aim of the study is to verify that tumour graft models of lung cancer maintain the same metabolic and histological characteristics of the original tumour.

Methods: Tumour graft models of lung cancer were obtained by subcutaneous direct transplant of primary lung cancers originated from patients undergone surgery after [18F]FDG-PET. Groups of animals (n=6 per group) with difference in patients' SUV and in CSC expression performed weekly [18F] FDG-PET studies for a month starting at about 130 mm3 tumour dimension. For the quantification, a 3D ROIs (Region of Interest) analysis was performed and, after PET images calibration and correction for F-18 half-life, max radiotracer uptake values expressed as Standardized Uptake Value (SUVmax) were calculated and compared to correspondent patient's SUVmax.

Results: First of all, we observed a tendency at engrafment of the tumour obtained from patients with medium-high SUV values. Then, tumour graft models showed different growth and maximum [18F]FDG uptake. Moreover there wasn't correlation between the speed of growth and SUVmax, in particular tumour with the highest SUVmax value showed a slow time of growth. SUV values of mice were lower than those of patients; despite a good correlation between patients SUVmax and mice SUVmax ($r2=0.81$, $p<0.05$) indicating that tumour glucose metabolism didn't vary in mouse model. The tumor grafts recapitulate the phenotype of primary tumour concerning the presence of CD133 positive cancer cells. We observed no correlation between [18F]FDG uptake and CD133 expression, justifying low proliferating cancer stem cells characteristics.

Conclusions: Lung cancers obtained from patient and implanted in mice maintain their glucose metabolism. Further metabolic pathways like cellular proliferation and regional hypoxia will be analyzed. Tumour graft model is an interesting preclinical model to study efficacy of new therapies.

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QUANTIFICATION OF VIABLE GD-LIPOSOME LABELED CELLS IN VIVO WITH MRI MAPPING WITH BIOLUMINESCENCE VALIDATION

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Purpose: To determine the R1 and R2 relaxation rate of viable Gd-labeled MSCs in vivo as a function of cell concentration, validated by bioluminescence imaging.

Materials and methods: Gd-DTPA was incorporated in the water phase of cationic liposomes using a mixture of cholesterol, DOTAP and DPPC at molar ratios 2.35:1.65:1.0. Liposomes were analyzed for phosphate content and size by spectrophotometer analysis and dynamic light scattering respectively. Rat mesenchymal stem cells (MSCs; Millipore, Billerica, MA, USA) were transduced to constitutively express firefly luciferase and labeled with liposomes (125μM) for 4 h. Cells were harvested and a serial dilution of viable Gd-Fluc-MSCs was injected in several spots in rat musculoskeletal tissue. MRI T1 mapping (TR variation) and T2 mapping (TE variation) were performed on a 3T clinical scanner. Bioluminescence imaging was performed after intraperitoneal injection of D-Luciferin (100 mg/Kg). R1 and R2 relaxation rate was correlated to cell concentration. Rats were imaged with BLI as well, correlating the number of photons with cell concentration.

Results: Both the longitudinal R1 and the transverse R2 relaxation rate were shown to correlate linearly with Gd-liposome labeled cell concentration. The r1 and r2 relaxivity per cell were determined from the slopes of the regression lines of the R1 and R2 relaxation rates against cell concentration (r1= dR1/d[cell concentration] and r2= dR2/d[cell concentration]). Bioluminescence imaging signal showed a linear increase with cell concentration, confirming the cell viability in the various locations, thereby supporting the MR findings.

Conclusion: Viable Gd-labeled MSCs can be recognized by MRI and quantified according to their concentration using MR mapping, yielding a representative r1 and r2 relaxivity per cell. BLI serves as an excellent tool for validation of MRI measurements. Clinical relevance/application: Quantification of the viable cell amount may aid in therapy evaluation of cell transplantation, detecting gross changes in cell number e.g. due to cell death/proliferation.

poster number: 111

SOLID LIPID NANOPARTICLES TARGETING ATHEROSCLEROTIC PLAQUES FROM BRACHIOCEPHALIC ARTERY IN APOE KNOCK OUT MOUSE MODEL

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Introduction: Solid Lipid Nanoparticles (SLNs) are considered excellent vehicles for the targeted delivery of diagnostic and therapeutic agents, since they possess several advantages, including good tolerability, high bioavailability, low toxicity and large-scale production processes. The bioavailability of SLNs at pathological sites like atherosclerotic plaques, is strictly related to the permeability of the lesion, due to an induced endothelium leakage triggered by several inflammation factors. Indeed the assessment of the inflammation state of both the plaque and the surrounding tissues is a key factor suitable for the identification of unstable plaques. In this study fluorescent SLNs were intravenously administered to ApoE (-/-) mice to probe the permeability properties of the endothelium in atherosclerotic plaque at different developmental stages. Fluorescence microscopy was used to quantify and localize fluorescent SLNs after their extravasation into the plaques.

Moreover microscopy was also used to histologically identify and classify the variety of collected plaques validating the correlation between murine and human lesions progression. Histology was then used to characterize the morphological parameters associated with the vulnerability conditions, and the possibility to target vulnerable lesions using SLNs.

Methods: The brachiocephalic artery was excised from ApoE (-/-) mice after 5 to 40 weeks of hyperlipidic diet, then it was frozen and cut for labelling. Three analytical protocols were performed as follows: i) histological characterisation with hematoxilin and eosin staining in order to identify the lesion architecture and composition; ii) localization of fluorescent SLNs to characterize their interaction with the cellular components into the plaques; iii). immunohistochemical labelling with specific antibodies against macrophages (anti-CD11) and vessels (CD31) to confirm the nanoparticles distribution in plaque tissues.

Results: The atherosclerotic lesions were characterised according to the quantity of foam cells, percentage of stenosis and thickness of fibrous cap. The ApoE (-/-) model was completely characterised and the degree of plaque evolution was assigned to one of three levels. Morphological features associated to the plaque vulnerability were identified for a complete and exhaustive description of the animal model. Data obtained from the SLNs labelling evaluation confirmed the ability of nanoparticles to target atherosclerotic lesions with an efficiency which is dependent by the developmental stages of the pathology. Moreover the data clearly demonstrated that the foam cells are the main accumulation sites of SLNs.

Conclusions: Histological analysis allowed us to describe the ApoE (-/-) mouse model in great details characterizing the typical plaque architecture and modification during its development. Our results demonstrate that fluorescent SLNs are able to efficiently identify atherosclerotic lesions at early stage of development but they can not distinguish between vulnerable and stable plaques, due to the reduced permeability of the fibrous cap in the more advanced plaques. Therefore, we can conclude that SLNs, if labelled with an appropriate imaging probe, have the potential to become a useful diagnostic tool to identify atherosclerotic lesions at very early stages of pathology progression.

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FULLY AUTOMATED ATTENUATION MEASUREMENT AND MOTION CORRECTION IN FLIP IMAGE SEQUENCES

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Introduction: Fluorescence loss in photobleaching (FLIP) [1] is a method to study compartment connectivity in living cells. A FLIP sequence is obtained by alternatively bleaching a spot in a cell and acquiring an image of the complete cell. Connectivity is estimated by comparing fluorescence signal attenuation in different cell parts [2]. The measurements of the fluorescence attenuation are hampered by the low signal to noise ratio of the FLIP sequences, by sudden sample shifts and by sample drift [3].

Methods: We propose an automated method that estimates the attenuation by modeling photobleaching as exponentially decaying signals. Sudden motion artifacts are minimized by registering the frames of a FLIP sequence to target frames (similar to [4]) based on the estimated model and by removing frames that contain deformations. Linear motion (sample drift) is reduced by minimizing the entropy of the estimated attenuation coefficients.

Results: Experiments on 16 in vivo FLIP sequences of muscle cells in Drosophila show that the proposed method results in fluorescence attenuation estimates similar to the manually identified gold standard, but with standard deviations of approximately 50 times smaller. As a result of this higher precision, cell compartment edges and details such as cell nuclei become clearly discernible.

Conclusions: The main value of this method is that it uses a model of the bleaching process to correct motion and that the model based fluorescence intensity and attenuation estimates can be interpreted easily. The proposed method is fully automatic, and runs in approximately one minute per sequence, making it suitable for unsupervised batch processing of large data series.

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A THREE-DIMENSIONAL MRI BRAIN ATLAS OF THE MOZAMBIQUE TILAPIA (OREOCHROMIS MOSSAMBICUS)

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Introduction Today, non- rodent animal models are extensively studied in biological research. E.g. (a) Drosophila is used as a model organism in genetics, cell-biology, biochemistry, and especially developmental biology; and (b) zebrafish is used in developmental biology, cancer, pharmacology and toxicology, disease modeling, and neuroscience. Also the African cichlid Oreochromis mossambicus, has been used as a model system in a wide range of studies. The increasing number of genetic tools available for this species, together with the emerging interest in its use for neurobiology studies, increased the urgency in an accurate hodological mapping of the tilapia brain to complement the available histological data. The goal of our study was to elaborate a three-dimensional, high-resolution digital atlas of the Tilapia brain using magnetic resonance imaging.

Methods Specimen preparation: three adult tilapia females (standard length: 10.7±1.8cm) were perfused transcardially (PBS 0.2 M - Paraformaldehyde (2 %) in Dotarem® (1 %)). For scanning, the brains of the fish were removed from the skull and transferred to a polypropylene tube filled with Fluorinert®. Ex vivo scans: MRI scanning was performed on a 9.4T Bruker MRI system (Ettlingen, Germany). Horizontal images of the Tilapia brain were acquired using a fat-suppressed T2-weighted 3D RARE sequence: TE/TR=30/350ms, RARE factor=2, 8 averages, a field of view of (13.5×8×10)mm3 and an acquisition matrix of (270×160×200), resulting in a nominal spatial resolution of (50×50×50)µm3. The total acquisition time was 12.6 h. Manual segmentation: Brain and nuclei delineation was done manually using Amira software (Mercury Computers Systems, USA). Segmentation was done slice-by-slice in a coronal perspective and posteriorly confirmed systematically in the two other orthogonal views (axial and sagittal). Major brain subdivisions (Telencephalon, Diencephalon, Mesencephalon, Rombencephalon) were first delineated, followed by structures which presented more distinct boundaries (e.g. olfactory bulbs, optic tectum and corpus cerebellis), which helped identifying smaller nuclei. In addition, histology slices were used as guidelines for the location and boundaries of smaller structures. In this atlas, we adopted the nomenclature for brain structures from Wullimann et al. [1] and Meek and Nieuwenhuys[2].

Results Using high resolution MRI, we acquired a detailed digital tilapia brain atlas, depicting several major and minor structures. Supported by the acquired histological map of this species, we identified 55 structures. Using the intrinsic three-axis nature of MRI-based atlases, we established a stereotaxic coordinate system which gives the centre coordinates for each structure with respect to the reference point – i.e intersection between the mid-sagittal and the mid-horizontal planes and the Anterior Commissure.

Conclusions This high resolution tilapia brain atlas is expected to become a very useful tool for neuroscientists using this fish model and will certainly expand their use in future studies of the CNS.

Acknowledgment: This study was supported by the Portuguese Foundation for Science and Technology (FCT – Fundação para a Ciência e Tecnologia), project PTCD/PSI/71811/2006

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poster number: 114

COMBINED PREOPERATIVE LYMPHATIC MAPPING WITH REAL TIME INTRAOPERATIVE FLUORESCENCE GUIDANCE TO THE SENTINEL LYMPH NODE IN HEAD AND NECK MELANOMA PATIENTS

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Introduction: Sentinel lymph node (SLN) biopsy in the head and neck is often more difficult than in other nodal basins due to the complex anatomy and the variable drainage patterns in this region. SLN surgery is based on the combination of gamma probe detection and blue dye mapping. Unfortunately, the signal of the injection area can cause difficulty in localizing nearby SLNs with the gamma probe. Furthermore, patent blue migrates quickly and SLNs in the head and neck region are less frequently stained blue. The incorporation of another visual element in sentinel node surgery of the head and neck may facilitate the procedure. The purpose of this study was to assess the feasibility of combining pre- and intraoperative sentinel lymph node imaging using a multimodal tracer that is both radioactive and fluorescent [1].

Methods: 10 patients scheduled for SLN biopsy in the head and neck region were included. Approximately 4 hours prior to surgery, the multimodal tracer (ICG-99mTc-NanoColl) was injected intracutaneously in 4 deposits around the scar of the primary melanoma excision. Subsequent lymphoscintigraphy and SPECT/CT was performed to preoperatively localize the SLNs. During surgery, a portable gamma camera was used to guide the incisions. After injection of blue dye, SLNs were acoustically localized with a gamma-probe and their exact contour was visualized using a dedicated fluorescence camera system. The portable gamma camera was then used to confirm complete excision of all SLNs.

Results: In total, 25 SLNs were preoperatively identified on SPECT/CT. All preoperatively identified SLNs could be intraoperatively localized by acoustic gamma tracing and/or fluorescence imaging. Fluorescence particularly improved surgical guidance in areas with a high radioactive background signal such as the injection site. In the patients where blue dye was used, merely 23% of the SLNs stained blue, whilst all of them were fluorescent. Ex vivo analyses validate the strong intensity correlation of the radioactive and fluorescent content in all excised LNs. No metastases were found at histopathological examination in all excised LNs.

Conclusion: Preliminary data indicate that fluorescence guidance toward the preoperatively identified SLNs surgery is technically feasible using a multimodal tracer. This technique may improve intraoperative localization and excision of sentinel nodes in the head and neck region.

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poster number: 115

REAL-TIME IMAGING OF 2-STEP ULTRASOUND-MEDIATED TEMPERATURE-CONTROLLED DRUG DELIVERY USING FIBERED CONFOCAL FLUORESCENCE MICROSCOPY

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Introduction: Recently, thermosensitive liposomes (TSLs) have been explored for temperature controlled ultrasound (US)-mediated intracellular delivery of cell-impermeable molecules (1, 2). Here we propose to use fibered confocal fluorescence microscopy for real-time imaging of this 2-step protocol.

Methods: TO-PRO-3, a cell-impermeable intercalating fluorophore that exhibits a 100-fold increase in signal intensity upon binding to nucleic acids, was used as a 'model drug' for intracellular delivery. TSLs incorporating 50µM TO-PRO-3 were previously characterized in DNA solution during heating (Tm =41.5°C). U87 human glioma cells were seeded in an OpticellTM cell culture system and pre-stained with DiD (plasma membrane stain, Ex/Em=644/665nm) to delineate cells. After adding TSLs together with 400µL (107) Sonovue microbubbles (Bracco, Italy), Opticell was installed in the waterbath at 37°C. A setup for the imaging of sonoporation consisted of a monoelement transducer (d=5.8 mm) positioned 8mm below the cell surface at the acoustical focal distance and the microprobe tip of the fibered confocal fluorescence Cellvizio® (MaunaKea Technologies, France) microscope (M/100 -Typical FOV= 240x200µm2 -working distance= 100µm) that was placed in the same axis as the transducer but above the cell surface. Baseline real-time image acquisition (frame rate=8.5Hz, laser excitation=660nm, spectral sensitivity 680-900nm, laser power=1.0mW) lasted for 10s followed by application of US (power=1W, pulse repetition frequency=1kHz, duty cycle=20%, duration=30sec); recording continued during 2 minutes followed by 5 s acquisition every 30s during 5minutes. The recording was continued in the same mode after heating from 37°C to 42°C (6minutes in total) during 20 more minutes (experiment repeated in triplicate for different areas). Identical recordings were made in the absence of heating and/or sonication and corrected for photobleaching. After fixing with 4%-PFA Opticells were imaged with the epifluorescence microscope Leica DM R equipped with CCD camera and appropriate for TO-PRO-3 set of filters.

Results: Spatio-temporal evolution of TO-PRO-3 was monitored in a real-time mode. US application led to a slight displacement (1-5µm) of some cells but no cell detachment was observed. No change of the signal intensity / localization was noticed in the absence of consecutive heating or when heating was applied alone. However, when US application was followed by heating to 42°C, circular hyperintense areas appeared in some cells (typically 3-8 per FOV). Shape and size (about 10µm) corresponded to nuclei. Fluorescence microscopy showed DiD staining present at all the experimental conditions, however, a typical nuclear staining of TO-PRO-3 was observable only in the areas of sonication followed by heating.

Conclusions: In vitro real-time imaging of 2-step ultrasound-mediated temperature-controlled drug delivery was performed for the first time with fibered fluorescence microscopy (Cellvizio®). This imaging modality may significantly contribute to the understanding of the concept of temporal window and US-mediated delivery as well as protocol development and optimization.

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DETECTION OF HEAD AND NECK SQUAMOUS CELL CARCINOMA AND CERVICAL LYMPH NODE METASTASIS IN A MOUSE MODEL USING ACTIVATABLE NEAR-INFRARED FLUORESCENCE PROBES

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Introduction: Intraoperative assessment of tumor-free margins in head and neck cancer surgery is critical to completely remove the primary tumor and to improve prognosis. Currently, preoperative tumor staging is performed using different imaging modalities. However, in the operating room, the surgeon is confined to visual appearance and palpation of the tumor. Optical imaging has the potential to traverse the gap between radiology and surgery by providing real-time visualization of tumor tissue, warranting image-guided surgery. In order to gain optimal tumor-to-background ratios (TBR), new near-infrared (NIR) fluorescence agents have been designed that contain a cleavage site specific to targeted proteases. These agents are injected in a quenched (i.e. non-fluorescent) state, resulting in minimal fluorescence at the time of administration. Only when cleaved by the specific enzyme, the agent becomes dequenched (i.e. fluorescent). We performed a study to assess the possibilities of optical imaging of head and neck squamous cell carcinoma (HNSCC) and cervical lymph node metastasis. To our knowledge, this is the first study that describes the use of activatable NIR fluorescence agents in an HNSCC mouse model.

Methods: An HNSCC xenograft mouse model was developed using 6 x 104 luciferase-bearing OSC-19 human HNSCC cells that were injected directly into the tongue of 10 Balb/C nu/nu mice. Tumor progression was followed by bioluminescence imaging (BLI) using the IVIS-100™ (Caliper) and by visual inspection of the tongue. At day 21, mice were randomly allocated to administration of one of two activatable NIR fluorescence agents: ProSense680TM (PerkinElmer; activated by cathepsins) and MMPSense680™ (PerkinElmer; activated by matrix metalloproteinases). Fluorescence imaging (FLI) of the mouse and organs (after surgical removal) was performed using the Maestro™ (CRI) and Odyssey™ (LI-COR). Two control groups, each containing 5 Balb/C nu/nu mice without tumor, were treated with an injection of physiological saline in the tongue and later systemically injected with the activatable agents.

Results: Seven days after injection of OSC-19 cells, tongue tumors had developed in all 10 mice. After 11 days, all mice had developed cervical lymph node metastasis, which was confirmed by an increased BLI signal. The primary tumor and cervical lymph node metastases were successfully detected by the use of both activatable NIR fluorescence agents. The difference between the FLI signal intensity of the tumor versus the control mice was statistically significant for both agents.

Conclusions: This preliminary study shows the establishment of an HNSCC mouse model, in which tumor growth and regional metastases could be assessed by BLI. Moreover, this is the first time that visualization of HNSCC and cervical lymph node metastases was achieved by FLI using activatable NIR fluorescence agents. The technique of image-guided surgery has the potential to be translated into the clinic in order to improve the complete removal of HNSCC.

poster number: 117

INITIAL EXPERIENCE WITH 3D INTRAOPERATIVE FREEHAND SPECT PROBE IMAGING FOR RADIOGUIDED TUMOR EXCISION AND SENTINEL NODE BIOPSY IN BREAST CANCER

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Introduction: The wide-spread use of breast imaging has increased the numbers of impalpable breast lesions. More than 1/3 of the excised breast lesions are clinically occult. Precise localisation is an important factor in the surgical management of these small lesions. Freehand SPECT is a 3-D tomographic imaging modality based on the imaging concepts of SPECT, but with the major difference of being based on data acquisition by a freehand scan using hand-held detectors instead of gantry-based gamma cameras. The present study aims to evaluate the feasibility of 3-D intraoperative imaging with a freehand SPECT probe to guide tumor excision and sentinel node biopsy in patients with breast cancer.

Methods: This pilot study was designed to evaluate 10 patients with breast cancer scheduled for radioguided excision of the primary tumor (ROLL) with or without sentinel node biopsy. Following ultrasound guided administration of 99mTc-nanocolloid in a single injection of 40 MBq (ROLL) or 120 MBq (ROLL + sentinel node biopsy) in 0.2 ml into the tumor static gamma camera planar images were performed at 15 min. For sentinel node detection images were also obtained at 2 en 4 hours. SPECT-CT was performed subsequently to the planar images. For the intraoperative procedure a device combining a spatial localization system and two tracking targets respectively fixed on a gamma probe and on the skin of the thorax of the patient was used. 3-D images were generated and displayed in real time following a protocol based on freehand SPECT probe movements.

Results:The first experience was acquired in 1 patient (age 63 years) scheduled for ROLL combined with sentinel node biopsy. In this patient the primary tumor as well as a sentinel node in level I of the axilla were adequately excised using the freehand SPECT probe. In another patient (age 58 years) both the primary tumor and an intramammary lymph node were excised. In-vivo and ex-vivo real time display of the radioactivity in the tumor in relation to the margins of the specimen was possible in both patients. Histopathologic margins were tumor negative in both cases (15 mm and 4 mm respectively), in accordance with the ex vivo images.

Conclusion: 3-D intraoperative imaging using a freehand SPECT appears to refine radioguided surgery in breast cancer. This device may be of special value in other surgical applications involving tumor excision and sentinel node biopsy.

poster number: 118

TUMOR-SPECIFIC TARGETING VERSUS THE ENHANCED PERMEABILITY AND RETENTION (EPR) EFFECT FOR OPTICAL IMAGING OF CANCER

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Introduction: Near-infrared (NIR) fluorescence optical imaging is a promising technique to assess the tumor-free margins during cancer surgery. Specific targeting by NIR fluorescence agents is essential to differentiate tumor from normal surrounding tissue. This can be performed by targeting specific characteristics of the cancer cell, including upregulation of the epidermal growth factor (EGF) receptor, GLUT-1 receptor or avß3 integrins. However, a different targeting strategy can be used by means of the enhanced permeability and retention (EPR) effect. Defective architecture of tumor angiogenesis and the lack of adequate lymphatic drainage lead to the EPR effect, resulting in non-specific accumulation of fluorophores in the tumor. In the current study, in vivo detection of oral squamous cell carcinoma (OSCC) was studied using three tumor-specific NIR fluorescence agents that target the EGF receptor, GLUT-1 receptor or avß3 integrins. Furthermore, a non-specific NIR fluorophore was used to detect the tumor using the EPR effect.

Methods: In vitro, specificity of the targeted agents was assessed with competition studies for the targeted tumor characteristic. In vivo, OSCC was induced in BALB/c nu/nu mice by submucosal inoculation of human OSC19-luc cells into the tongue. Tumor growth was followed with bioluminescence imaging. Four NIR fluorescence agents (800CW EGF, LI-COR Biosciences; 800CW 2-DG, LI-COR Biosciences; IntegriSense680, PerkinElmer; 800CW PEG, LI-COR Biosciences) were injected and fluorescence imaging was performed at various time-points. Mice without tumor were injected with the agents to determine background signal. After in vivo imaging, tumors were removed and ex vivo imaging was performed on tissues and histological sections.

Results: Specificity of the targeted agents was demonstrated in vitro. In vivo, OSCC was successfully detected using the targeted agents and the agent targeting the EPR effect. Significantly higher fluorescence was found in tongue tumors compared to control animals. Fluorescence correlated with histopathology. After 24 hours, tumor-to-background ratio in the tongue was 13.8 (SD=6.1) for 800CW EGF, 4.6 (SD=2.1) for 800CW 2-DG, 67.6 (SD=24.8) for IntegriSense680 and 13.4 (SD=10.6) for 800CW PEG. On histopathology, intratumoral fluorescence signal of the targeted agents was found, whereas the agent targeting the EPR effect showd fluorescence signal directly around the tumor within a margin of 3 mm.

Conclusions: This study demonstrates the feasibility of in vivo optical imaging of OSCC based on targeting of EGF receptor, GLUT-1 receptor and avß3 integrins, as well as by targeting of the EPR effect. Histological analysis demonstrated difference in localization between the tumor-specific targeted agents and non-specific agent. Once these NIR fluorescence agents become available for clinical testing, optical image-guided surgery could improve the complete resection of oral cancer.

poster number: 119

DEVELOPMENT OF CLINICALLY RELEVANT ANIMAL MODELS FOR IMAGE-GUIDED SURGERY OF COLORECTAL CANCER

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Background: In most solid cancer types, surgical resection of the primary tumor is the only available curative treatment option. A complete tumor resection is of great importance, in order to prevent local recurrence. As the surgeon can only rely on palpation and visual inspection, real-time visualization of cancer cells at the time of surgery is needed to increase the number of complete tumor resections. Near-infrared fluorescence (NIRF) imaging is a promising technique to assess the extent of disease during surgery. To validate the ability of NIRF imaging to visualize all tumor cells, a secondary imaging modality is used for confirmation. This study aimed to use bioluminescent imaging as a co-localization factor for NIRF imaging in clinically relevant animal models, by inducing tumors or metastases orthotopically.

Methods: Human (SW480, SW620 and HT29) and rat (CC531) colorectal cancer cell lines stably expressing the luciferase 2 gene under the control of a CAGGs-promoter were generated. From each cell line, 3 clones were injected subcutaneously on the back of nude mice to assess in vivo tumorigenicity and bioluminescence. After in vivo selection of the optimal clones, animals were inoculated at relevant sites (in the cecal wall for the human cell lines and subcapsular in the liver for CC531S). Tumor growth was monitored weekly by bioluminescent imaging. Four to six weeks after tumor inoculation, animals were sacrificed and tumor presence was confirmed macroscopically and histologically.

Results: For all cell lines, tumors rapidly developed subcutaneously from at least 2 clones. When these cell lines were tested orthotopically in the cecal wall (human) or in a syngenic liver metastasis model (rat), not all cell lines were tumorigenic. We have established 1 human (SW480) and 2 rat cell lines, which in an orthotopic transplantation model show significant bioluminescence. These cell lines are now used to test NIRF probes.Clones from the SW620 and HT29 cell lines are now being tested orthotopically for tumorigenicity.

Conclusion: We have developed both human and rat clinically relevant colorectal cancer animal models which can now be used to test NIRF-probes for imaging of these tumors during surgery.

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IMAGING in DRUG DISCOVERY and DEVELOPEMENT

poster number: 140

EVALUATION OF CY5.5-LABELED INHIBITORS FOR IN VIVO OPTICAL IMAGING OF MMP ACTIVITY IN TUMORS AND METASTASES

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Background: Matrix Metalloproteinases, especially MMP-2 and -9, were consistently up-regulated and activated in inflammatory processes or in malignant tissues and are associated with tumor aggressiveness, metastatic potential and poor prognosis [1]. A pyrimidine-2,4,6-trione (AF443) and a hydroxamic acid (AF489) were selected as two lead structures to develop non-peptidic low-molecular weight MMP inhibitors (MMPIs). For *in vivo* optical imaging the amino precursor of each MMPI was conjugated to a Cyanine dye (Cy5.5) via a short polyethylene glycol. Both MMPIs showed strong inhibition potency of gelatinolytic activity *in vitro* and both Cy5.5-labeled probes bound specifically to highly MMP-2/-9 expressing tumor cells and also tumor sections [2, 3]. Aim: The aim of the present study was to verify whether both MMP-targeted probes are applicable for the specific visualization of MMP-2/-9 activities of tumors and metastases *in vivo*.

Methods: Human cancer cell lines (A-673 Ewing sarcoma; HT-1080 fibrosarcoma; MDA-MB 231 adenocarcinoma; BT-20 breast carcinoma) were implanted subcutaneously into the mammary fat pad of nude mice and grown up to 5 mm. For imaging, 2 nmol of each optical probe was injected intravenously and mice were imaged by Fluorescence Reflectance Imaging and Fluorescence Mediated Tomography at multiple time points post injection. After the 72 h-image mice were sacrificed, tumors and cancer cell-infiltrated organs were excised and analyzed for their MMP-2/-9 expressions (qRT-PCR), protein levels (immunohistochemistry) and activities (*in situ* zymography).

Results: All tumors could be rapidly and clearly delineated from the surrounding tissue already at 1.5 up to 6 h after injection. A strong correlation was found between *in vivo* NIRF signal intensities and tumors *ex vivo* MMP-2/-9 activities. High NIRF intensities were related to tumor aggressiveness as determined by the growth rate up to a typical tumor size and the potential to metastasize spontaneously to distant sites. Metastases with minimal sizes down to 0.1 mm were clearly definable as distinct high fluorescent spots. Probe binding specificity could be demonstrated by predosing A-673 tumor bearing mice. Both MMPIs suppressed the uptake of the Cy5.5-labeled compounds in a time- and dose-dependent manner.

Conclusions: These novel optical tracers allow specific, non-invasive *in vivo* imaging and quantification of MMP-2/-9 activities expressed in tumors and their metastases. Both may have important future applications in other disease models where MMP activity plays an important role in disease activity and progression, such as arthritis, atherosclerosis, and myocardial infarction.

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poster number: 141

DEVELOPMENT AND CHARACTERIZATION OF NANOPARTICLES FOR TUMOR IMAGING: EPR EFFECT AND IN VIVO FRET APPROACH

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Introduction: Nanoparticles are designed to safely deliver therapeutic and imaging contrast agents to the tumor for cancer diagnostics and therapy. Nanoparticles (NP) can target cancer by simply being passively accumulated in tumors; this phenomenon is called the EPR effect (enhanced permeability and retention effect). This phenomenon occurs in solid tumors only and is related to their anatomical and pathophysiological differences from normal tissues. The EPR effect mainly depends on the high vascular density and the large gaps between endothelial cells in tumor blood vessels, leading to selective extravasation and retention of macromolecular compounds. Furthermore, NP can also target specifically the tumor through recognition of a couple receptor/ligand, in our case, integrin $a_v ß_3$/RGD-peptide.

Methods - Results: To better understand the EPR effect and ideally to predict its importance on tumor targeting, we investigated experiments using two imaging modalities on different tumor types-bearing mice: MRI and fluorescence imaging. Fluorescence imaging was used to observe the passive accumulation of fluorescent nanoparticles due to the EPR effect. MRI was used to determine the vascular characteristics of the tumors, as vascular density, blood volume fraction, diffusion constant and permeability. Immunohistochemical analysis of the tumor samples allowed confirming the MRI parameters. Nanoparticles accumulation varied between the four tumor types chosen, HUH-7 (human renal carcinoma), HEK 293 $ß_3$ (human renal carcinoma), U87MG (glioblastoma) and TS/A-pc (murine melanoma), from 1 to 10 respectively. Permeability, determined by MRI, fitted with the optical data. A significant correlation was obtained between MRI parameters and optical imaging (R^2 = 0.63, p = 0.0012**).

NP stability and specificity were determined using FRET (Förster resonance energy transfer) studies *in vitro* and *ex-vivo* on tumor slides. NP specific internalization, intracellular trafficking and degradation were determined. *In vivo*, the NP biodistribution, stability and specifity were also followed-up using noninvasive fluorescence imaging.

Conclusions: The development of new NP dedicated to tumor diagnostic, therapy or theranostic is on prime interest. Prediction of EPR effect might contribute to their investigation and use in preclinical and clinical studies. NP biodistribution and stability are crucial for their development. In this context, fluorescent imaging is a major tool to develop new targeted treatments.

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TARGETED SOLID LIPID NANOPARTICLES CHARACTERISATION BY BIOLAYER INTERFEROMETRY AND MICROSCOPY

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Introduction: Solid Lipid Nanoparticles (SLNs) represents a very well established platform for drug delivery applications. SLNs can be uploaded with both hydrophilic and hydrophobic drugs and functionalised at their surface with targeting moieties to amplify their accumulation in pathological tissues when characterized by an enhanced endothelial leakage of blood vessels triggered by inflammation processes. The bioavailability of the targeting moieties at the surface of SLNs is a key feature which regulates the efficacy of the molecular recognition processes at the target pathological site. The availability of a simple in-vitro experimental assays measuring the affinity of the functionalized nanoparticle to the target receptor, enables the preparation of more standardized formulations resulting in a better reproducibility of further in vivo experiments. Biolayer Interferometry (BLI) is a label-free optical technique that is used to measure biomolecular interactions, in connection with specific biosensors. In this work we used this technique to develop a suitable assay to test the accessibility of folic acid (FA) residues in FA decorated SLNs. In addition fluorescence optical imaging and microscopy were also used to assess the different behaviour between targeted and untargeted SLNs, both in cancer cells and xenografted animal tumor models.

Methods: The solid core of SLNs was composed by a lecithin stabilized crystalline structure of tripalmitin. The stealth agent was a flexible poly(ethylene glycol) (DSPE-PEG-2000). In order to analyse and screen different SLNs preparation, the anti-Folate antibody FA2 was immobilised on Protein-A coated biosensors in a ForteBio Octet QK instrument. Various SLNs formulations of similar composition were tested, using an untargeted SLNs preparation (without FA) as negative control. Targeted and untargeted SLNs were further tested in Igrov-1 cells and in human ovarian cancer xenografted mice assessing their specificity using both in vitro and ex vivo fluorescence microscopy. Labelling with specific antibodies against macrophages (anti-CD11) and folate receptor alpha (anti-FRa) was also adopted to confirm the nanoparticles distribution in the tumor tissues.

Results: Binding kinetics varied in agreement with the concentration of targeting ligand uploaded into the SLNs, while no binding occurred after incubation with ligand-free SLNs. These data were consistent with a specific molecular recognition process occurring at the surface of the nanoparticles between the FA moiety and the corresponding anti-Folate antibody.Moreover these experiments demonstrated the suitability of BLI to study the binding of SLNs to specific immobilized targets. In cancer cells and tissues, differences were highlighted in terms of signal intensity and distribution between targeted and untargeted SLNs.

Conclusions: BLI, as applied in the ForteBio Octet QK instrument, is a convenient and fast technique to screen targeted nanoparticles. Moreover, this method can be applied to several targets depending upon the availability of suitable antibodies. At the best of our knowledge, this work represent the first application of the BLI in the affinity characterization of targeted SLNs.Pathological tissue analysis by microscopy confirmed the ability of the nanoparticles to recognise and target specific cell types depending on their composition and functionalisation.

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poster number: 143

IMAGING GASTROINTESTINAL TRANSIT USING GASTROSENSE 750

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Introduction: The ability to accurately determine gastric emptying rates is crucial for understanding the physiological mechanisms underlying alterations in gastric motility in both clinical and animal studies. A pharmacological agent's effects on gastric emptying can impact its potential as a therapeutic or limit its dose or dosing regimen in patient treatment. Currently, the methods used for preclinical determination of gastric emptying rates involve terminal assessment of dye or radioactive tracers within the stomach, requiring multiple cohorts of animals incurring high financial and time cost. Using a novel, near-infrared fluorescent (NIR) fluorescent imaging agent, GastroSense 750, gastric emptying rates in in vivo murine models can be monitored and quantified non-invasively and in real time.

Methods: The imaging agent (GastroSense 750) is comprised of an acid-stable NIR fluorophore conjugated to a non-absorbable pharmacokinetic modifier designed to be formulated in liquid or incorporated into a solid meal. Using female 8 week old BALB/c mice recipients, 0.25 nmol GastroSense 750 was orally gavaged in 200 uL of PBS (Control) or fed as a mixture in egg yolk, an experimental approach designed to better mimic changes in gastric emptying in the presence of solid diet. All mice were imaged in vivo on the FMT 2500 Fluorescence Molecular Tomography (FMT) System at multiple time points and the resulting images were analyzed to determine the quantification of agent fluorescence in the stomach and its transit through the intestines.

Results: In vivo 3D fluorescent imaging and quantification using the FMT 2500 at multiple time points revealed a gastric emptying time of approximately 55 minutes for the control (liquid) meal and a delay to 135 minutes in the presence of solid food, a significant change. Clonidine (an alpha-2 adrenergic agonist, 1 mg/kg) further inhibited gastric emptying time, halting the process completely between 2 and 4h and ultimately delaying the completion of gastric emptying to >6-7h. GastroSense 750 signal was also detected and quantified as it moves into the intestines, providing a tool for quantifying intestinal transit and generating tissue contrast.

Conclusions: These findings reveal that GastroSense 750 and 3D FMT fluorescent in vivo imaging can be used successfully to quantify gastric emptying rates of both liquid and solid diets in small animals and can be used to measure the pharmacological effect of drugs that affect gastric motility.

poster number: 144

PHARMACOKINETIC STUDIES OF AN INTACT ANTIBODY AND FRAGMENTS THEREOF IN HUMAN XENOGRAFT BASED ON NEAR-INFRARED FLUORESCENCE IMAGING

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Background: Preclinical studies aimed to analyze drug distribution and accumulation into the desired location are conventionally performed by quantification of drug in plasma/ serum and in lysates of the different organs (tumor tissue) by analytical methods (e.g. ELISA, SEC, HPLC). In order to receive statistically meaningful data, 3 to 5 mice are used for each time point. Alternatively non-invasive imaging methods (PET) using radiolabeled compounds have been used to address these questions. We propose a method which allows continuous monitoring of organ distribution over a time period of at least 4 days. Application of planar optical reflectance imaging facilitates distribution studies and we demonstrate the utility of this approach by correlation analysis with fluorescence microscopy of tissue slides of explanted organs.

Material and Methods: Antibody GA201 (RG7160) and fragments F(ab')2 and Fab targeting EGFR were labeled with the Cy5 fluorophore in a protein : label ratio of 1 : 2.7 (median) by established methods. Xolair a humanized IgG1 monoclonal antibody targeting human IgE and a Fc fragment (from a humanized IgG1 mab) were used as negative controls. In order to check labeled compounds for biological identity binding to the antigen EGFR was in-vestigated by surface plasmon resonance (SPR) using a Biacore T100 instrument (GE Healthcare, Sweden) and by cellular binding assays. Human H322M non small cell lung cancer cells expressing EGFR were implanted s.c. (5x10e6 in 100µl) in female BALB/c nude mice (Charles River, Germany). When tumor size was 370 mm³ labeled compounds were injected i.v. in equimolar amounts. Imaging was performed using the MAESTRO System (CRi, USA) and blood was collected at different time points thereafter. At termination tumors were explanted, fixed in formalin and embedded in paraffin. Slides were examined by multi-spectral analysis using the NUANCE system (CRi, USA).

Results: SPR-analysis reveals no significant changes in binding characteristics of Cy5 labeled proteins compared to the non-modified versions and binding to H322M cells in vitro was not affected by fluorophore labeling. Optical imaging measurements indicate accumulation of intact antibodies in the tumor within 2 and 6 hrs after application, whereas the anti-EGFR F(ab')2 and Fab fragment was immediately detected in the kidney. Histological analysis of explanted tumor tissue indicates unspecific accumulation of Xolair and Fc fragment in stroma.

Conclusions: Planar reflectance optical imaging is an excellent method to analyze accumulation of labeled proteins to organs. Ex vivo analysis is mandatory to confirm the in vivo imaging data.

INFLAMMATORY and METABOLIC DISORDERS

poster number: 145

TOWARD DEVELOPMENT OF TARGETED NONSTEROIDAL ANTIANDROGEN COMPLEX FOR PROSTATE CANCER DIAGNOSTICS

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Introduction: Androgen receptors are present in most advanced prostate cancer specimens, having a critical role in development of this type of cancer. For correct prognosis of patient conditions and treatment monitoring, noninvasive imaging techniques have great advantages over surgical procedures.

Methods: We developed synthetic methodologies for preparation of novel androgen receptor-targeting agents in an attempt to build a versatile platform for prostate cancer imaging and treatment. The structure of these compounds comprises of a lanthanoid metal ion, gadolinium-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (Gd-DOTA)-based binding fragment and, connected to it by a flexible linker, bicalutamide-derived nonsteroidal antiandrogen moiety. A representative gadolinium complex 15 was evaluated as a magnetic resonance imaging (MRI) agent in C57/bl6 male mouse bearing orthotopic TRAMP C2 prostate tumor.

Results: Our investigations present unprecedented synthesis of prostate-targeted nonsteroidal antiandrogen-DOTA-gadolinium complex designed for prostate cancer magnetic resonance imaging. Binding studies clearly affinity toward AR.

Conclusions: We believe that use of prostate-targeting ligand complexes, containing appropriate radioactive metal ions, could be also very promising for PET- and SPECT-based imaging of prostate tumors. Our study paves the way for the introduction of novel 'dual-purpose' receptor-targeted pharmaceutical precursors (metal chelators), which depending on a dosage and utilized metal ions, are designed to be suitable for diagnostics or therapy. To further evaluate this type of compounds performance as potential therapeutic agents, additional experiments are planned to study these materials stability, target-specificity, and metabolism.

Acknowledgment: The Israel Science Foundation and the Raymond and Beverly Sackler Institute of Biophysics at Tel Aviv University for purchasing the MRI scanner and to the Strauss Institute of Computational Imaging at Tel Aviv University for data processing. We thank the Tel Aviv University for their financial support and to the Maiman Institute for Proteome Research of Tel-Aviv University for their contribution in performing mass spectrometry analyses.

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poster number: 146

MONITORING *IN VIVO* FLUORESCENCE MOLECULAR IMAGING OF LUNG INFLAMMATION AFTER ACUTE EXPOSURE TO TOBACCO SMOKE IN MOUSE STRAINS WITH DIFFERENT SUSCEPTIBILITY

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Introduction: Pulmonary matrix metalloproteinases (MMPs) are extracellular proteolytic enzymes involved in acute lung inflammation in response to cigarette smoke exposure (CSE) *(1)*. We present in vivo detection of MMPs activity in mouse strains with different susceptibility to developing smoking-induced emphysema to track time of CSE-induced acute inflammation in order to determine molecular differences between these two strains in the early stages of the disease.

Methods: To accomplish this task, susceptible (C57BL/6j) *(2)* and resistant (129S2/SvHsd) *(3)* mice were exposed to acute CSE using a whole-body exposition 24, 48 and 72h after CSE, MMPs activity was assessed by optical imaging system using a MMPs-sensitive activatable fluorescence probe *(4)*. Furthermore MMPs protein levels in the lung tissue were analyzed by Western Blot analysis. We also measure inflammatory mediator levels (TNF-a) and NF-?B activity by EMSA.

Results: In vivo semiquantitative optical imaging analysis of pulmonary inflammation revealed a significantly increase of MMPs activity in the lung of smokers measured at 24 and 48h after acute-CSE than in nonsmokers C57 mice while 129S2 did not observed changes at all times studied. Ex vivo imaging of the lungs of each group of mice confirmed the same in vivo experimental results obtained for both strains of mice. In the biochemical study of lung tissue, only in susceptible mice most important MMPs protein levels were significantly increased in the lung tissue of smokers compared with the non-smokers group. Respect to the studied of NF-?B, significantly increased at 24 and 48h, in correlation with an increase in the levels of TNF-a, only in C57 mice.

Conclusions: Fluorescence molecular imaging via MMPs-activatable probe provides a useful, effective, and rapid technique for in vivo monitoring and semiquantitative analysis of lung inflammation and MMPs expression. We are able to distinguish between susceptible (C57) and resistant (129S2) mice strains in terms of the profile of MMPs activity in the early stages of pulmonary disease after acute CSE. The results of our study suggest that mechanisms underlying different susceptibilities to CSE can be tracked at the beginning of the disorder and over time and can be explained in terms of inflammatory mediators and transcription factors.

Acknowledgement: This work is supported in part by the Spanish 'Ministerio de Ciencia e Innovación'(SAF2008-05412-C02- 02) and CIBERES (CB06/06/0009).

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poster number: 147

15O--WATER AND 68GA-DOTA PET IMAGING FOR ASSESSMENT OF BLOOD FLOW AND VASCULAR PERMEABILITY IN A RAT MODEL OF INFLAMMATION - COMPARISON WITH ULTRASOUND IMAGING

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Introduction: Increased vascular permeability and blood flow are among key events of inflammation. Based on the fact that Gd-DOTA is commonly used in MR imaging, we evaluated ^{68}Ga-DOTA for PET imaging of vascular permeability and perfusion.

Methods: Rats with turpentine oil induced sterile skin/muscle inflammation were evaluated under rest and adenosine (120 µg/min/kg) induced stress with ^{15}O-water and ^{68}Ga-DOTA PET, and contrast-enhanced ultrasound (US) imaging with non-targeted microbubbles. Rat blood pressure was measured using a tail-cuff non-invasive method. For quantification of PET data, ROIs were drawn on left ventricle (LV), myocardium, inflammation focus and healthy muscle. Blood flow was computed using 1-compartmental model taking input function from LV PET imaging data. Maximum standardized uptake values (SUV_{max}) were calculated for both tracers. From the US imaging studies, maximum peak intensities were determined for inflammation and muscle.

Results: Adenosine infusion decreased blood pressure on average of 15 mmHg and heart rate of 20 bpm. High focal uptake of both ^{15}O-water and ^{68}Ga-DOTA was seen at the site of inflammation. According to ^{15}O-water PET, adenosine increased myocardial blood flow (MBF, rest 0.37±0.01 and stress 0.52±0.08 (ml/min/g, P = 0.035) but not blood flow of inflammation or muscle (rest 0.66±0.23 and stress 0.69±0.13 and rest 0.14±0.03, stress 0.12±0.04, respectively). The blood flow of inflammation was significantly higher compared to muscle (P = 0.014). At rest, the SUV_{max} of ^{15}O-water was 3.1-fold higher in inflammation compared to muscle (P = 0.009). According to ^{68}Ga-DOTA PET, adenosine had no effect on K_1 of inflammation or muscle (rest 0.34±0.11, stress 0.29±0.05 and rest 0.17±0.03, stress 0.10±0.01, respectively). At rest, the SUV_{max} of ^{68}Ga-DOTA was 2.9-fold higher in inflammation compared to muscle (P = 0.03). Enhancement with intravascular US contrast agent confirmed at rest increased blood flow at the site of inflammation as compared with contralateral side.

Conclusion: Adenosine induced changes in rat MBF were revealed with ^{15}O-water PET. However, the adenosine induced stress had no apparent effect on blood flow of inflammation focus or healthy muscle. The uptake of ^{68}Ga-DOTA was significantly higher at the site of inflamed skin/muscle compared to healthy muscle, suggesting that inflammation induced changes in vascular permeability may be detected with ^{68}Ga-DOTA.

poster number: 148

DENDRITIC POLYGLYCEROLSULFATE NIR FLUORECENT DYE CONJUGATE TO VISUALISE INFLAMMATORY PROCESSES IN THE LUNGS IN A MOUSE MODEL OF ACUTE ALLERGIC ASTHMA

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Introduction Dendritic polyglycerol sulfates (dPGS) are a class of compounds with both anticoagulant and anti-inflammatory activities (1, 2). They bind L-selectin on leukocytes and P-selectin on inflamed vascular endothelium reducing leukocyte extravasation by shielding the adhesion molecule. In the present study, we investigated the biodistribution of dPGS and dPG conjugated with a near infrared (NIR) dye chromophore (dPGS-NIR and dPG-NIR) using a mouse model of acute allergic asthma. Time Domain (TD) Optical Imaging analysis were undertaken upon intravenous administration of the active and control polymers to evaluate accumulation at the site of lung inflammation.

Methods A total of 37 mice were sensitized intraperitoneally at days 0 and 21 with ovalbumin (OVA). We then administered a solution of OVA intranasally on days 28 and 29. At day 31 mice were injected intravenously with either with dPGS-NIR or the control dPG-NIR conjugates and scanned at 0, 4 and/or 24 hrs by using the small-animal TD Optix MX2 preclinical imager. After the last in vivo imaging session, animals were sacrificed and ex vivo optical imaging of lungs was performed. Asthmatic and naive lungs were fixed in 10% buffered formalin and embedded in paraffin for histopathological evaluation.

Results Two groups, each consisting of asthmatic and control mice, were analyzed, one at 4 hrs and the second 24 hours after dPGS-NIR injection. An additional two groups were analyzed 4 hrs and 24 hrs after dPG-NIR control probe injection. In vivo TD Optical Imaging revealed a significant difference in relative average intensity (RAI) over the lung areas between control and asthmatic mice treated with dPGS-NIR probe 4 hrs post injection, while no significant difference in RAI was observed between control and asthmatic mice treated with the dPG-NIR control conjugate both in living mice and in ex vivo analysis.Inflammation sites and accumulation of the dPGS-NIR probe within the lung of mice during acute onset of asthma was confirmed by NIRF microscopy and histological analysis. These data show accumulation of polyglycerol sulfates in lungs of animals with severe lung inflammation.

Conclusions We found a higher concentration of polyglycerol sulfates NIR dye conjugate in asthmatic lungs compared to healthy lungs, suggesting a specific accumulation of polyglycerol sulfates in the inflammation site. Therefore, this novel dendritic polyglycerolsulfate NIR fluorecent dye, in combination with TD optical imaging might be a novel approach for the non-invasive visualisation of inflammatory processes in the lungs of mice during the course of inflammation and in response to therapy in preclinical studies.

Acknowledgement Funding for the P3AGI project is provided by the European Commission through an FP7- IAPP Marie Curie Action - (GA 230739).

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poster number: 149

OPTICAL IMAGING OF ACTIVATED MMPS IN THE PANCREAS OF NON-OBESE DIABETIC (NOD) MICE AS A MEASURE OF RESIDUAL ß-ISLET FUNCTION

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Type 1 Diabetes (T1D) is an autoimmune disease of the pancreas which results from T-lymphocyte mediated destruction of the insulin-producing ß-cells. Disease progression is asymptomatic, with induction of insulitis only when 90 % of the ß-cells are destroyed at late stages of disease development. Hence, to be able to non-invasively image the extent of functional ß-cell mass would provide a powerful diagnostic tool in families predisposed to diabetes and an efficient means of following disease progression. Towards this end, we have explored the use of MMP-targeted probes in fluorescent reflectance imaging (FRI) of pancreases of NOD mice, which spontaneously develop T1D, as a measure of residual ß-cell function. Our choice of MMPs as a biological target is based on high gelatinase activity in healthy ß-islets, as determined by in situ zymography [1], which colocalizes with insulin producing ß-islet cells.

During progression of T1D, the pancreatic islets are invaded by leukocytes resulting in progressive ß-islet loss and associated loss of gelatinase positive ß-cells. The aim of the present study was to verify whether the small synthetic hydroxamate-based (CGS 25966) [2] and barbiturate based (RO 28-2653) MMP-targeted probes [3] specifically recognize activated gelatinases in ß-islets and, if so, whether these probes can be employed to image ß-islet loss and disease progression in NOD mice.

Cy5.5-labeled CGS 25966 and RO 28-2653 were tested for their ability to recognize activated gelatinases in tissue sections and by means of fluorescent reflectance imaging (FRI). Both probes bound to frozen sections of healthy pancreases, revealing a similar pattern as observed with in situ zymography. In sections of diabetic NOD pancreases, both probes recognized only the non-leukocyte infiltrated areas where residual gelatinases occurred. Intravenous injection of the probes into different aged non-diabetic and diabetic NOD mice and analysis of excised pancreases by FRI, revealed an accumulation of both probes in pancreases and reduced probe accumulation correlating with increased disease progression.

These results demonstrate that Cy5.5-labeled hydroxamate-based as well as barbiturate-based MMP-targeted tracers are suitable probes for in vivo imaging of ß-islet loss in NOD mice. The next step is to translate these probes into clinical imaging modalities such as PET and SPECT, as radiotracers for non-invasive assessment of human T1D.

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poster number: 150

FUNCTIONAL PHASE CONTRAST X-RAY LUNG IMAGING IN A PRECLINICAL ASTHMA MODEL

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Introduction X-ray based imaging is the major imaging method utilized in clinical practice. It outranges all other modalities in terms of spatial resolution. Nevertheless, along with the radiation dose applied to the patient it suffers from a poor soft tissue contrast and a low sensitivity hampering all functional imaging approaches. Application of phase contrast x-ray imaging enables strong tissue interface enhancement accompanied with reduced dose due to the facts that phase shift is proportional to the used x-ray energy and about 100 times stronger than absorption in soft tissues. Current developments show that its limitation to Synchrotron sources can be overcome and therefore it will be a big leap forward in next generation medical x-ray imaging [1,2]. Here we combine this technique with the application of barium labeled macrophages as functional contrast agent targeting inflammation sites inside the lungs of an acute asthma mouse model. Demonstrating the feasibility of both high resolution and functional x-ray imaging.

Method Ovalbumin treated and challenged BalbC mice were intra tracheal injected with double barium and DiD (a NIR fluorescence dye) stained macrophages taken from an immortalized mouse alveolar macrophages (MHS cell line, 3) cell line grown in vitro. 24h after macrophage administration and 48h after the last challenging mice were imaged in-vivo with a NIR fluorescence imager to demonstrated accumulation of the macrophages in the asthmatic lungs. Wild type mice injected with the same amount of macrophages as well as a native mouse without injected macrophages were used as controls. After the fluorescence imaging mice were sacrificed, the lungs inflated in-situ with air under a constant pressure to maintain comparability, and imaged with the phase contrast x-ray microCT at the SYRMEP beamline of the Elettra Synchrotron (Trieste, Italy).

Results We could clearly depict morphological alterations of the lung tissue caused by the inflammation by analyzing the phase effects in comparison with the control group. After utilizing a novel phase retrieval algorithm phase and absorbance information were decoupled enabling the detection of macrophage home-sites in the lung which otherwise are cloaked by the phase effects.

Discussion In the here presented study we successfully combined the remarkable features of phase contrast x-ray imaging with labeled macrophages as carrier of contrast agent to inflammation sides. We show that medical x-ray imaging in the near future will produce sharper images even in soft tissue applications, can dramatically reduce the applied radiation dose to the patient and could also support functional imaging approaches.

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poster number: 151

IN VIVO MRS ANALYSIS OF NON-ALCOHOLIC FATTY LIVER DISEASE IN A TRANSLATIONAL MODEL FOR THE METABOLIC SYNDROME

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Introduction : The metabolic syndrome is characterized by the co-occurrence of several risk factors, such as obesity, insulin resistance and dyslipidemia. Prominent pathology herein is the currently untreatable liver cirrhosis, which is preceded by non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis. Since histopathological evaluation of liver biopsies is clinically not-feasible, non-invasive Magnetic Resonance Spectroscopy (MRS) is used to assess liver fat content as a surrogate marker for liver pathology. The aim of this study was to investigate whether NAFLD could be quantified in a translational mouse model for the metabolic syndrome by repeated MRS measurements using a clinical 3T MR scanner, and whether MRS could be used to evaluate the effect of anti-diabetic and hypolipidemic drugs on disease progression.

Methods : Male APOE*3Leiden.CETP mice (n=4-5 per group) were fed a High Fat Diet (HFD) for 3-4 months to induce obesity, insulin resistance, hyperlipidemia and hepatosteatosis. The latter was confirmed by in vivo MRS measurements on a clinical 3T scanner adapted with a dedicated animal coil. Thereafter, the mice were fed the HFD alone (HFD control) or were treated with either rosiglitazone (10 mg/kg/d) or ezetimibe (3 mg/kg/d). 5 Mice on a chow diet were included as healthy controls. Effects on plasma lipid and glucose levels, as well as hepatic fat content by means of MRS were assessed before and after 4 weeks of treatment. The in vivo MRS data was compared to ex vivo liver analyses by histology, HPTLC, Magic Angle Spinning-NMR and near infra red (NIR) based diffuse optical spectroscopy.

Results : Hepatic fat content could be accurately quantified using MRS. Dietary treatment resulted in hepatosteatosis with a hepatic fat content of 14.3 ± 4.9% in the control group (vs 2.7 ± 0.7% in the chow group), which significantly increased to 25.8 ± 10.6% ($P<0.05$) during the study. Rosiglitazone reduced the hepatic fat content by 76% ($P<0.01$) as compared to control, and even reduced hepatic fat content as compared to before treatment by 56% ($P<0.05$). Ezetimibe did not significantly reduce hepatic fat content as compared to control, but prevented further disease progression in time. Data obtained in vivo by MRS highly correlated with the ex vivo analyses histology, MAS-NMR, HPTLC and NIR spectroscopy ($P<0.001$).

Conclusion : We showed that hepatic fat can accurately be measured in vivo using a clinical 3T MR scanner in a translational mouse model for the metabolic syndrome. Rosiglitazone and ezetimibe showed similar effects on NAFLD as was observed in humans. Therefore, we conclude that this mouse model using a clinical relevant imaging method is a highly translational setting to investigate the effects of new drugs on risk factors of the metabolic syndrome and its complications like NAFLD.

MOLECULAR NEUROIMAGING

poster number: 152

CB1 RECEPTOR [125I]SD7015 RADIOLIGAND IMAGING IN MOUSE BRAIN

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Introduction: We aimed to evaluate a high-affinity (KI = 3.4 nM) and moderately lipophilic (cLogD = 4.14) CB1 receptor (CB1R) radioligand [125I]SD7015 ([125I]1-(2-iodophenyl)-4-cyano-5-(4-methoxyphenyl)-N-(piperidin-1-yl)-1Hpyrazole-3-carboxylate)1 for SPECT imaging of mouse brain CB1R in vivo. The multiplexed multipinhole SPECT system, NanoSPECT/CTPLUS, is capable of quantitative, high-resolution imaging of 125I-labelled compounds in small animals in vivo.

Methods: We determined the biodistribution of radioactivity in CB1R knock-out (-/-) mice (n = 3), control wild-type (+/+) mice (n =2), and control C57BL6 mice (n = 3) under urethane narcosis after injecting about 1 MBq of [125I]SD7015 radioactivity systemically with SPECT/CT imaging. A mouse MRI atlas was fused to the SPECT/CT images by using a combination of rigid and non-rigid algorithms. After SPECT imaging, animals were killed and their excised brains, livers and bodies were measured for radioactivity in a well-type counter. Phosphor imager plate autoradiography (ARG) was performed on 4 µm-thin cryostat sections of the excised brains.

Results: The fuse of images show SPECT/CT/MRI-atlas coronal brain sections from CB1R(+/+) mice with corresponding ex vivo ARG plates. ARG of identically taken sections from CB1R(-/-) mice showed very low radioactivity uptake. Brain radioactivity was 0.11±0.05% (average ± SD) of injected activity in (+/+) mice and 0.01±0.00% in (-/-) mice at 2 h after injection. Some asymmetry was present in all positive in vivo images. Only noise and blood/liquor signal was observed in (-/-) mouse SPECT images. High radioligand uptake regions in SPECT images obtained in control animals correspond well to CB1R-rich areas.

Conclusions: [125I]SD7015 readily enters mouse brain to enable SPECT imaging. Brain radioactivity distribution largely matches that of known CB1R expression. [125I]SD7015 appears to be a promising SPECT radioligand for studying mouse brain CB1R.

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Reference: Donohue S.R. et al. (2009) Bioorg. Med. Chem. Lett. 19, 6209-6212.

poster number: 153

EFFECT OF ANESTHESIA ON FUNCTIONAL CONNECTIVITY NETWORKS OF THE RAT BRAIN

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Introduction: Functional Magnetic Resonance Imaging of the brain's 'resting state' (rsfMRI) has evolved into an important tool to investigate intrinsic functional networks of the brain [1] and various neurological disorders have been found to correlate with changes in functional connectivity. Applied to animal models, rsfMRI has an enormous potential to track progression, recovery or therapy of various diseases on and individual and longitudinal basis. Anesthesia required for animal MRI, however, may confound functional connectivity results. We therefore assessed functional connectivity networks in the rat under Medetomidine (MED) sedation and Isoflurane (ISO) anesthesia respectively, both protocols allowing for survival experiments.

Methods: Resting state fMRI data were obtained from male Wistar rats (n=17) under 1.5% ISO anesthesia and - subsequently in the same session - under MED sedation [2]. Data was acquired using gradient echo planar imaging on an 11.7 T Bruker BioSpec system. After pre-processing (co-registration, filtering), functional data underwent independent component analysis (ICA) using FSL. Independent components were classified through hierarchical clustering. A seed based analysis was performed to complement ICA. For this purpose, functional data was pre-processed the same way and additionally regressed for physiological noise [3]. Seed regions were defined based on ICA results and pair wise correlations between these regions were calculated before and after removing global signal fluctuations.

Results: ICA found 10-19 independent components (ICs) in each dataset (Median/SD: 15/3 ISO, 13/3 MED). Reproducible and distinct bilateral networks were identified under MED sedation, three in the cortex (medial, intermediate, lateral) and two in the striatum (dorsal / ventral). Under ISO, however, similar networks were observed in only a few datasets and were less distinct. Seed based analysis revealed that initially high interhemispheric connectivity in both, cortex and striatum, was substantially driven by low frequency global signal fluctuations under ISO. Under MED, in contrast, strong interhemispheric connections were less affected by removal of global signal fluctuations. Interestingly, global signal removal revealed negative correlations along cortico-striatal connections in particular under MED.

Conclusions: Our results show that connectivity networks in the rat brain revealed via ICA show significant differences in MED sedation vs. ISO anesthesia. Connectivity networks identified in the MED regime are stronger, more reproducible and spatially more coherent, which is supported by a recent report using a seed-based analysis approach [4] and comparison to other studies [5,6]. The origin of low frequency global signal fluctuations under ISO could be related to effects of synchronicity as observed in burst-suppression EEG. We conclude that MED sedation of the rat is to favor over ISO anesthesia whenever functional connectivity networks are to be studied in their complexity and greater level of detail.

Acknowledgements: This work was upported by the German Federal Ministry of Education and Research (BMBF 0314104).

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MULTIMODAL CHARACTERIZATION OF TRANSGENIC BACHD RATS WITH FULL-LENGTH HUMAN MUTANT HUNTINGTON

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Background: Huntington`s disease (HD) is the most common neurodegenerative disorder in Western Europe affecting muscular coordination leading to cognitive decline, dementia and finally to complete incapacitation. The disease is supposed to be caused by an autosomal dominant mutation of the gene huntingtin (htt) and is accompanied by a widespread loss of neurons, which is earliest seen in the caudatus putamen (CPu). Although recent research has intensively focused on HD, only few promising approaches achieved clinical relevance, because of the lack of appropriate animal models. Most rat models mirror different aspects of HD, but lack the full-length mutant htt protein. Therefore some aspects of the human condition might be imperfectly replicated. In order to overcome this potential disadvantage, a transgenic rat expressing full-length mutant human htt (tg5) was generated to study the early onset of the HD like phenotype including reduced binding potential of ^{11}C-raclopride in the striatum as well as progressive motor deficits and impaired motor skill learning.

Methods: Our aim was to characterize the D2-receptor's loss/dysfunction and the behavioural deficits of tg5 rats. For the longitudinal imaging studies we inject 25.9MBq of the specific radiolabeled D2-receptor ligand ^{11}C-raclopride i.v.. Simultaneously we started a dynamic 60min PET scan, followed by a 15min attenuation correction. Data was repeatedly acquired at rat's (n=5) age of 4, 12 and 18 months. The same rats were analyzed once a month during aging in a rotarod test to detect progressive motor deficits and impaired motor skill learning. In parallel we analyzed rat's behaviour in an elevated plus maze (EPM). On the EPM test day rats were adapted to the environment prior to behavioural testing. After 30min rats were placed consecutively in the middle of the EPM. One run lasted 5min while rats were allowed to explore the maze. For subsequent analyzes the percentage of time spend in the open arms was calculated. Additionally a DARPP32 as well as an immunhistological staining using a specific anti-htt antibody S830 was performed, to visualize and correlate the neurodegenerative effects caused by the htt-mutation.

Results: In imaging studies we observed a significant decrease in binding potential of ^{11}C-raclopride in the striatum of tg5 compared to WT rats during aging (only 60% of WT binding potential could be observed in 18 month old tg5 rats), indicating a strong involvement of the dopaminergic system. This observation is supported by ex vivo histological datasets, revealing a manifested neurodegeneration most prominent in the striatum shown by DARPP32 staining as well as in all behavioural models tested. Here tg5 rats showed massive impairments in the EPM and significant motor deficits already at the age of 1month. Tg5 rats also had difficulties in learning a motor skill task and thereby did not improve even after 12 training trails.

Conclusion: The neuropathological changes in addition to the early onset of the observed behavioural deficits makes this transgenic rat valuable for fundamental research and therefore better understanding of HD pathogenesis leading to future preclinical therapy studies.

poster number: 155

A PET STUDY OF TSPO IMAGING IN PATIENTS WITH MULTIPLE SCLEROSIS USING [18F]FEDAA1106

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Introduction: Inflammation in the CNS is characterized by activation of microglia cells. This process includes the up-regulation of the translocator protein (18 kDa) (TSPO) system (1). TSPO imaging may provide useful information in various CNS neuroinflammatory diseases, such as Multiple Sclerosis (MS). Recent studies using TSPO PET radioligands such as [^{11}C]PK11195 and [^{11}C]PBR28 have indicated their potential to distinguish between healthy subjects and patients with MS (2,3). [^{18}F]FEDAA1106 is a recently developed PET radioligand for in vivo quantification of TSPO (4). Considering the advantages of F-18 labeled TSPO radioligands to PET facilities without access to cyclotron, we investigated the diagnostic potential of [^{18}F]FEDAA1106 in patients with MS.

Methods: Nine patients with relapsing-remitting MS in acute relapse and gadolinium (Gd) enhancing lesion(s) on MRI (3 on interferon beta therapy and 6 without immunomodulatory therapy; 7F/2M; age 34.2 ± 9.1 y.o.) and 5 healthy controls (4F/1M, age 38.0 ± 9.7 y.o.) were investigated at Karolinska Institutet. Dynamic PET acquisitions were performed with a ECAT EXACT HR system (Siemens Medical Solutions) for a total of 150 min with a 30 min break, after injection of 153.4 ± 10.2 Bq of [^{18}F]FEDAA1106. Metabolite-corrected arterial plasma samples were used as the input function. Image analysis in MS patients and healthy controls was approached in several ways. 1. Regions of interest (ROIs) in several cortical and subcortical regions were analyzed using 2-tissue compartment kinetics model in order to estimate binding potentials (BP_{ND}) and distribution volume (VT). 2. The possibility of estimation of BP_{ND} for MS lesions was investigated. 3. VT parametric images estimated by Logan plot and standard uptake value (SUV) images were generated. The images were visually compared with corresponding MRI, focusing on MRI-identified MS lesions.

Results: Kinetic modelling analyses in patients with MS and healthy controls showed no significant difference of BP_{ND} or VT in any examined ROI. Robust BP_{ND} values could not be obtained for MS lesions as time activity curves of most MS lesions were noisy due to their small size. VT and SUV images did not demonstrated higher uptake than surrounding areas in MRI-identified MS lesions. In MRI-identified MS lesions close to cortical regions, the uptake could not be differentiated from the accumulation in cortical regions.

Conclusions: This study did not support [^{18}F]FEDAA1106´s potential to differentiate between patients with MS and healthy controls or to detect MS plaques in patients with MS. Possible reasons for this finding could be low robustness of outcome measures of [^{18}F]FEDAA1106 or biological heterogeneity in TSPO expression.

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poster number: 156

THE SYNTHESIS AND EVALUATION OF 5-HT1A AGONISTS AS PET IMAGING AGENTS

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Introduction: The monoamine hypothesis postulates that reduced serotonergic tone is pivotal in the pathogenesis of mood and anxiety-related disorders. Although, now widely accepted that such disorders are multifactorial in origin and progression, serotonergic dysfunction remains a key mediator. The serotonergic system is the primary target for investigative research into the etiology and therapy of these disorders. The main focus being on the g-protein coupled 5-HT1a receptor. Molecular neuroimaging techniques, such as Positron Emission Tomography (PET), provides a means for studying changes at the 5-HT1a receptor and could provide a practical diagnostic tool for these mood disorders. Currently, only antagonist radioligands for the 5-HT1a receptor are available, labelling both high and low affinity states of the receptor. Studies carried out to date with these ligands show contradictory results. It has been suggested that these difficulties may be overcome by exclusively considering the high affinity binding state with an agonist radioligand. The present study outlines attempts to develop a suitable fluorinated agonist compound with the aim of demonstrating in vivo imaging efficacy.

Methods: A series of compounds were synthesised based on a generic structure. Compounds were assessed for affinity, selectivity and degree of agonism in a panel of in vitro assays. Those with favourable characteristics were evaluated in vivo. Here, we present findings from compounds with ranging degrees of agonism to the 5-HT1a receptor and compare with the antagonist [18F]MPPF. Conscious male Sprague-Dawley rats were administered radioactivity (i.v.) and then sacrificed at discrete timepoints between 2 and 60 minutes post injection (p.i.) by cervical dislocation. Brains were immediately dissected into subregions chosen to represent high, medium and low 5-HT1a receptor density. Specific binding was calculated using cerebellum as a reference region. Competition studies were conducted at the 5-HT1a, dopamine (D2) and alpha 1 adrenergic receptors by predosing animals with unlabelled WAY (1mg/kg), Raclopride (2mg/kg) and Prazosin (1mg/kg) respectively 5 minutes before radioligand.

Results: Despite very good in vitro profiles, in vivo the [18F]-radiolabelled agonists failed to show significant retention in 5-HT1a receptor rich brain areas. However, the 18F radiolabelled partial agonists did show higher uptake and better retention in vivo than the full agonists, although attenuated when compared to [18F]MPPF. Further assessment of the compounds showed that the agonists elicited a potent serotonergic response in vivo compared to the partial agonists confirming the compounds were able to bind to the 5-HT1a receptor invivo, although a specific signal could not be attained when rodents were administered the [18F]-radiolabelled compound.

Conclusions:A range of possible explanations for the lack of an in vivo specific signal is discussed and we conclude that high affinity 5 HT1a receptor agonists may not make suitable imaging agents, although our partial agonists do hold some promise.

poster number: 157

IN VIVO DETECTION OF AMYLOID-B DEPOSITS USING HEAVY CHAIN ANTIBODY FRAGMENTS

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Introduction: This study investigated the in vivo properties of two heavy chain antibody fragments (V_HH), ni3A and pa2H, to differentially detect vascular or parenchymal amyloid-ß deposits characteristic for Alzheimer's disease and cerebral amyloid angiopathy.

Methods: Blood clearance and biodistribution including brain uptake were assessed by bolus injection of radiolabeled V_HH in APP/PS1 mice or wildtype littermates. In addition, in vivo specificity for Aß was examined in more detail with fluorescently labeled V_HH by circumventing the blood-brain barrier either via direct application or intracarotid co-injection with mannitol.

Results: Both V_HH showed rapid renal clearance (10 - 20 min). Regarding brain uptake pa2H resulted in a significant higher brain uptake 24 hours post injection in the APP/PS1 animals. In vivo specificity for Aß was confirmed for both fluorescent V_HH, where pa2H remained readily detectable for 24 hours or more after injection. Furthermore, both V_HH showed affinity for parenchymal and vascular deposits, in contrast to human tissue, where ni3A specifically targeted only vascular Aß.

Discussion: Despite a brain uptake that is as yet too low for in vivo imaging, this study provides evidence that V_HH detect Aß deposits in vivo, with high selectivity and favorable in vivo characteristics, making them promising tools for further development as diagnostic agents for the distinctive detection of different Aß deposits.

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REGENERATIVE MEDICINE

poster number: 175

NOVEL FUNCTIONAL OPTICAL IMAGING OF CRANIOFACIAL BONE DEFECT REPAIR

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Introduction: The establishment of new therapeutic approaches for tissue repair and regeneration could be accelerated if adequate technologies to monitor the repair process are developed. In cranio-maxillofacial surgery, 96,000 bone grafts are performed each year to regenerate bone loss due to trauma or disease. Autologous bone, usually used to treat these lesions, is not always available and necessitates additional surgery for its harvest. Alternatively, there is a large potential supply of craniofacial allografts, which are composed of non-vital bone. Yet, these grafts often fail to integrate due to the formation of scar tissue around them. We hypothesized that functional fluorescence imaging (FLI) of osteogenesis, angiogenesis and osteoclastogenesis in calvarial defects treated with allografts would provide new insights that would enable the development of novel therapies for allograft bio-integration.

Methods: A critical-size calvarial defect was created in FVB/n mice. Groups included transplantation of: allografts, autografts, and untreated defects (used as control). For blood flow and angiogenesis imaging the mice were injected with AngioSense 750 or Integrisense 750 on Days 5 and 12-post transplantation. FLI of angiogenesis was done using epi-illumination 24 hours after probe injection. Signal quantification eliminated autofluorescence. For osteogenesis imaging the mice were injected with OsteoSense 680 or 800. Imaging with Osteosense was performed on Day 20, in vivo and then ex vivo following mice sacrifice. Finally, bone resorption activity was imaged using Cathepsin-K 680 probe. Bone formation and vasculogenesis were analyzed as well using Micro CT imaging and histological analysis.

Results: Functional FLI of blood flow showed significantly higher fluorescence on Day 6 than on Day 13 in both treated groups and was greater in both treated groups than in the control group. Imaging of OsteoSense 680 on Day 20 in vivo and ex vivo showed no difference. Thus, although epi-illumination FLI is known for high tissue attenuation, in this model the signal is preserved. MicroCT and histological analysis performed on the calvaria samples corroborated the results of osteogenesis and angiogenesis detected by functional FLI.

Conclusion: Our findings clearly indicate that functional FLI is a reliable tool for monitoring the repair of a calvarial bone defects. An evaluation and validation of new therapies involving allografts for the repair of calvarial injuries may be obtained using this system. Furthermore, a better understanding of the healing process in craniofacial defects treated with allografts may be obtained.

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MR IMAGING OF A NOVEL 19F LABELED HYALURONIC ACID HYDROGEL

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Introduction: Hydrogels are widely used for growth factor delivery and tissue replacement. Hyaluronic acid (HA) hydrogels are of interest because of their role in wound repair and development of cultured cells. Moreover, the architecture, mechanics and degradation of HA hydrogels can be manipulated for special purposes*. However, the biodegradation of HA hydrogels and biodistribution of their degradation products can differ drastically for different implantation site. Therefore, proper imaging approaches are required, to know about the residence time of the HA hydrogels at given injection site. The aim of this research is to investigate the MR imaging possibilities of the HA material in order to assess its biodistribution and monitor its degradation and disappearance at the injection site. Since ^{1}H MRI is limited for this purpose we considered that labeling with ^{19}F atoms would be a good option to obtain high selectivity imaging with quantitative information at low cytotoxicity. Hence, we chemically linked ^{19}F to HA hydrogel, and then investigated the material with MR imaging.

Material and Methods: Injectable ^{19}F-immobilized HA hydrogel was obtained by mixing ^{19}F-labeled HA polymer containing carbazate groups with another HA derivative containing aldehyde groups. The reaction between the carbazate and aldehyde group causes cross-linking of the polymer chains into three-dimensional matrix*. The concentration of polymer was 2%(w/v) and the amount of fluorine was1.9 mass% per solid content of the hydrogel. The MR imaging and spectroscopy were performed on a 7T MR system (Bruker BioSpin MRI) with a home-built $^{1}H/^{19}F$ double tuned solenoid coil. ^{1}H images are obtained with a Gradient Echo (GE) Flash 2D, with a FOV = 60x60 mm, Matrix = 128x128, slice thickness= 20 mm while TR = 100 ms, TE = 10 ms, TA = 13 sec. ^{19}F MRS was performed with a pulse-acquire sequence at TR = 1500 ms, TA = 1.5 sec with 1 average. ^{19}F images were acquired by GE Flash 2D, with a FOV = 60x60 mm, Matrix = 32x32, slice thickness =20 mm while TR = 88 ms TE = 1.2 ms TA = 12:03 min.

Results: In vitro and in vivo experiments (subcutaneous and intramuscular injection of hydrogel in rat legs) showed that the solid content HA hydrogel can be visualized by ^{1}H MR imaging. However, the lesions seen on MR images not necessarily fully coincides with the presence of the hydrogel and thus are not seem to be specific. The hydrogel showed a strong ^{19}F signal by MR spectroscopy, indicating that ^{19}F MR imaging would be possible. Indeed by 2D19F GE imaging it was possible to obtain good ^{19}F images of the material.

Conclusion: The novel ^{19}F HA hydrogel appears to have proper MR imaging possibilities for the visualization of the material in vivo. Experiments in which we employ these features to monitor the degradation and biodistribution of the HA hydrogels in vivo are ongoing.

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Reference: *.D. A. Ossipov, X. Yang.et al. Chem Commun (Camb), 2010, 46, 8368-8370.S2

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MOLECULAR IMAGING OF STEM CELL-MEDIATED THERAPY FOR VERTEBRAL COMPRESSION FRACTURES

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Introduction: Vertebral compression fractures are the most common fragility fractures accounting for approximately 700,000 injuries per year. Since open surgery involves morbidity and implant failure in the osteoporotic patient population, new minimally invasive solution are being developed. These methods include injection of synthetic nonbiological material that does not resorb and remains a permanent foreign-body fixture. Therefore there is a clear clinical need for a biological solution for vertebral bone repair. We have previously shown that BMP-transfected adipose-derived stem cells (ASCs) are capable of inducing spinal fusion in vivo. We hypothesized that direct injection of ASCs, transiently expressing BMP6, to a vertebral bone defect would accelerate bone regeneration leading to fracture repair.

Methods: Porcine ASCs were isolated and labeled with a lentiviral vector that encodes for two reporter genes, Luciferase (Luc) under constitutive and tissue-specific (Osteocalcin) promoters. Labeled ASCs were transiently transfected with a BMP6 plasmid using nucleofection. 24-hours later bone void defects were created in coccygeus vertebra of Nude rats. The cells were suspended in fibrin gel and injected into the bone void. A control group was injected with fibrin gel only. The regeneration process was monitored in vivo using µCT, while cell survival and differentiation were monitored using bioluminescent imaging (BLI). The operated vertebrae were harvested after 12 weeks, and analyzed using histology and immunohistochemistry against porcine vimentin.

Results: In vivo BLI detected the Luc-expressing cells at the implantation site for 12 weeks. The gradual decay of the signal probably indicates cell death and growth arrest due to MSC differentiation. µCT scans showed that starting from 4 weeks post surgery, considerable defect repair was seen in the group treated with ASC-BMP6. Complete repair was achieved 12 weeks post cell injection. ASCs contributed to new bone formation, shown by immuno-staining against porcine vimentin.

Conclusions: In this study we have shown the potential of injected, BMP-transfected, ASCs to repair vertebral bone defects in a rat model. These results could pave the way to a novel approach for a biological treatment of traumatic and osteoporosis-related vertebral bone injuries.

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LIVER PERFUSION AFTER IRON LABELED INTRA-HEPATIC PANCREATIC ISLETS TRANSPLANTATION AND GRAFT OUTCOME: PRELIMINARY RESULTS FROM A MAGNETIC RESONANCE IMAGING CLINICAL STUDY

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Introduction: Vascular phenomena as islets revascularization or portal microthrombosis could play an important role in pancreatic islets engraftment after intraportal transplantation. Aim was to assess the functional modification of liver perfusion related to intrahepatic iron labeled islet transplantation (SPIO-islet-tx) using dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI).

Methods:Three diabetic patients (#1,#2,#3) were enrolled until now and received SPIO-islet-tx. Islets were labeled with a clinically approved contrast agent composed by superparamagnetic iron oxide nanoparticles (Ferucarbotran) [1]. MRI with T2* sequences was performed before islet-tx and 1, 2, 3, 7 days, and each month after islet-tx; before islet-tx and 1 day and 7 days after transplantation DCE-MRI was also performed with a 3D-T1w-TFE dynamic sequence (temporal resolution: 4 sec; 45 dynamic scan) acquired during gadolinium bolus injection. Both T2* sequences and DCE-MRI were performed on a 1.5T magnet (Achieva Nova, Philips Medical System) encompassing the entire liver. Semiquantitative analysis of DCE-MRI was performed on a dedicated software (ViewForum, Philips) to calculate the maximum enhancement (ME) and area under curve (AUC) for each liver segment and each time point.

Results: Patients #1 and #3 achieve good mid-term graft function; patient #2 experienced graft failure during the first month. In patient #2 the complete graft function loss was associated with all hypointense spots disappearance during the first month of MRI follow-up. In patients #1 and #3 the good graft function was associated with MRI evidence of an hypointense spots loss between 60% and 80% during the first month followed by minimum progressive loss in subsequent months. Liver perfusion parameters strongly decreased from basal to 7 days DCE-MRI in the patient with graft failure (Pt#2: ME=-30%; AUC=-28%). In the other two patients the same parameters showed an increase in the liver perfusion at 7 days post-tx (Pt#1: ME=+5%; AUC=+13%. Pt#3: ME=+17%; AUC=+38%). The changes in perfusion parameters after islet-tx were significantly different comparing Pt#2 with patients with working graft ($p<0.01$).

Conclusions: Liver perfusion impairment in the earliest phases after islets-tx may impact on the islets engraftment. Our preliminary data provides the first evidence in human of an association between early changes in liver perfusion and graft outcome. Data on largest population are needed to confirm this hypothesis. These data, if confirmed with the enrollment of more patients, suggest that microvascular changes occurring after islet-tx could influence and predict clinical outcome.

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REPORTER SYSTEMS for MOLECULAR IMAGING

poster number: 179

MN LOADED APOFERRITIN: A MRI SENSOR OF CELLULAR REDOX CAPABILITIES.

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Introduction. MRI has reached a prominent position among the imaging modalities thanks to its outstanding spatial and temporal resolution. The mains drawback deals with the low sensitivity of its contrast enhancing probes that limits the potential applications in the field of molecular imaging. Therefore great efforts are currently devoted to design probes endowed with an enhanced sensitivity. It has been shown that a naturally occurring system such as apoferritin can be an excellent candidate for generating a high sensitive contrast agent. Firstly, apoferritin has been loaded with Gd-HPDO3A to yield a system endowed with a relaxivity of ca. 800mM-1s-1 per particle. Next, an increase of almost one-order of magnitude (r1 ca. 7000mM-1s-1) was obtained when apoferritin inner cavity was filled up by Mn^{2+} ions. The preparation of the latter system relies on a chemical reduction step that transforms partially the previously loaded solid ß-MnOOH into Mn^{2+} ions that remain entrapped in the apoferritin cavity. The chemical transformation that enables the T_1 silent Mn-Apo to convert into a powerful relaxation agent prompts us to seek whether an analogous transformation may be operated by a naturally occurring reductant. At physiological pH, the redox potential of Mn(III)/Mn(II) pair is 1.5V. Although several naturally occurring processes may display suitable redox characteristics, it appear necessary to deal with processes that operate massive transformation inside the cell in order to overcome the above mentioned sensitivity issues of the MRI technique. Melanin formation in melanocytes has been selected to test the ability of Mn-Apo to act as sensor of the massive oxidation of tyrosine derived products that yield the formation of the black insoluble macromolecule.

Methods. Mn-Apo was incubated in the presence of melanogenic and non-melanogenic B16-F10 obtained by growing cells in a humidified atmosphere containing 5 or 10% CO_2 , respectively. Melanogenesis was evaluated by measuring the absorbance of cells lysate at 490 nm.

Results. It is well established that starting from tyrosine, successive oxidation steps lead to dopamine 5,6 DHI and dopachrome that readily polymerize to yield melanin. First the reduction of Mn-Apo has been carried out ‘in vitro’ by adding to its solution, aliquots of L-DOPA and tyrosinase. Proton relaxation rate of Mn-Apo solution steadily increases in parallel with melanin formation as assessed spectrophotometrically. Next the Mn-Apo ability to act as reporter of melanin formation was assessed on B16-F10 melanoma cells. Melanogenesis is sensitive to extracellular pH and ionic strength. Therefore, at relatively acid pH (6.7-7.2) the melanogenic process occurs slowly or is completely inhibited as confirmed by absorbance measurements of cell lysates. Only melanogenic cells showed a marked enhancement of their MRI signal intensity.

Conclusions. Mn-Apo can act as ‘in vivo’ sensor of massive oxidative process responsible for the melanin formation in melanocytes. The almost undetectable SI enhancement observed in non-melanogenic cells demonstrated the lack of other oxidative metabolic pathway that can significantly affect the Mn-Apo reduction.

poster number: 180

IMAGING THE PH EVOLUTION IN AN ACUTE KIDNEY INJURY MODEL BY MEANS OF IOPAMIDOL, A NEW MRI-CEST PH-RESPONSIVE CONTRAST AGENT

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Introduction: Pathological altered renal physiology resulting from acute kidney injury (AKI) or tubular acidosis is associated with a perturbation of renal pH [1]. Clinical biomarkers of kidney damage, like blood urea nitrogen (BUN) and serum creatinine denote kidney damage only after a significant loss (50%) of renal function has occurred [2]. Therefore, newer approaches able to provide reliable and non-invasive surrogate biomarkers of kidney injury as pH values would have considerable clinical relevance. Magnetic Resonance Imaging is a primary diagnostic and imaging technique. Recently, we proposed that Iopamidol, a clinical-approved radiopaque X-Ray contrast agent (CA), can be used as a pH-responsive CA for MRI-CEST (Chemical Exchange Saturation Transfer) investigations [3]. In this study, we investigated the use of Iopamidol to monitor the disease evolution 'in vivo' by imaging pH variations in glycerol-induced AKI model.

Methods: AKI model was induced in BALB/c mice (n=8, Charles River) by intramuscular injection of a 50% glycerol solution (8 mL/kg body weight) into the inferior hind limbs. Healthy mice (n=6) were used to assess the pH base levels. A clinical dose of Iopamidol (0.75 g Iodine / kg b.w.) was injected via a catheter into the tail vein. CEST images were acquired on a 7 T scanner Avance 300 (Bruker BioSpin) using a fast spin-echo sequence preceded by a saturation pulse (3 μT, 5 sec). CEST spectra were interpolated by smoothing splines and the ratio of the saturation transfer effects at 4.2 and 5.5 ppm was used for the calculation of the pH values. Animals were imaged at the following time points: day 0 and after the glycerol injection at days 1, 3, 7, 14 and 21. After mice were sacrificed and paraffin-fixed biopsy kidney samples were sectioned (5 μm); luminal hyaline casts, tubular damage and dilatation were assessed by light microscopy to evaluate renal histology.

Results: In control mice we observed, 15 min after Iopamidol injection, a mean pH value of 6.7. In AKI mice, pH evolution follows a slight increase in the first days (mean pH values 7.1 and 7.3 after 1 and 3 days, respectively). After pH values start to decrease (pH = 6.9 after 1 week, pH = 6.8 after 2 weeks) reverting to control values (pH = 6.7 after 3 weeks). This evolution closely followed the time evolution BUN levels with a peak after 3 days, a recovery starting after one week and with the complete restoration at day 21. A good inverse correlation was also observed between the number of pixels highlighted by the presence of Iopamidol in post-contrast images in kidney regions with the lesion stage associated with the tubular injury.

Conclusion: Iopamidol, a MRI-CEST CA allowed to image in vivo renal pH and to follow the kidney damage evolution in a glycerol-induced AKI model.

Acknowledgements: Economic support from regional government (NanoIGT project)

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poster number: 181

DEVELOPMENT AND *IN VITRO* VALIDATION OF A LENTIVIRAL VECTOR FOR MULTI-MODALITY CELL TRACKING USING BIOLUMINESCENCE, FLUORESCENCE AND RADIONUCLIDE IMAGING

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Introduction: Multimodality visualisation of transduced cells in vivo is of interest to quantify and localise these cells over longer periods of time. In this study we developed a lentiviral vector displaying a fusion protein of gaussia luciferase (Gluc) and Yellow Fluorescent Protein (YFP) on its surface. This combination allows FACS validation of the YFP expression, bioluminescence imaging (BLI) of cell survival both in vitro and in vivo and SPECT/Fluorescence tomographic imaging using ^{99m}Tc and red fluorescent labeled anti-YFP Nanobodies.

Methods: Fusion protein expression was driven by a CMV-promoter and membrane anchorage, allowing nanobody binding, was ensured by the in-frame cloning of the PDGFR-transmembrane domain at the 5' end of the reporter. HEK 293T cells were transduced with these lentiviral vectors and the tranfection efficiency was evaluated by flow cytometry. The YFP-positive fraction was subcloned and cell-membrane specific expression of YFP was evaluated using fluorescence microscopy (Evos FL). GLuc expression was evaluated in vitro using BLI (Biospace Photon Imager) in cell concentrations ranging from 10e3 up to 10e10 cells per well. Anti-YFP nanobodies were labeled with ^{99m}Tc using tricarbonyl chemistry and the labeling efficiency was assayed on instant thin layer chromatography (ITLC). In vitro binding studies of ^{99m}Tc labeled anti-YFP nanobodies were performed and binding of fluorescently labeled anti-YFP nanobodies was qualitatively scored using fluorescence microscopy.

Results: The YFP-positive HEK 293T cells were subcloned by flow cytometry. We demonstrated that the fluorescent signal was confined to the cell membrane on fluorescence microscopy. Next, BLI was performed and was found to be significantly higher in the transduced group, compared to the dsRed-transduced control group. Our results showed these significantly higher values throughout our dilution series (10e3 to 10e10 cells). 99mTc labeling of anti-YFP nanobodies yielded a labeling efficiency of 99%. Binding studies of ^{99m}Tc labeled nanobody showed receptor-specific binding as it was blocked by an excess of cold anti-YFP nanobody. Binding of fluorescent dye labeled anti-YFP nanobodies was successfully visualised in vitro using fluorescence microscopy.

Conclusion: We developed a functional lentiviral vector combining fluorescence, bioluminescence and SPECT imaging possibilities of transduced cells in vitro. The next step is to test the functionality of this vector in an in vivo model.

poster number: 182

ELECTRON PARAMAGNETIC RESONANCE AS A NEW SENSITIVE TOOL TO ASSESS THE IRON CONTENT IN CELLS AND TISSUES FOR MRI CELL LABELING STUDIES

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Introduction: The ability to track the migration of metastatic cells when leaving the seeding tumor should become an invaluable tool to understand this phenomenon and propose new therapeutic anti-cancer strategies. Magnetic cell labeling is a promising technique to detect metastatic cancer cells with magnetic resonance imaging. Usually, cells are incubated with iron oxides (T2 contrast agent) in order to uptake the particles before being injected in vivo. In addition to MRI protocols, there is generally a need for complementary techniques able to confirm and quantify the cells that have migrated inside a host tissue. We propose here to implement Electron Paramagnetic Resonance (EPR) as a very sensitive method to quantify iron oxide concentration (in cells and tissues) and to optimize labeling protocols. Iron oxide particles exhibit an EPR spectrum, which directly reflects the number of iron oxide particles in a sample. EPR spectroscopy has already been proposed as a method of quantifying the accumulation of iron oxide inside tissues (1) and macrophages (2). In order to compare EPR with existing methods (Perl's Prussian blue reaction, and fluorimetry), we labeled tumor cells (melanoma B16F10-luc, renal adenocarcinoma RENCA) and fibroblasts 3T3 with fluorescent iron oxide particles, and defined the limit of detection of the different techniques.

Methods: Cell labelling: B16F10, RENCA and 3T3 were grown in full medium. One day before the experiment, particles of iron oxide (Molday Ion Rhodamine B, MIRB supplied by Biopal) were incubated overnight. Incorporation of MIRB in cells: Cells were rinsed three times with PBS, trypsinized, and counted. For EPR measurements, the cells were resuspended in pure water in order to be analyzed by EPR. Cells were also analyzed by fluorescence cytochemistry. Colorimetric assays were performed using an OD 700 nm . Fluorescence quantification of the MIRB accumulated inside the cells was done using a ?emission= 550-650 nm and a ?excitation = 555 nm. Limit of detection was defined as the standard deviation of the calibration curves multiplied by three.

Results: MIRB iron oxide particles accumulated intracellularly with no remaining particles in the plasma membrane for all the three cell lines. EPR was able to give an objective and accurate information about labeling efficiency (requiring a very limited amount of sample). Iron oxide quantification studies with EPR show that all the cells uptake from 10 to 20 pg Fe/cell. Moreover, this technique was implemented for kinetics studies of iron oxide uptake in cells. With the present setup, the limit of detection of MIRB particles was 104 ng/ml (7.8 ng detected) using EPR, 860 ng/ml (172 ng detected) using Perl's reaction, and 624 ng/ml for fluorimetry.

Conclusions: EPR is a fast, easy, and highly sensitive method to quantify iron oxide content after magnetic labelling. Moreover, EPR allows ex vivo Fe quantification, a good way to confirm and quantify MRI results after cell tracking experiments.

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poster number: 183

NOVEL GD(III)-BASED PROBES FOR MR MOLECULAR IMAGING OF MATRIX METALLOPROTEINASES

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Introduction: Matrix Metalloproteinases (MMPs) are a family of zinc dependent endoproteases that degrade proteins in the extracellular matrix (ECM) and have a key role in tissue remodeling in normal (angiogenesis, tissue repair) as well as pathological conditions (cancer, stroke, neurodegeneration). Different pathological states are characterized by a specific profile of MMP expression, and such profile may be regarded to as a sort of signature of a given pathology. For instance, the level of MMPs expression and activity in malignant cancers is generally higher than in normal or premalignant tissues, with maximum activity occurring in areas of active invasion at the tumor-stroma interface.1 The visualization by MRI of MMP activity in a given tissue or microenvironment would be of great value for the grading of the pathological process and for the choice and calibration of therapy.2 We are developing new probes for the MR molecular imaging of MMP activity, based on a gadolinium-based contrast agent undergoing to an amphiphilic-to-hydrophilic transformation after cleavage by MMPs.

Methods: Two novel Gd-based contrast agents (CAs) for the molecular imaging of MMPs have been synthesized through solid phase peptide synthesis. The two tested CA are based on the PLG*LWAR peptide sequence, known to be hydrolysed between Gly and Leu by a broad panel of MMPs. A Gd-DOTA chelate has been conjugated to the N-terminal position through an amide bond, either directly to proline (compd Gd-K11) or through a hydrophilic spacer (compd Gd-K11N). Both CAs were made strongly amphiphilic by conjugating an alkyl chain at the C-terminus of the peptide. The compounds were characterized in vitro by NMRD profiles and enzyme kinetics were measured for MMP-1/2/9/12. Compound Gd-K11 was systemically administered to mice bearing a subcutaneous B16.F10 melanoma, either pre-treated or not with the broad spectrum MMP inhibitor GM6001 to follow the washout kinetics from the tumor microenvironment.

Results: The relaxivity of Gd-K11 (20 MHz, 25 °C, pH 7.4) was 8.5 mM-1s-1, while that of Gd-K11N was dependent upon the concentration of the complex (in the range 5.4-12.7 mM-1s-1) because of the formation of polydisperse aggregates. Both CAs have an affinity in the order of hundreds of uM for serum albumin. Efficient cleavage by MMPs was confirmed by MMP-12 and MMP-2. Upon MMP-dependent cleavage, the CAs loose the C-terminal tetrapeptide and the alkyl chain, thus undergoing to an amphiphilic-to-hydrophilic transformation altering tissue pharmacokinetics. As a result, the washout of Gd-contrast enhancement from subcutaneous melanoma (mouse model) was significantly slower for subjects treated with ilomastat (MMP activity inhibited) with respect to untreated animals (native MMP activity).

Conclusions: Washout kinetics from the tumor microenvironment of Gd-contrast agents are responsive to the local activity of MMPs.

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OTHERS

poster number: 184

EVALUATION OF DIFFERENT BIFUNCTIONAL CHELATORS FOR THE DEVELOPMENT OF 177LU-LABELED NANOBODIES

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Introduction: Nanobodies show favourable pharmacokinetic characteristics for tumor targeting. Nanobodies, labelled with a therapeutic radionuclide, may be used for the treatment of cancer. Within this project, we evaluated four different bifunctional chelators to identify an optimal connection between the therapeutic radionuclide Lutetium-177 and the nanobody.

Methods and materials: We conjugated the nanobody with the different bifunctional chelators p-Bn-SCN-DOTA, DOTA-NHS-ester, DTPA-CHX-A and 1B4M-DTPA. Mass Spectrometry (MS) was performed to assess the degree of conjugation. 177Lu labeling was performed during 45 min at 55 °C for the DOTA-analogues, while an incubation of 30 min at room temperature (RT) was used for the DTPA-based chelates. Purification was done using disposable gel filtration columns. Radiochemical purity was analyzed with Instant Thin Layer Chromatography (ITLC-SG) and High Performance Liquid Chromatography (HPLC). Stability in PBS at RT and human serum at 37°C was evaluated with Instant Thin Layer Chromatography (ITLC-SG) and size-exclusion chromatography up to 72 h pi. Affinity and specificity were evaluated in binding assays on specific cancer cell lines. Ex vivo biodistribution was studied in tumor bearing nu/nu mice. After 1, 3, 24 and 48 h, the animals were dissected and major organs and tumors were collected and counted. Radioactivity was expressed as % injected activity per gram tissue (% IA/g). Finally, In vivo SPECT/micro-CT was performed using tumor bearing nu/nu mice.

Results: MS results revealed a conjugation of 1,5 chelator average per nanobody. After labeling, HPLC and ITLC showed a radiochemical purity >95%, indicating a successful labeling procedure. Stability studies of the different constructs showed >95% intact nanobody in PBS and >95% in human serum after 72h. In vitro results revealed nanomolar affinity of the 177Lu-labeled nanobodies towards their target. Accumulation of the activity in the tumors was the highest at 1 h pi, with 4.17 ± 0.45 % IA/g, 3.03 ± 0.51 %, 2.41 ± 0,40 % IA/g and 2.45 ± 0.53 % IA/g when using DTPA-CHX-A, DTPA-1B4M, DOTA-NHS and p-Bn-SCN-DOTA, respectively. Tumor accumulation then gradually decreased in time with 1.64 ± 0.44 % IA/g, 1.32 ± 0.32 % IA/g, 1.15 ± 0.24 % IA/g and 1.34 ± 0.23 % IA/g at 3 h p.i., and below 1 % IA/g at 24 and 48 h. Kidney uptake was high for all four constructs, up to 150 % IA/g 1h pi. The DTPA-analogues showed improved tumor-to-muscle values compared to DOTA-analogues, with 42.50 ± 8.69 %, 29.91 ± 7.32 %, 7.84 ± 1.02 % and 10.41 ± 1.73 % at 1 h pi to 14.94 ± 2.98 %, 16.00 ± 0.60 %, 8.58 ± 1.90 % and 2.84 ± 1.36 % at 48 h pi.

Conclusion: We showed a successful labeling of cancer targeting nanobodies with 177Lu by using different bifunctional chelators. The nanomolar affinity of the nanobody was maintained after labeling. Stability up to 72 h was comparable for all four chelates. In terms of in vitro specificity and ex vivo biodistribution, the DTPA-chelate analogues are preferential over the DOTA-based chelates for development of 177Lu-labeled nanobodies.

poster number: 185

THE IMAGING PROBE DEVELOPMENT CENTER AT THE NATIONAL INSTITUTES OF HEALTH: PRODUCTION OF A WIDE RANGE OF MOLECULAR IMAGING PROBES

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Introduction: The Imaging Probe Development Center (IPDC) was established as part of the National Institutes of Health (NIH) Roadmap for Medical Research Initiative (http://nihroadmap.nih.gov/), under the 'Molecular Libraries and Imaging' section of the 'New Pathways to Discovery Program'. IPDC's set up as a core resource recognizes that molecular imaging, and the probe chemistry that underlies it, constitute key technologies. The IPDC produces and provides to collaborators known and novel imaging probes including optical, radionuclide and magnetic resonance agents and may produce any type of contrast agent. IPDC has already collaborated with over fifty investigators in providing probes.

Methods: Organic syntheses, radiolabeling, bioconjugation, nanoparticle fabrication.

Results: Probes synthesized at IPDC include small organic compounds, biologicals and complex nanoparticles. More specifically, they encompass, by way of examples, fluorogenic enzyme substrates, fluorescent dyes, radiolabeled drug candidates, caged dyes that become fluorescent upon uncaging, labeled antibodies, liposomes and dendrimers. Details of probes already prepared or under preparation will be described in order to illustrate the breadth of the molecular imaging chemistry at IPDC, accompanied by a brief summary of the diverse biological applications for various probes.

Conclusions: As a trans-NIH effort IPDC core synthetic chemistry serves all Institutes and Centers. Its influence is expected to impact widely different subject matter spanning biological research on molecular imaging. IPDC may selectively offer its synthetic, radiochemistry and bioconjugate chemistry services to the broader imaging community, particularly to those imaging scientists who may be limited by a lack of chemistry resources.

To learn more readers are encouraged to visit the IPDC website at http://www.ipdc.nih.gov

poster number: 186

IMAGING OF IRON OXIDE (ENDOREM®) LABELLED TRANSPLANTED PANCREATIC ISLETS BY MAGNETIC RESONANCE IMAGING, FROM BENCH TO BEDSIDE.

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Introduction Islet transplantation is one of the most effective procedures for restoring normoglycemia in type-1 diabetic patients, but long-term islet graft function is achieved only in a minority of cases. Developing a reliable, non-invasive imaging modality to trace over time the fate of islets transplanted within the liver would help the interpretation of functional parameters monitored in the periphery. Non-invasive magnetic resonance imaging of pancreatic islets is an attractive option for the 'real-time' monitoring of graft evolution. The aim of the present study was to translate into the clinic the imaging approach optimized in the animal model for monitoring non-invasively islet graft persistence after transplantation.

Methods Human and mouse islets were labelled according to the standardized protocol developed previously (Malosio et al, 2009) with the clinically approved contrast agent Endorem®, which is composed of superparamagnetic iron oxide nanoparticles. Labelling efficiency, insulin content and secretion, viability, proinflammatory status and cytokine release was monitored in labelled islets and compared to unlabeled ones. In the animal model intrahepatic transplanted labeled islets were comparatively imaged at 7T and 1.5T. In humans a fraction of transplanted islets were labeled and following intrahepatic transplantation monitored by MRI at 1, 2, 3, 7 days and once a month up to one year after transplantation. In parallel metabolic measurements of islet function (glycemia; C-peptide; insulin need; HbA1c) were acquired at the same time points of MRI scans.

Results The ratio of Endorem®/islet is crucial for reproducible labeling. Labeled islets are neither inflamed nor more susceptible to inflammatory insults than unlabeled ones. In mice transplanted labeled syngenic islets revert diabetes and can be detected by MRI as hypointense spots, both at 1.5T and 7.0T magnetic fields, up to 6 months after intrahepatic infusion. Also in diabetic patients labeled islets are clearly visible by MRI as hypointense spots scattered within the liver parenchyma after transplantation. In one patient complete loss of hypointense spots accompanied islet graft failure (undetectable c-peptide) occurring at one month after transplantation. The other patients showed an important loss of hypointense spots during the first week and the first month (60-80% loss) after transplantation, despite partial graft function.

Conclusions Islet monitoring by MRI following transplantation is feasible and safe in patients. The complete loss of islet-associated signals in the patient with complete graft failure suggests that MRI monitoring reflects the condition described by the metabolic measure (c-peptide; insulin need). The reason why the other patients showed discrepancy between reduction of islet signals and partial graft function is still under investigation. Since the syngenic murine model analyzed and the transplanted diabetic patients, have a different immunological condition, imaging by MRI labelled islets in the clinical setting of autotransplantation would be very important to understand the potential of this approach for the follow-up of islet transplantation.

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[111IN]SARGASTRIN 2 VS. [111IN]SARGASTRIN 1: EFFECTS OF GLU(6-10) / DGLU(6-10) REPLACEMENT ON RENAL ACCUMULATION AND CCK2-R+ TUMOR-TARGETING IN SCID MICE

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Introduction: Radiolabeled minigastrin (MG) and cholecystokinin (CCK) analogs have been proposed for application in the diagnostic imaging and radionuclide therapy of CCK2-R^+ human tumors, such as medullary thyroid cancer (MTC) [1]. We have previously reported on Sargastrin-1 ([(DOTA)Gln^1,Nle^{15}] Gastrin I) a DOTA-functionalized analog of the heptadecapeptide Gastrin-I (pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2) which can be labeled with a wide range of clinically relevant metallic radionuclides. [^{111}In] Sargastrin-1 was able to effectively target CCK2-R^+-tumors in experimental animals, but exhibited unfavorable high accumulation in the kidneys [2]. Radiolabeled minigastrin analogs undergone (Glu)5 / $(DGlu)_5$ substitutions have been recently shown to accumulate significantly less in mouse kidneys [3]. We decided to adopt this promising strategy and accordingly, we synthesized Sargastrin-2 ([(DOTA)Gln^1,$DGlu^{6-10}$,Nle^{15}]Gastrin I), wherein Glu^{6-10} have been replaced by $DGlu^{6-10}$. The success of these structural modifications was tested by direct comparison of [^{111}In]Sargastrin-1 and [^{111}In]Sargastrin-2 in mouse models.

Methods: Rat pancreatic cancer AR4-2J cells spontaneously expressing the CCK2-R were cultured in a humidified-5% CO_2 atmosphere at 37^{0}C in Ham's F-12 nutrient mixture supplemented with 10%FBS, 100 U/mL penicillin / 100 µg/mL streptomycin. Inocula (~1 x 10^7 cells suspended in 150 µL normal saline) were subcutaneously injected in the left flank of young SCID mice. After 12 days well-palpable tumor masses developed at the inoculation site and biodistribution was conducted. Animals received either [^{111}In]Sargastrin-1 or [^{111}In]Sargastrin-2 by bolus injection (100 µL, 2 µCi, 10 pmol total peptide) in the tail vein. Animals were sacrificed in groups of four at 4 and 24 h postinjection (pi) and tissues were excised, weighed and counted in a gamma-counter. Values were calculated as percent injected dose per gram (%ID/g) tissue and are expressed as mean ± SD.

Results: [^{111}In]Sargastrin-2 displayed a significantly reduced kidney uptake *vs*. [^{111}In]Sargastrin-1 (46.3±9.8 %ID/g *vs*. 104.4±15.7 %ID/g at 4 h pi). [^{111}In]Sargastrin-2 showed less accumulation than the parent radiopeptide in the CCK2-R^+-implants (2.55±0.93%ID/g *vs*. 5.74±1.62%ID/g of [^{111}In]Sargastrin-1 at 4 h pi), as well as in the gastric mucosa of mice (1.65±0.47 %ID/g *vs*. 3.6±0.5%ID/g of [^{111}In]Sargastrin-1 at 4 h pi).

Conclusions: Replacement of Glu^{6-10} by $DGlu^{6-10}$ in the [^{111}In] Sargastin-1 motif led to a ~2.5-fold reduction of renal accumulation, but also to an analogous reduction of CCK2-R targeting in experimental animals. Further comparative evaluation of the two analogs in CCK2-R^+-models is currently in progress.

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INFLUENCE OF ANIMAL HANDLING AND IMAGE RECONSTRUCTION ON 18-FDG BIODISTRIBUTION IN BALB/C MICE USING PET

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Introduction: Obtaining dynamic parameters of in-vivo tracer-distribution is very important for studying and optimizing targeted imaging and therapy. The aim of this study was to determine optimal animal handling and image reconstruction to study the biodistribution of 18FDG in mice using dynamic scans.

Methods: In total 30 experiments were performed using 6 BALBc mice, each mouse was used in 5 different setups: 1. Fasted overnight, pre-warmed 30 degrees, iv. cannula (isoflurane) 2. Fasted overnight, pre-warmed 30 degrees, iv. needle (awake) 3. Fasted overnight, pre-warmed 30 degrees, ip. (awake) 4. 60 min awake after iv. needle (awake) 5. Pre-warmed 30 degrees, iv. cannula (isoflurane). On average 7.4±0.4 MBq 18FDG was administered per experiment. Mice were anesthetized using 2.5% isoflurane and scanned until 90 min at 36-38°C after intravascular (iv) or intraperitoneal (ip) injection using a Siemens Inveon animal PET scanner. Images were reconstructed using filtered back projection (FPB) and ordered subsets expectation-maximization (OSEM3D) with standard settings for noise reduction, voxel size and iterations. The 3rd group served as reference; this group was found to give the best results after ip. injection at 30°C by Fueger et al. in 2006. For analysis, acquisition data obtained between 60-90 min post injection were reconstructed and the standard-uptake-value (SUV) at 75min were compared. Two observers independently performed manual segmentation of the the brain, brown fat, heart, kidneys, muscle, abdomen and bladder. Where possible, the full-with-half-maximum (FWHM) SUV was used to define the metabolic boundary of the tissue/organ.

Results: Between groups biodistribution varied largely. Compared to group 3, all groups showed increased heart and brown fat uptake, with less brain and kidney uptake. However, due to the intraperitoneal administration method, group 3 showed additional inhomogeneous abdominal radioactivity, potential hampering assessment of metastasis in tumor-bearing mice. Muscle and brown fat uptake were higher when animals were awake during administration (applied for easier injection, group 2 and 4). OSEM3D reconstruction resulted in less image noise and reconstruction artifacts. The overall quantification was comparable for both reconstructions. Using the described segmentation method there was a large consistency in the data, on average the difference was 0.2±0.02 SUV implying that using the FWHM method results in a low inter-observer variability.

Conclusion: Depending on the region of interest, animal handling should be adjusted such that maximal contrast is achieved between the target and surrounding tissue activity. Especially with metastasis models, no ip injection should be performed. Although the results between filters were in general comparable, further research has to be performed to validate the quantification by either FBP or OSEM3D reconstructions. Using well defined method for tissue/organ delineation, intra-observer variability can be minimized.

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IN VIVO IMAGING OF TSPO IN MOUSE PERIPHERAL TISSUES AND BRAIN USING MICROPET-CT AND [18F]DPA-714

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Background: In vivo binding of [18F]DPA-714 to TSPO has been characterized with PET/ microPET in normal primate(1) and in rat models of neuroinflammation (2). However, only limited data are available on [18F]DPA-714 binding in peripheral tissues, despite the interest of its application to the study of TSPO function in extracerebral regions. Moreover, the feasibility of in vivo [18F]DPA-714 imaging in mice has never been reported. Here we evaluated with microPET the in vivo biodistribution of [18F]DPA-714 and the specific binding in extracerebral regions in normal mice.

Methods: [18F]DPA-714 has been synthesized as described elsewhere (3). C57BL mice (age: 8-9 weeks; weight: 30-20g; anesthesia: i.p. ketamine-xylazine) were injected in the tail vein (9.4 ± 2.4;MBq; SRA: 163 ± 89 GBq/μmol). Dynamic PET acquisition was performed using the μPET-CT GE Explore Vista (spatial resolution: PET=1.6 mm). Six 10 min frames were acquired starting 10 min after radiotracer injection. To evaluate the specificity of radioligand binding, unlabeled DPA-714 (1mg/kg) was injected 10 minutes prior the radiotracer injection. Parametric images of SUV were obtained and ROIs defined for lungs, heart, spleen, kidney and brain.

Results: After [18F]DPA-714 injection, the uptake was high in lungs, heart, kidney and spleen. Between 10-30 minutes post [18F]DPA-714 injection, SUV values were higher in the lung (3.7±0.6) and spleen (2.9±0.4) followed by the kidney (2.1±0.7), heart (1.6±0.7) and brain (0.17 ±0.03). Between 30-60 min, SUV values were decreased in the lung (2.7±0.4), remained stable in the heart (1.6±0.6) and brain (0.18±0.08), whereas increased in the spleen (3.5±0.3) and kidney (3.0±0.4). Pretreatment with unlabelled compound inhibited almost completely the uptake of [18F]DPA-714 in all extracerebral structures at 30 minutes post-radiotracer injection. At later times there was an increase of SUV values more evident in the spleen and kidney and a redistribution of radioactivity to the intestine and to the renal medulla.

Conclusions: These preliminary results suggest that [18F] DPA-714 allows in vivo imaging of TSPO in mice. Biodistribution of [18F]DPA-714 follows the well known distribution of TSPO receptors in brain and peripheral tissues and the specific binding of [18F]DPA-714 is high in TSPO reach regions. Radioactive metabolites might account for increased SUV values at later times after the radiotracer injection mostly in the kidneys and spleen and deserve further investigation.

Aknowledgement This work is supported in part by DiMI, LSHB-CT-2005-512146

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AUTOMATED SEGMENTATION AND BONE VOLUME AND THICKNESS MEASUREMENTS IN SMALL ANIMALL WHOLE-BODY MICROCT SCANS

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Introduction: Quantification of osteolysis is crucial for monitoring treatment effects in preclinical research and should be based on MicroCT data, rather than conventional 2D radiographs, to obtain optimal accuracy. However, data navigation is greatly complicated in case of three dimensional data. Here we present an automated method to assess the progress of osteolytic lesions quantitatively and visually in whole-body follow-up MicroCT data of mice. Based on a previously published approach for atlas-based whole-body registration of the skeleton and automated Volume of Interest determination for individual bones, this work presents functionality for highly accurate segmentation of a structure of interest. This enables the user to monitor disease related changes in bone volume over time. In addition, a method for measuring and visualization of cortical bone thickness is presented.

Methods: The method was evaluated using MicroCT datasets of fifteen animals bearing an osteolytic tumor in the right tibia. Scans were made at baseline, at three weeks and at seven weeks. MicroCT scans were made using a SkyScan-1076 MicroCT scanner (SkyScan, Kontich, Belgium). The datasets were reconstructed with voxel size 36.5 x 36.5 x 36.5 um. Tibial bone volumes were measured manually by two independent observers and by the fully automated system. To assess the agreement between the presented method and the human observers, Bland-Altman plots and Pearson's correlation coefficients are presented. A three-way repeated measures ANOVA was used for a more in-depth analysis of the dependency of the segmentation result on the observers.

Results: The Pearson's correlation coefficients, including all time points, are r=0.9996 (observer 1 vs. 2), r=0.9939 (observer 1 vs. auto) and r=0.9937 (observer 2 vs. auto). There was no significant difference the human and automated observers as tested with a three-way repeated measures ANOVA (p=0.10). There were significant changes in bone volume over time.

Conclusions: We described a fully automated method to measure bone volume. The correlation coefficients and Bland-Altman plots indicate a good agreement between human observers and the automated method. This conclusion is supported by the lack of a significant difference between the three methods. Changes in bone volume over time could be quantified using this new automated method. In addition, cortical thickness maps are generated giving the user an indication where in the bone the volumetric changes occur.

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ZINC-BASED FIXATIVE PROVIDES POSSIBILITY TO COMBINE IN SITU ZYMOGRAPHY, FLUORESCENCE IMMUNOHISTOCHEMISTRY AND IRON STAINING ON PARAFFIN-EMBEDDED TISSUE SECTIONS

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Introduction VSOP (very small iron oxide particles), developed for MR-angiography, are like other super-paramagnetic iron oxide particles used as potent contrast agents for molecular MRI. The particle accumulation in histological sections is usually shown by Prussian blue iron staining. Gelatin in situ zymography localizes activity of MMP-2/MMP-9 and other gelatin-degrading enzymes, which recently has been demonstrated in zinc-fixed paraffin-embedded tissues1. Here we demonstrate, that zinc-fixation of paraffin-embedded tissues allows besides zymography, fluorescence immunohistochemistry (IHC) and Prussian blue iron staining with excellent quality and preservation of morphology.

Methods and results Spleen tissue pieces of mice, treated with VSOP, were fixed in zinc-fixative (BD Pharmingen) for 24 hours at room temperature, dehydrated and embedded in paraffin through standard procedure. 6 µm sections were used for IHC and in situ zymography experiments with subsequent Prussian blue iron staining. At first, we performed Prussian blue iron staining on two parallel sections of the mouse spleen. On the first section, immunohistochemical staining revealed cells positive for MMP-2/MMP-9 fluorescence were found mainly in the red pulp and marginal zone of white pulp. Typically kidney-formed nuclei corresponded presumably with macrophages. On the second parallel section, enzymatic activity of both gelatinases was analyzed using in situ zymography, as described1. Highly defined FITC-fluorescence pattern of digested DQ-gelatin overlapped mainly with double immunostaining for MMP-2/MMP-9, reported before. After microscopic documentation, both sections were furthermore processed to subsequent Prussian blue staining. Good distinguishable iron deposits ensure compatibility of this staining technique on zinc-fixated tissue and provide possibility to detect enzymatic activity and iron-containing particles (VSOP) as well as show fluorescence-labeled proteins of interest and VSOP on one section. Single-cell analysis demonstrated also detailed morphology and precise spatial localization. Second, we tried to perform all three described staining techniques on one tissue section. For single-cell investigation, at higher magnification, combining of IHC and in situ zymography provided defined results after subtracting relatively strong background. Subsequent iron staining of the same slice yielded also well detectable results with good overlap.

Conclusion In summary, zinc salt-based fixation of paraffin sections provides an excellent method to combine IHC, in situ zymography and iron staining with very good morphology for investigations of molecular targets of iron oxide particles. Compared to frozen sections, this type of fixation better preserves tissue morphology. Protein antigens for IHC and activity of enzymes are preserved without cross-linking of functional groups, in contrast to widely used aldehydes.

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NORMALIZED VOLUME OF INTEREST SELECTION AND MEASUREMENT OF BONE VOLUME IN MICROCT SCANS

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Introduction At present, osteolytic lesions are quantified using 2D X-ray radiographs. The scoring of X-ray radiographs is performed by manual drawing of a region of interest (ROI) around the lesion, a method prone to observer bias. MicroCT scans provide 3D structural information which allows a more exact assessment of the disease induced changes. In contrast to conventional radiography, MicroCT scans can be used to quantify both the decrease in bone volume in case of osteolysis as well as the increase in bone volume in case of osteoblastic lesions. However, analyses of MicroCT scans are difficult and time consuming.

Methods For the image processing of the MicroCT scans, first a centerline through the bone was defined. Next, regular spaced planes perpendicular to the centerline were extracted. These planes were stacked into a new volume. In this new volume, cut-off planes were defined. The cut-off planes were transformed back into the original space after which a region grower, initiated at a single or multiple seed point(s) was used to select bone material. The region grower was set to stop when no further connected voxels met the bone criterion or at the cut-off planes indicated by the user.

Results The result is a method which allows section of a region of interest relative to anatomical features of the bone. We show that this method is reproducible. Using this method, we show the quantification of changes in bone volume, both loss and gain. In addition, we give an example of bones where 3D volume measurements give a better representation than 2D measurements.

Conclusions Here we present a new method to select volumes of interest in a normalized way regardless of curving, fractures or dislocations within the bone. In addition, this method enables the user to visualize normalized cross sections in an exact 90 degree angle or along the longitudinal axis of bone, at any given point. As a result, the user can compare measurements of diameter, volume and structure between different bones in a normalized manner.

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VALIDATION OF AN EASY NEW METHOD TO QUANTIFY *IN VITRO* MINERALISATION USING BONETAG OR OSTEOSENSE

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Throughout life, bone is constantly being remodelled. This process is a balance between bone resorption and bone formation. In osteoporosis, this balance is disturbed, leading to bone loss and an increased risk of fractures. Compounds that can stimulate bone formation would be of great importance in the treatment of osteoporosis. KS483 cells are multipotent mouse mesenchymal progenitor cells that can differentiate into chondrocytes (cartilage), adipocytes (fat) and osteoblasts (bone). These cells, therefore, provide an excellent tool to study compounds which stimulate bone formation *in vitro*. To monitor osteogenic differentiation in these cultures, Alizarin Red S staining is frequently used to indicate and quantify mineralisation and differentiation. Bonetag (Li-COR) and Osteosense (Perkin Elmer) are mineral binding compounds, labeled with a near infrared fluorescent tag, and are used for *in vivo* imaging of bone. Here we validate a method to easily quantify *in vitro* mineralisation of KS483 cells using these fluorescent probes. KS483 cells were cultured under osteogenic conditions in the presence of different concentrations of compounds that either stimulate (BMP-6) or inhibit mineralisation (NH2-olpadronate, or PTHrP). After 21 days of differentiation, the cells were incubated with Bonetag 800 or Osteosense 800 for 24h. Fluorescence was measured at 800nm with an Odyssey scanner (Li-COR). For comparison, differentiated cells were stained with Alizarin Red S, which was quantified by spectrophotometry after solubilization of the staining.

Our results indicate a good correlation between Alizarin Red S staining and fluorescent labeling by Bonetag and Osteosense. Also, changes in differentiation caused by BMP-6, NH2-olpadronate, or PTHrP can be visualized and quantified by these tags.

In conclusion, both Bonetag and Osteosense can be used to easily study the effects of compounds on differentiation and subsequent mineralisation of mesenchymal progenitor cells into bone forming osteoblasts.

poster number: 194

ESTIMATION OF HUMAN EFFECTIVE ABSORBED DOSE OF 67GA-DTPA-GONADORELIN BASED ON BIODISTRIBUTION RAT DATA

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Background: In this investigation, we used a robust description of organ biodistribution owning to large amount of biodistribution data (source organs in dosimetry calculations); we tried to estimate the effective radiation absorbed dose into human organs following i.v. administration of 67Ga-labeled Gonadorelin by using biodistribution data in normal rats.

Methods: Four rats were sacrificed at exact time intervals (0.25, 0.5, 1, 2, 4, 24 and 48 hour post injections) and the percentage of injected dose per gram of each organ was measured by direct counting from rat data. The Medical Internal Radiation Dose (MIRD) formulation was applied to extrapolate from rat to human and to project the absorbed radiation dose for various organs in the human [1]. In order to use the tables presented in the Medical Internal Radiation Dose (MIRD) No.11 [2] which are used to compute the absorbed dose, it is necessary to calculate the cumulated source activity (Ah) in each organ according to the equation below: $A=?^{8}_{t1}A_{h}(t)dt$ The Medical Internal Radiation Dose (MIRD) formulation was applied to calculate the absorbed radiation dose for various organs [2]. $D(r_k)=SA_hS(r_k \text{ to } r_h)$ Where the mean absorbed dose stated in (rad) to a target organ r_k from a radiotracer distributed identically in a source organ r_h has bean formulated by MIRD committee. The expressed in (rad/μCi-H) represented the specific absorbed fraction of energy for the target organ rk for particles emitted in source organ rh [2]. For Radiation Absorbed Dose Estimation: Methods consistent with those recommended by the MIRD Committee of the Society of Nuclear Medicine were used to determine absorbed doses into the normal organs and whole body [2].

Results: The results showed that most of the activity was accumulated in the urine and lungs. Nearly all excretion of activity occurred by the renal system, and hepatobiliary excretion was negligible. From rat data we estimate that a 185-MBq injection of 67Ga-cDTPA-GnRH into the human might resulted in an estimated absorbed dose of 5.26 mGy to the whole body and the highest effective absorbed dose was in lung with 2.73 mSv and the organs received the next highest doses were the bladder wall , liver, bone marrow, gastrointestinal, spleen, kidneys, testis and adrenals which received (1.59 mSv), (0.80 mSv), (0.53 mSv) , (0.41 mSv), (0.31 mSv), (0.21 mSv), (0.19 mSv) and (0.15 mSv), respectively.

Conclusion: The biodistribution of 67Ga-cDTPA-GnRH in rats showed high breast uptake and low muscle and blood uptake. These results suggest that it should be possible to perform early imaging of the breast anomalies and GnRH receptors indicating potential malignant lesions.

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poster number: 195

FROM BOG TO BENCH: PALEOPATHOLOGICAL ASPECTS OF HIGH RESOLUTION COMPUTED TOMOGRAPHY IMAGING

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Introduction: Bog bodies are intriguing archaeological finds of the northern half of Europe and are often the only preserved witnesses of the pre-Roman Iron Age because burial rites in this time were solely restricted to cremation. In an interdisciplinary approach, several mortal remains of four north-german bog bodies were analyzed by high resolution non-destructive volume computed tomography to get access to new morphological as well as functional information of these individuals.

Methods: Several bones and also the left and right calcaneus of the bog body 'Uchter Moor' (pre-Roman Iron Age) were scanned using a high-resolution clinical computed tomography (GE Healthcare). The left tibia of the bog body 'Kayhausen boy' (pre-Roman Iron Age) and the mummified skin of bog body 'Bareler Moor' (Roman Iron Age) were analysed by a flat panel detector based cone beam volume computed tomography (VCT) (GE Global Research Niskayuna, NY, USA). From bog body 'Esterweger Dose' (Middle Ages) a tibia fragment of 1 cm3 was scanned by micro-computed tomography (µCT, GE Healthcare), which achieves an isotropic resolution of 8 µm. A modified Feldkamp algorithm was used for image reconstruction resulting in isotropic high resolution volume data sets. Axial images as well as multiplanar reconstruction and volume rendering images were obtained and further analyzed with voxtools 3.0.64 Advantage Workstation 4.2 (GE Healthcare, Buckinghamshire, UK).

Results: Due to the demineralization of all bones, special adapted presentation protocols were generated to depict and analyze these selected bones two- and three-dimensionally. The CT-data revealed healed fractures of the frontal bone and a tumor localized at the sphenoid bone in the bog body 'Uchter Moor'. Additionally, the functional analysis of the two calcanei showed differences in the distribution of bone material density and therefore suggested the probable left-handedness of this individuum. VCT-data of the 'Kayhausen boy' offered for the first time the three-dimensional depiction of 'HARRIS LINES', providing evidence of physical stress during lifetime. VCT-analysis of the mummified skin from 'Bareler Moor' demonstrated different preserved layers which supported the assumption that this specimen is most probably part of the chest. µCT-data of the 'Esterweger Dose' uncovered intravital osteolytic destructions and reactive bone formations as typical features of an osteomyelitis, which appears to be the cause of death in this young individuum.

Conclusions: High resolution non destructive CT techniques allow the accurate detection of the skeletal microstructure and skin as morphological features and furthermore the evaluation of bone densities. High resolution CT imaging is therefore a valuable paleopathological tool alongside recently used destructive histological and scanning-electron microscopic techniques.

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