day one: sunday 19 june 2011

INAUGURAL LECTURE BY

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chaired by

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METABOLIC IMAGING IN HEALTH AND DISEASE

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Imaging is no longer just a way of studying anatomical structures but also provides observations about physiological function, biochemical and metabolic processes, molecular and cellular events in vivo in animal models for human disease and in human investigations.

Molecular imaging is a set of emerging technologies at the life science/physical science interface which is set to revolutionize our understanding and treatment of disease.

The term molecular imaging is used in a variety of ways and includes imaging or observing specific molecules which are present in living systems, the use of externally added reporter molecules or signals that can be activated by a particular process such as gene expression. Molecular imaging techniques span the electromagnetic spectrum from ultrasonic to gamma ray and X-ray frequencies. Within this spectrum Magnetic Resonance (Imaging and Spectroscopy), optical imaging and nuclear imaging are the key ways of observing molecular event and interactions in living systems, including man.

To answer a significant biological or clinical question we need to select the most appropriate technique, often requiring the combination of more than one method, use chemical and biological approaches to design and evaluate specific imaging probes and develop advanced computational techniques for analyzing the images and interpreting the information.

In this presentation I shall illustrate the use of Magnetic Resonance Spectroscopy (MRS) and Imaging (MRI) to study heart disease in diabetes and in heart failure, of optical and MR imaging to study, insulin release from pancreatic beta-cells in models for diabetes. In addition I shall describe how new approaches, including Hyperpolarised 13C Spectroscopy, the use of fluorescent probes generated by diversity oriented fluorescence libraries for cellular and metabolic targets and Mass Spectrometric Imaging provide novel in vivo markers for biochemical functions.

Molecular imaging is a key tool in the translation of basic and pre-clinical research findings to clinical situations aimed at leading to the improvement of human health.

PARALLEL SESSION 1:

MOLECUAR NEUROIMAGING

chaired by

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YIA applicant

IMAGING NEURAL STEM CELL POPULATIONS IN THE DEVELOPING MOUSE BRAIN USING MAGNETIC RESONANCE MICRO HISTOLOGY

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Introduction: Advanced methods for labelling neural stem cells (NSC) and progenitor cells (NPC) are fundamental for monitoring brain development under normal and pathological conditions. Magnetic resonance (MR) histology is an emergent technique utilising MR microscopy and active staining to provide enhanced tissue contrast for anatomical characterisation of transgenic mice [1]. This approach employs contrast agents to reduce scanning time and increase signal-to-noise. Recent studies [2,3] demonstrated they may also be used to differentially enhance contrast in specific tissues in the adult mouse, suggesting MR histology could provide an array of staining options to highlight distinct cellular structures. Here, the bio-distributions of two agents were investigated in the embryo. We identify previously undetected substructures and delineate regions of NSC and NPC within the intact embryo brain.

Methods: C57BL/6 embryos (E15.5) were fixed for 2 weeks in 4% formaldehyde doped with 8mM Gd-DTPA or Mn-DPDP and embedded in 1% agarose doped with the corresponding agent. High-resolution MR microscopy was performed using a Varian 9.4T VNMRS system on each embryo set with a 3D gradient echo sequence (FOV 27x27x27mm3, matrix 512x512x512) using optimised scanning parameters (Mn-DPDP: TR=20ms, TE=4ms, FA=46°, NSA=4, Gd-DTPA: TR=20ms, TE=9ms, FA=60°, NSA=7 [4]). Mn-DPDP embryos were sectioned and stained with H&E (marks nuclei and cytoplasm i.e. cell density), and Nestin (stain for NSC and NPC).

Results: Marked tissue-specific differential staining was observed between Mn-DPDP and Gd-DTPA-stained embryos (e.g. brain/heart T1 ratio is 1.79 for Mn-DPDP and 1.05 for Gd-DTPA). In particular, differential contrast enhancement was observed in the cerebellum, midbrain, eye, pituitary gland and cortex. The agents may evenly distribute throughout all tissues and either: i) the inherent tissue relaxation properties have a marked effect at these very short T1s/T2*s; or ii) the contrast agent relaxation rates vary between different tissues. However, the most apparent reason is the agents preferentially distribute to certain tissue and cellular structures leading to local changes in relaxation and enhanced contrast. Our findings indicate there are possible uptake and/or tissue dependent affinity differences between Mn-DPDP and Gd-DTPA in the embryo. Furthermore, Mn-DPDP appears to accumulate in regions of densely packed cells and clearly highlights the NSC and NPC in the ventricular zone of the cortex, midbrain and cerebellum, which was confirmed by H&E- and Nestin-stained histological sections, suggesting their cytoarchitecture influences the site-specific distribution of Mn-DPDP.

Conclusion: Distinguishing brain tissue sub-structure in ex vivo embryos may be achieved using specific MR contrast agents, demonstrating MRI could offer flexibility and target specificity for phenotyping studies. Mn-DPDP staining enables visualisation of NSC and NPC populations, which may allow greater sensitivity for phenotypic characterisation of mutant mouse models. Further investigation of how bio-distribution and relaxation mechanism contribute to this phenomena will enable development of new contrast agents that possess enhanced selective staining properties for studying developmental and disease processes.

Acknowledgements: EPSRC and BHF. References: [1] Johnson G.A. et al. Radiology 2002, 789-793; [2] Huang S. et al. Neuroimage 2009, 46:589-599; [3] Cleary J.O. et al. Neuroimage 2011 In print; [4] Cleary J.O. et al. NMR Biomed 2009, 22:857-866.

YIA applicant

PET-CT IMAGING OF TRANSPORTER MEDIATED DRUG-DRUG INTERACTIONS AT THE BLOOD-BRAIN BARRIER USING [18F]-GEFITINIB

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Introduction. Reaching sufficiently high therapeutic concentrations of drugs acting in the brain is often hampered by the activity of the efflux transporters P-glycoprotein (P-gp, ABCB1) and Breast Cancer Resistance Protein (BCRP, ABCG2) at the blood-brain barrier. The broad substrate specificity of P-gp and BCRP can further cause transporter-mediated drug-drug interactions (DDIs), for example when co-medicated drugs compete for efflux by these transporters. This can lead to increased brain concentrations of drugs in treated patients, potentially resulting in unexpected brain toxicity. It is therefore highly important to determine whether a drug may be involved in transporter-mediated drug-drug interactions at the blood-brain barrier. PET-CT imaging is especially suited for such a purpose, since it is a quantitative and non-invasive technique, thereby allowing repeated measurements, which can potentially reduce the number of laboratory animals needed for this type of studies. Furthermore, PET-CT imaging is a highly translational technique and could therefore be used to assess drug transporter activity in patients before treatment.

Methods. To detect possible P-gp and/or BCRP mediated DDIs at the murine blood-brain barrier, we set up an automated synthesis for the PET tracer [^{18}F]-gefitinib and imaged the pharmacokinetics of [^{18}F]-gefitinib using a preclinical PET scanner in wild-type, *Bcrp1*$^{-/-}$, *P-gp*$^{-/-}$ and *Bcrp1;P-gp*$^{-/-}$ mice in the presence and absence of the P-gp and Bcrp1 inhibitor elacridar (10 mg/kg). To assess the quality of the method, a validation study with the same mouse strains was performed using [^{14}C]-gefitinib.

Results. We showed that P-gp and Bcrp1 together limit the brain penetration of [^{18}F]-gefitinib when administered at a dose of 1 mg/kg. Furthermore, the DDI between the Bcrp1 and P-gp inhibitor elacridar (10 mg/kg) and [^{18}F]-gefitinib (1 mg/kg) at the blood-brain barrier could be quantified using PET-CT imaging. Highly comparable results were obtained when the pharmacokinetics of [^{14}C]-gefitinib (1 mg/kg) in the presence and absence of 10 mg/kg elacridar in wild-type, *Bcrp1*$^{-/-}$, *P-gp*$^{-/-}$ *and Bcrp1;P-gp*$^{-/-}$ mice were analyzed.

Conclusions. [^{18}F]-gefitinib is a useful tool to non-invasively analyze potential P-gp and Bcrp1 mediated DDIs *in vivo*. Combining such quantitative PET-CT imaging data with *in vitro* human drug transporter assays and PBPK-modeling could provide a powerful approach to predict drug pharmacokinetics in humans. Furthermore, this method may in the future be used to asses drug transporter activity in patients before treatment with drugs that are transporter substrates.

MULTISPECTRAL OPTOACOUSTIC TOMOGRAPHY BRAIN IMAGING AND CHARACTERIZATION OF GLIOBLASTOMA

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Introduction: Imaging plays a vital role in brain research for assessing function and disease, yet each modality comes with particular strengths and limitations. X-ray CT and MRI attain high spatial resolution but are limited by molecular specificity. PET and FMT have target specificity, but are limited by low resolution. Intravital microscopy has specificity and resolution, but is limited by shallow tissue penetration. Instead we examine herein a novel technology, Multispectral Optoacoustic Tomography (MSOT) that offers high resolution optical imaging deep inside tissues [1]. MSOT illuminates tissue with light pulses at multiple wavelengths and detects the acoustic waves generated by the thermoelastic expansion following light absorption. Using spectral analysis of the data collected, MSOT can then differentiate the spectral signatures of oxy-/deoxy-hemoglobin and of photo-absorbing agents and quantify their concentration. By being able to penetrate through several millimeters to centimeters it represents an ideal modality for brain imaging, providing anatomical, hemodynamic, functional, and molecular information.In this work we examine the MSOT capacity in cross-sectional imaging of the mouse brain and contrast the in-vivo results with ex-vivo brain imaging to validate the in-vivo findings.

Methods: 8 week old nude CD-1 mice were used for brain imaging and stereotactic implantation of U87 glioblastoma cells ($3x10^5$ cells in the striatum). Imaging of hemoglobin contrast and ICG bio-distribution was examined using a small-animal real-time imaging MSOT system previously described [2].

Results: *In-vivo* MSOT of the intact mouse head yielded unprecedented performance in cross-sectional imaging of the mouse brain by visualizing the overall brain outline and anatomy, and imaging temporal arteries and blood vessels beneath the skull. Additionally, gold nanorods, packaged in fluorescently labeled liposomes, were injected into the 3rd ventricle, with an excellent correlation between MSOT and fluorescence imaging of cryoslices. This demonstrated the capacity of MSOT to localize NIR probes in the brain through intact skin and skull with high accuracy. In addition, spectral decomposition of hemoglobin confirmed the MSOT ability to visualize well perfused and ischemic brain conditions. Additionally, MSOT accurately visualized ICG bio-distribution injected into the tail vein, and followed in real time the ICG kinetics and clearance. Finally, we observed the growth of U87 tumor cells injected into the striatum to demonstrate the MSOT ability to be used for studying various brain pathologies in mouse and rat models.

Conclusions: The application of MSOT in in-vivo brain imaging is demonstrated. MSOT can be used to follow changes in blood oxygenation, as well as the distribution of near-infrared probes. With the advent of new molecular probes, MSOT could also track molecular features of neurological disease and cancer in mouse models

Acknowledgements: We acknowledge support from the German Federal Ministry of Education and Research (BMBF) through the GO-Bio program.

References[1]: Ntziachristos and Razansky. Chem Rev., 2010, 110, 2783-2794.[2] Taruttis et al. Optics Express, 2010, 18, 19592-19602.

EARLY DETECTION OF BRAIN METASTASIS USING NOVEL MRI CONTRAST AGENT TARGETING VCAM-1

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Introduction: Metastatic spread of a primary tumour to the brain remains one of the greatest hurdles in cancer therapy, and prognosis is poor. Contrast-enhanced magnetic resonance imaging (MRI) is currently the most sensitive method for brain metastasis detection, but relies on blood-brain barrier (BBB) compromise and, consequently, is sensitive to late-stage metastases only when therapeutic options are limited. We have developed an MRI-detectable contrast agent targeted specifically at the endothelial adhesion molecule VCAM-1 (VCAM-MPIO) and have shown that this agent enables detection of endothelial activation early in brain pathology [1]. Based on our recent findings that brain metastases develop in close association with existing brain vessels [2], we hypothesised that VCAM-1 is upregulated during metastasis development and that our VCAM-MPIO may enable early detection of brain metastases.

Methods: Female balb/c mice were injected intracardially with 1x105 4T1 cells, a metastasising murine mammary carcinoma line. Puri?ed monoclonal rat antibodies to mouse VCAM-1 (clone M/K2, Cambridge Bioscience) or control IgG-1 (clone Lo-DNP-1, Serotec) were conjugated to myOne tosylactivated MPIO (1µm diameter; iron content 26%; Invitrogen) [1]. At day 5, 10 or 13 after 4T1 cell injection animals were anaesthetised and injected intravenously with either VCAM-MPIO or IgG-MPIO. After 1h animals underwent MRI on a 7T Varian Inova system and a T2*-weighted 3D gradient-echo dataset was acquired. Subsequently, post-gadolinium T1-weighted images were acquired to assess BBB integrity. A second metastasis model was also studied; female SCID mice were injected intracardially with 1x105 MDA-231BR cells, a metastasising human breast adenocarcinoma line. These animals were imaged at 21 days post-cell injection, as described above. In all cases T2*-weighted images were processed into a 3D isotropic dataset and the brain was manually masked. Quantification of VCAM-MPIO binding (defined as focal hypointensities) was performed using Image-Pro software. Following MRI animals were transcardially perfusion-fixed and the brains sectioned and assessed immunohistochemically for VCAM-1 expression associated with tumour growth.

Results: Immunohistochemically, upregulation of VCAM-1 was co-localised to brain metastases in both models studied, and in many cases the presence of the VCAM-MPIO in VCAM-positive vessels was observed. Similarly, MRI revealed focal areas of signal hypointensity throughout the brain, indicating VCAM-MPIO binding, in both 4T1 and MDA-231BR injected animals. Assessment of the co-localisation of MRI hypointensities and metastases detected immunohistochemically indicated >90% positive identification by VCAM-MPIO MRI above a tumour diameter threshold of ca. 100?m. Quantitatively, the volume of VCAM-MPIO-induced hypointensities in 4T1 injected animals was greater than in IgG-MPIO-injected animals (i.e. background) and increased with disease progression. No 4T1 injected animals showed BBB breakdown.

Conclusions: Upregulation of VCAM-1 during metastasis development enables earlier detection of metastases in the brain, using our novel VCAM-1-targeted contrast agent, than is currently possible clinically. We believe that this new approach will open a therapeutic window in brain metastasis that currently does not exist.

Acknowledgment: This work was funded by Cancer Research UK (Programme Grant C28461/A10158). References: [1] McAteer MA et al. Nat Med. 2007;13:1253-1258. [2] Carbonell WS et al. PLoS One. 2009;4:e5857.

RESPONSIVE MAGNETIC RESONANCE IMAGING PROBES TO MONITOR SYNAPTIC GLUTAMATE FLUCTUATION IN THE BRAIN

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Introduction: The neurotransmitter glutamate is the major mediator of excitatory signals in the nervous system and is involved in nearly all aspects of normal brain functioning (cognition, memory, learning). Our idea was to develop glutamate 'responsive' magnetic resonance imaging (MRI) contrast agents (CAs) to image changes in specific brain regions upon neural activation. As CAs directly responsive to glutamate would not be feasible due to the very short half-life of glutamate in the extracellular space, we chose CAs that bind to glutamate receptors instead (to be specific metabotropic glutamate-receptor subtype 5 (mGluR5)), by this increasing image contrast. Ideally, upon glutamate-binding to the receptor (e.g. after glutamate-release at the synapse) the CA will be released, hence leading to a reduction in image-contrast, followed by a restoration of equilibrium and re-binding of the CA to the receptor. These events are believed to occur over a period of seconds allowing data acquisition using modern FLASH pulse techniques[1]. Here, we present a proof-of-concept study for such 'indirect' glutamate-responsive MRI CAs.

Methods: We have designed and synthesized different prospective CAs derived from various potent mGluR5-receptor antagonists (alkynes like MPEP, MTEP and dipyridyl/heterobiaryl amides) coupled to DOTA-derived macrocyclic lanthanide-chelates. The CAs were evaluated in cultured primary cortical rat astrocytes, expressing mGluR5 (verified by immunofluorescence). MRI-measurements to examine the ability of the CAs for cellular labeling were done with a 3T human whole body scanner. Antagonistic potency of the CAs was assessed with a calcium fluorescence assay, by which glutamate induced intracellular calcium-transients mediated by mGluR5 were measured. Antagonistic activity of the CAs was calculated as changes in EC50 of glutamate. Receptor binding was measured for the dipyridyl derivaties, as these compounds have an inherent fluorescence that changes upon binding. Commercially available receptor membrane preparations containing human mGluR5A were used for these experiments.

Results: Two of the gadolinium complexes retained significant antagonistic activity, one in each structural class. For the alkyne-derivative, about a threefold increase of the EC50(glutamate) (100µM CA, 15min, $P<0.001$) was found while under similar conditions the cellular relaxation rate R1,cell increased to 126% of control (100µM, 45 minutes incubation time, $P<0.001$). The CA derived from dipyridyl amides increased the EC50(glutamate) about fourfold ($p<0.001$) and the R1,cell to 115% ($p<0.05$). Fluorescence measurements of the latter CA showed enhanced emission upon binding to mGluR5-membrane preparations. This was reversed when increasing concentrations of glutamate were added, consistent with the a reversibility of CA-receptor binding

Conclusions: Using primary rat astrocytes as cellular model system to investigate newly developed glutamate-responsive MRI contrast agents, we were able to identify two promising candidates. These CAs are based on the structures of antagonists to mGluR5 and our studies establish the validity of the concept, by which it might be possible to use MRI to image transient changes in the neurotransmitter glutamate.

Acknowledgement**:** This work was supported by the Max-Planck Society, the German Ministry for Education and Research (BMBF, FKZ:01EZ0813), by a Marie Curie Fellowship (PIEF-GA-2009-237253) and was performed in the frame of COST action D38. Reference: Logothetis, Nature 453(2008)869

68GA-DOTA-4-FBN-TN14003 AS A NOVEL PET TRACER FOR THE IMAGING OF CXCR4 EXPRESSION

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Introduction: The expression of the chemokine receptor CXCR4 by tumors is associated with tumor aggressiveness and poor prognosis for the patient and also contributes to metastatic spread. Therefore it is of high interest to find a specific PET tracer for the imaging of CXCR4 expression in tumors and metastases. The aim of this study was the synthesis, ^{68}Ga labeling and first evaluation of DOTA-4-FBn-TN14003 as a potential PET tracer for this purpose using human lymphoma and breast cancer cell lines.

Methods: DOTA-4-FBn-TN14003 as well as the parent peptide 4-FBn-TN14003 were manually constructed by Fmoc-based solid phase peptide synthesis. The versatile DOTA-conjugated labeling precursor was labeled with ^{68}Ga, which was obtained from a ^{68}Ge/^{68}Ga generator. Purification of the radiotracer was conducted by solid phase extraction and the radiochemical purity was analyzed by radio-HPLC. The affinity of Ga-DOTA-4-FBn-TN14003 and 4-FBn-TN14003 to CXCR4 was determined in an inhibition assay using 125I-SDF-1a. For these evaluations Jurkat (T-cell lymphoma) as well as MDA-MB 231 (breast cancer) cells of human origin were used. For the evaluation of the CXCR4 expression levels of both cell lines immunohistochemical staining was performed.

Results: ^{68}Ga-DOTA-4-FBn-TN14003 was reproducibly obtained in radiochemical yields of 61.1 ± 3.5 % with an excellent radiochemical purity of > 99.5 % and a specific activity of 15.4 ± 1 GBq/µmol. The overall time for synthesis and purification was 35 min, which is compatible with the half-life of ^{68}Ga (68 min). While using Jurkat cells, the IC50 value of the parent peptide 4-FBn-TN14003 was 3.07 ± 0.76 nM and thus comparable to previously determined IC_{50} values of this peptide (0.99 nM [1] and 2.5 nM [2]. The IC_{50} value of Ga-DOTA-4-FBn-TN14003 was comparable (2.27 ± 0.04 nM) to that of the parent peptide. When using MDA-MB 231 cells, the maximal binding of both compounds to the cells was reduced significantly (e.g. for Ga-DOTA-4-FBn-TN14003: 8.44 ± 0.32 % vs. 18.52 ± 0.76 %, respectively). In accordance with these results immunohistochemical staining indicated a higher CXCR4 expression in Jurkat cells as compared to MDA-MB 231 cells.

Conclusion: Here we show that Ga-DOTA-4-FBn-TN14003 has favorable properties as a tracer for the imaging of CXCR4 using two human tumor cell lines with different expression levels of this chemokine receptor. ^{68}Ga-DOTA-4-FBn-TN14003 is a promising novel PET tracer for imaging of CXCR4 expression in tumors and its metastases to be applied in further *in vivo* studies.

Acknowledgement: This work was supported by Deutsche Forschungsgemeinschaft (BA 4027/4-1; UM and TB). ; References: [1] Tamamura et al., (2003), FEBS Letters, 550, 79-83, [2] Jacobson et al., (2010), J. Nucl. Med., 51, 1796-1804.

YIA applicant

BIOLUMINESCENCE IMAGING OF LENTIVIRAL VECTOR-LABELED HIPPOCAMPAL NEURAL STEM CELLS IN MOUSE BRAIN

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Introduction The hippocampus is one of the main brain areas where resident neural stem cells (NSCs) give rise to new neurons throughout life. Stimulation of these endogenous NSCs, and subsequently boosting adult neurogenesis, is a promising new approach for slowing down or possibly reversing the age- or disease-related decline in memory function. In vivo bioluminescence imaging (BLI) would be a valuable tool to monitor hippocampal adult neurogenesis. We previously showed the feasibility to track and follow the proliferation and migration of NSCs from the subventricular zone (SVZ) to the olfactory bulb using BLI, after stereotactic injection of a lentiviral vector (LV) encoding firefly luciferase (Fluc) in the SVZ of mice (1). When using conditional LV, we were able to specifically label NSCs in the SVZ of Nestin-Cre transgenic mice, and to follow these labeled cells and their progeny over time with BLI (2). We now aimed to use these conditional LV for specific labeling and non-invasive visualization of NSCs in the other main neurogenic region of the brain: the dentate gyrus (DG) of the hippocampus.

Methods We produced conditional 'FlexSwitch' LV in which the cDNA of enhanced green fluorescent protein (eGFP) and Fluc are present in antisense orientation and flanked by two mutually exclusive lox sites. Upon Cre recombination the cDNA between 2 lox sites will be inverted and thereby inducing transgene expression. 4µl of FlexSwitch LV was stereotactically injected in the DG of 8 week old Nestin-Cre mice. After intravenous injection of D-luciferin, mice were imaged using an optical CCD-camera (IVIS 100).
Results We first validated the FlexSwitch LV in cell culture. Efficient expression of both transgenes was demonstrated only in the presence of the Cre recombinase. Next, the FlexSwitch LV was injected in the DG of Nestin-Cre mice, where expression of Cre recombinase is driven by the rat nestin promoter and enhancer, ensuring specific expression of the transgenes in the NSCs. A BLI signal was detected from 2 days post injection onwards. There was a clear difference in BLI signal and pattern of GFP positive cells between Nestin-Cre mice injected with the FlexSwitch LV compared to WT mice injected with a constitutive LV encoding eGFP and Fluc. In the Nestin-Cre mice, the BLI signal was stable for at least 3 months. Confocal histological analysis was performed at different time points post injection and showed labeling of NSC and their progeny.

Conclusions Our previously designed viral-vector based system for specific labeling of NSCs in the SVZ can also be used to label NSCs in the DG. This technique can be used to non-invasively follow up NSCs and their progeny and potentially monitor biological effects of genes and small molecules on adult hippocampal neurogenesis.

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References: 1. Reumers V., et al., Stem Cells, 2008. 26(9): p.2382-90 2. Reumers V., et al., in preparation

PARALLEL SESSION 2:

IMAGE GUIDED THERAPY

chaired by

Silvio Aime, Torino

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IMAGE-GUIDED THERAPY USING MR-HIFU

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Diagnostic imaging is indispensible in clinical routine for diagnosis and staging of diseases. Over the last years, diagnostic imaging moved also into the therapeutic setting to guide therapeutic interventions. Examples range from x-ray guided placement of stents, radiofrequency or high intensity focused ultrasound (HIFU) based thermal ablation of tumors to catheter-based embolization procedures.

Besides these pure device based interventions, image guided interventions are immerging that comprise a drug formulation. In general the aim is to achieve as high as possible concentrations in the lesion, while sparing the healthy tissue. One example are temperature activatable drug formulations that were proposed already thirty years ago but are only reaching clinical approval. Here, a small molecule drug, doxorubicin, is encapsulated in a temperature sensitive liposome, which prevents the drug from leaving vascular system reducing for example cardiac toxicity. For tumor treatment, the lesion is heated with radiofrequency or high intensity focused ultrasound to temperatures around 42 oC. At this temperature, the liposomes become porous and quickly release the drug payload. This rapid release inside the tumor leads to high local concentrations and improves the therapeutic window. Magnetic resonance imaging plays a pivotal role in guidance of thermal ablation procedures, hyperthermia treatment of high focused ultrasound induced drug delivery as it provides morphological pictures with superb resolution and allows real time temperature mapping. The latter provides a feedback to ensure that an adequate thermal dose is applied. For image guided drug delivery, MRI is also able to provide a non-invasive way to quantify the drug concentration in vivio. This talk will give an overview of recent developments in image guided interventions with detailed examples of applications of MRI- guided high intensity focused ultrasound in thermal ablation and drug delivery.

TARGETED DRUG DELIVERY BY BLOOD-BRAIN BARRIER OPENING IN MICE USING LOW INTENSITY FOCUSED ULTRASOUND

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The goal of this project is to investigate the feasibility of improvement of chemotherapy of CNS tumors by transient blood-brain barrier (BBB) opening with low intensity focused ultrasound (LIFU). In general, treatment of pediatric brain tumors relies on effective tumor resection (gross tumor resection) followed by radiotherapy. Adjuvant chemotherapy is either used for inoperable tumors or to control tumor growth until radiotherapy is applicable, especially in infants who are particularly vulnerable to radiation-related morbidity. Reduction of long term side effects combined with increased efficacy may be achieved by increasing the concentration of chemotherapeutic agents in the tumor and its vicinity. It has already been shown in animal models that opening the BBB with low intensity focused ultrasound can overcome the limitation of the BBB.

Here, we report on the development of a suitable delivery protocols to replicate the current state of the art of LIFU-mediated BBB opening. A homebuilt FU system operating at 550 kHz was used to apply different ultrasound sonication schemes to the brain of C57BL/6 mice, through the intact skull, under image guidance of a clinical 3.0T high field MR-scanner. BBB opening was achieved in combination with intravenous injection of microbubbles (BR38®, Bracco, Geneva). The effective range of acoustic intensities was in agreement with the literature. Higher intensities caused cortical haemorrhage. Evaluation of the results was done by contrast enhanced T1-weighted MR-imaging. The region of BBB opening corresponded to the size of the transducer's focus.

To estimate the optimal parameters for therapy, a well established GL261 mouse glioblastoma model was implemented; tumor growth and its vasculature properties were assessed.

The MR-guided low intensity ultrasound (MRgLIFU) system allows non-invasive, safe, precise and reproducible opening of the BBB. This will allow us to study the impact of BBB opening on the standard and novels chemotherapies in mouse models.

YIA applicant

DUAL WAVELENGTH TUMOR TARGETING FOR DETECTION OF CANCER USING NEAR-INFRARED OPTICAL IMAGING IN AN ANIMAL MODEL

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Introduction: In current clinical practice, surgeons rely on palpation and visual inspection to discriminate between tumor and normal tissue and consequently determine an adequate tumor-free margin. Better postoperative results (e.g., survival, functionality) could be achieved by imaging techniques that help the surgeon discriminate between healthy and diseased tissues (e.g., cancer) and identify vital structures during surgery. Near-infrared (NIR) fluorescence imaging is a promising technique that can be used to visualize cancer tissue during surgery. In order to detect the tumor during surgery, a wide range of NIR fluorescence agents has been described that each have their specific targeting strategy.
Tumor detection can be improved by targeting multiple tumor-specific characteristics simultaneously with fluorophores that emit light at different wavelengths. In the 700 nm region, ProSense680 and MMPSense680 (PerkinElmer, Waltham, MA) are NIR fluorescence agents that detect increased activity of cathepsins and matrix metalloproteinases, respectively. These enzymes are mainly found in the invasive tumor border due to degradation of the extracellular matrix directly surrounding the tumor. NIR fluorescence agents that emit at 800 nm are 2DG CW800 (LI-COR Biosciences, Lincoln, NE), which detects tumor cells through uptake by the glucose transporter, and EGF CW800 (LI-COR Biosciences), which is internalized by the epidermal growth factor (EGF) receptor of tumor cells. In this study, dual wavelength targeting by NIR fluorescence technologies for in vivo cancer imaging is explored. We describe the use of two combinations of NIR fluorescence agents that each target specific tumor characteristics at different wavelengths for detection of hypopharyngeal cancer in an animal model.
Methods: Luciferase-bearing hypopharyngeal cancer cells were inoculated subcutaneously on the back of immunodeficient mice. Tumor growth was followed by bioluminescence imaging and caliper measurements. Subsequently, two combinations of agents that emit fluorescence signal at different wavelengths (ProSense680 combined with 2DG CW800 and MMPSense680 combined with EGF CW800) were systemically injected and fluorescence imaging was performed. Immunohistochemical staining was performed for each target: cathepsin, GLUT-1 receptor, matrix metalloproteinases and EGF receptor. In this way, co-localization of the fluorescence signal with the targeted tumor features was determined.
Results: Whole-body imaging revealed clear demarcation of tumor tissue using all 4 agents. The tumor-to-background ratio (standard deviation, p-value) was 3.69 (0.72, $p<0.001$) for ProSense680; 4.26 (1.33, $p<0.001$) for MMPSense680; 5.81 (3.59, $p=0.02$) for 2DG CW800; and 4.84 (1.56, $p<0.001$) for EGF CW800. Fluorescence signal corresponded with histopathology and immunohistochemistry, demonstrating signal of ProSense680 and MMPSense680 in the invasive tumor border, and signal of 2DG CW800 and EGF CW800 in the tumor tissue.
Conclusions: We demonstrated the feasibility of dual wavelength tumor detection using different targeting strategies simultaneously in an animal cancer model. Combined targeting at different wavelengths allowed simultaneous imaging of different tumor characteristics. The current technique of NIR fluorescence optical imaging has the potential to be translated into the clinic in order to improve the complete removal of tumors by real-time image-guided surgery.

YIA applicant

ASSESSMENT OF ULTRASOUND-MEDIATED DRUG DELIVERY BY REAL-TIME IMAGING USING FIBERED CONFOCAL FLUORESCENCE MICROSCOPY

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Introduction: Understanding conditions of drug incorporation into the cell and controlling its triggering are critical aspects of ultrasound (US)-mediated drug delivery [1]. Real-time imaging of a model drug uptake opens new means of investigation that may lead towards evaluation of pharmacokinetic parameters. Here we propose a novel setup that combines a fibered confocal fluorescence microscopy, an Opticell chamber and an ultrasonic transducer, and enables a direct real-time monitoring of US-induced permeability of the plasma membrane at the cellular scale.

Methods: C6 cells (rat glioma) were grown in an US-compatible OpticellTM culture chamber. 3.10^7 microbubbles (SONOVUE, Bracco, Italy) and 2µM Sytox Green (cell-impermeable fluorophore (Ex:504nm/Em:523nm) that exhibits fluorescence enhancement upon binding to nucleic acids) were added to the medium. A monoelement transducer (5.8mm o.d.) was positioned 8mm away from the surface of cell container at the acoustical focal distance. US parameters were as follows: main frequency=1.5MHz, pulse repetition frequency=1kHz, duty cycle=20%, effective electrical power 1W corresponding to acoustical pressure 0.88MPa p-p. The tip of the fibered confocal fluorescence Cellvizio® (MaunaKea Technologies, Paris, France) microscope (FOV=600x500µm², optical sectioning=70µm, working distance=100µm) was placed in the beampath. The whole set-up was immersed in water at 37.0°C. Optical focus was adjusted by finding an attached fluorescent dead cell. Real-time fluorescence images (frame rate=8.5Hz, laser excitation=488nm, spectral sensitivity=505-700nm-laser power=1.4mW) were acquired 10s before, during and 80s after US-exposure; afterwards, the follow-up was recorded during 10s every minute. Other areas were similarly investigated in absence of microbubbles and/or US. Photobleaching was measured for data correction. Signal evolution of each object was evaluated after data reconstruction using IDL (home made program). For each image, several parameters such as the number of objects, the average intensity per object, the average size per object were extracted after thresholding the image stack using ImageJ (Bethesda, USA).

Results: Temporal evolution of the signal in sonicated areas was characterized by the following pattern: before US exposure, 1-2 cells (presumably dead) with stable signal were identified in some images. US application was immediately followed by appearance of multiple circular areas of high fluorescence signal (corresponding to cell nuclei). When sonication was halted, the signal of each nucleus increased until 20-30 minutes post US-exposure. Individual and average profiles of the signal evolution were obtained. One hour after sonication, 110 +/- 18 Sytox Green labeled nuclei were detected. No signal variation was observed in control areas neither during nor after US application (apart from few dead cells seen initially).

Conclusions: Fibered fluorescence microscopy allowed real-time US-mediated intracellular delivery imaging in an Opticell chamber. Our set-up offers the possibility to monitor and quantify the internalization of a model drug such as cell impermeable fluorophore during and after sonication, opening promising perspectives for pharmacokinetic studies upon membrane permeabilization with cavitation.

Acknowledgement: We are grateful to Dr J.R. Cazalets and colleagues (INCIA UMR 5287 / University Bordeaux 2, France) for using cell culture facility. This work is supported by the EC-project FP7-NMP-2008-1-213706 SonoDrugs and Foundation InNaBioSanté-project ULTRAFITT. References: 1. Yudina et al, Mol Imaging Biol, 2011 Apr;13(2):239-49

YIA applicant

MR MONITORING OF HIFU-MEDIATED LOCAL DRUG DELIVERY WITH TEMPERATURE SENSITIVE LIPOSOMES

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Introduction. Temperature-triggered drug delivery is a promising treatment option in oncology, leading to an improved therapeutic efficacy and reduced toxicity profiles of the drug. Hyperthermia of the tumor can be accomplished using high intensity focused ultrasound (HIFU) under MR image guidance[1], while temperature-sensitive liposomes (TSLs) can serve as drug vehicles that release their payload upon heating. The co-encapsulation of a drug and an MRI contrast agent in the lumen of TSLs provides the ability to monitor the drug delivery process in vivo using MRI[2,3,4]. Here, TSLs incorporating both a chemotherapeutic drug (i.e. doxorubicin) and an MRI contrast agent (i.e. [Gd(HPDO3A)(H2O)]) were evaluated in vitro and in vivo for applications in MRI guided drug delivery.

Methods. TSLs containing doxorubicin and 250 mM [Gd(HPDO3A)(H2O)] were prepared[3]. Release of [Gd(HPDO3A)(H2O)] and doxorubicin from the TSLs during heating was studied in vitro by measuring the T1 and the intensity of fluorescence, respectively. In vivo experiments were performed on rats bearing a subcutaneous 9L tumor on the hind leg. Blood kinetics and biodistribution was studied for liposomes as well as for the liposomal contents ([Gd(HPDO3A)(H2O)] and doxorubicin) after intravenous injection of 111In-labeled TSLs. For MR-HIFU experiments, TSLs were injected intravenously while local hyperthermia in the tumor was induced for 30 minutes, using a 3T clinical MR-HIFU system. The local temperature-triggered release of [Gd(HPDO3A)(H2O)] was monitored with interleaved T1 mapping of the tumor tissue. At t=90 min after TSL injection the rats were sacrificed and tumors were analyzed for doxorubicin and gadolinium concentrations.

Results. In vitro studies showed a rapid and simultaneous release of the drug and the MRI contrast agent from the TSLs at 42 °C, while no leakage was observed over 1 hour at 37 °C. The combination of TSL administration with mild hyperthermia induced significant higher uptake of doxorubicin in the tumor as well as changes in the T1, whereas the T1 values of the surrounding muscle hardly changed. Control experiments with tumor bearing rats that received no HIFU showed only a minor uptake of doxorubicin going along with a subtle decrease in T1 upon injection of the TSLs. For all experiments, a good correlation was found between the ?T1 and the concentrations of doxorubicin and [Gd(HPDO3A)(H2O)] in the dissected tumors[4].

Conclusions. The good correlation between ?T1 and the uptake of doxorubicin in the tumor implies that the in vivo release of doxorubicin from TSLs can be probed in situ with the longitudinal relaxation time of the co-released MRI contrast agents. The combination of MR imaging of drug release from TSLs and pharmacokinetic modeling, may realize a way to quantify and monitor the drug delivery process and serve as a tool in clinical decision making to personalize treatments.

Acknowledgement: This project was funded in part by the EU Project Sonodrugs (NMP4-LA-2008-213706).

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PARALLEL SESSION 3:

PROBES/CHEMISTRY

(together with COST action D38)

chaired by

Eva Tóth, Orleans

Frédéric Dollé, Orsay

HYALURONIC ACID AS A VERSATILE CARRIER FOR DRUGS, CYTOKINES AND IMMUNOGENIC ANTIGENS: AN IMAGING APPROACH

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Introduction Hyaluronic acid (HA) is a biodegradable, biocompatible, non-toxic, non-immunogenic and non-inflammatory anionic biopolymer, which plays pivotal roles in wound healing, cell motility, angiogenesis as well as construction and integrity of extracellular matrix. Several receptors have been described for HA, such as CD44, the receptor for hyaluronate-mediated motility (RHAMM), HA receptor for endocytosis (HARE), and lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1), which have important biological roles in endocytosis, degradation and signal transduction. Because of various biological functions and excellent physico-chemical properties, HA and its derivatives are widely employed for biomedical and pharmaceutical applications. Other than for the linking of small drug molecules, HA has been also advanced for long-acting delivery applications of nucleotide, peptide and protein therapeutics. In this regard, aim of the present work was to exploit HA as a potential carrier of cytokines, in particular IFNalpha??and immunogens for the treatment of liver viral diseases and the induction of safer and more efficient immune responses, respectively.

Methods Recombinant IFNalpha?and a set of different model protein antigens were chemically linked to HA (200 kD). The cytokine-linked polymer was studied for antiviral and antiproliferative activity, and for the capacity to induce intracellular signalling. Antigen bioconjugates were used for immunization of groups of mice using different schedules of immunization. As controls, additional groups of animals received the same amounts of proteins, either free in PBS or emulsified in complete/incomplete Freund's adjuvant or admixed with clinical grade alum. At day 30, blood samples were collected from all groups of mice, and individual sera were titrated for their protein-specific Ig content by enzyme-linked immunosorbent assay (ELISA). In vivo biodistribution of fluorophore-labelled bioconjugates and free proteins was assessed by fluorescence optical imaging after administration through different routes.

Results HA-IFNalpha retained the antiviral and antiproliferative activity, induced STAT1 phosphorylation and gene modulation on PBMC and tumor cells similarly to the free cytokine, and exerted relevant therapeutic activity against susceptible target cells. Upon intravenous inoculation, the bioconjugate disclosed a rapid and almost complete accumulation in the liver, thus suggesting the possibility to realize an organ-focused therapeutic approach. With regard to the antigen conjugates, proteins alone were not generally sufficient to induce optimal antibody production, while adjuvants provided a strong boost for humoral responses. Notably, mice that received immunization with HA-based bioconjugates disclosed Ig titres that at least overlapped or were even superior to those observed in control groups. Biotolerability of HA-antigen bioconjugates was excellent. In vivo imaging revealed that intramuscular or footpad injection of HA-bound proteins was followed by accumulation in draining lymph nodes in higher amounts compared to the free form, thus indicating that the adjuvant properties of HA may rely on specific targeting of secondary lymphoid organs.

Conclusions In vivo imaging can provide important clues to define the biological behavior and targeting properties of new polymeric conjugates and allows to advance new strategies for optimized immunological and therapeutic interventions.

YIA applicant

MOLECULAR IMAGING OF HER2 EXPRESSION IN BREAST CANCER USING 68GA-NOTA-ANTI-HER2 NANOBODIES

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Introduction: Nanobodies are small (15 kDa) antibody fragments with beneficial pharmacokinetic properties that are ideally suited to be used as probes in molecular imaging [1, 2, 3]. Within this project, we develop new 68Ga-nanobodies labeled via the macrocyclic chelator NOTA, that could be used for in vivo radioimmunodetection of HER2 antigen using positron emission tomography (PET).

Methods: In this study, two nanobodies differing only in the C terminal 6-histidine tag, were chemically modified with S-2-(4-Isothiocyanatobenzyl)-1,4,7-triazacyclononane-1,4,7-triacetic acid (p-SCN-Bn-NOTA) and analysed by mass spectrometry. The resulting modified nanobodies, NOTA-nanobodies, were radiolabeled with 68Ga, an important PET radionuclide which half-life fits with the short biological half-life of the nanobodies. Labeling efficiencies were checked by Instant Thin Layer Chromatography (ITLC) and Reverse Phase-High Performance Liquid Chromatography (RP-HPLC). Stability of 68Ga-NOTA-nanobodies was evaluated in PBS, human serum and in the presence of excess EDTA. Biodistribution was evaluated in mice bearing HER2 positive or negative tumors.

Results: Mass Spectrometry results showed conjugation of 1 to 1,2 molecules of NOTA per nanobody. After incubation with 68Ga for 7 min at room temperature, ITLC and RP-HPLC showed a radiochemical purity >98%. The labeled nanobodies showed to be stable in PBS, human serum, and 500 fold excess EDTA presenting only one major peak of radioactivity (> 98 %), with the same retention time as that of intact 68Ga-NOTA-nanobodies. The uptake of 68Ga-NOTA-nanobodies in HER2 positive tumours was 3.13 ± 0.06 %IA/g for the His tag form and 4.34 ± 0.90 %IA/g for the no tag with a tumour-to-muscle ratio of 12.96 ± 1.88 and 28.49 ± 1.87, respectively, at 1 hour after injection. The uptake in the negative tumour was 0.45 ± 0.1 and 0.26 ± 0.07 for the His tag and no tag, respectively. The major difference between the two different nanobodies was the kidney retention, decreasing from 116.67 ± 1.83 to 45.00 ± 11.42 % IA/g for the no tag nanobody.

Conclusions: We showed for the first time the successful labeling of anti-HER2 nanobodies with 68Ga through the bifunctional chelator NOTA. High tumour uptake with high tumour-to-muscle ratio is observed early after administration. The removal of the 6-histidine tag showed a significant impact on kidney uptake being more than 60% lower. 68Ga-anti-HER2-Nanobody PET imaging of breast cancer should enable non-invasive assessment of HER2 receptor status in breast cancer patients.

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YIA applicant

IMAGING GABAERGIC NEURONS BY MRI: USE OF A RESPONSIVE CONTRAST AGENT FOR GLUTAMIC ACID DECARBOXYLASE

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Introduction: Responsive contrast agents are novel tools for molecular imaging. By overcoming the limitations of conventional contrast agents, they offer the possibility to highlight not only anatomical details, but to gain also functional information about cell viability, metabolism or differentiation. We developed and tested a contrast agent which can be activated by glutamic acid decarboxylase (GAD), the specific enzyme for inhibitory neurons in the brain [1]. GAD specifically cleaves a glutamate moiety linked to a long hydrocarbon backbone fused with Gd-DOTA. Upon cleavage the hydration of the paramagnetic metal ion increases, leading to a higher r1 relaxivity.

Methods: Rat B35 neuroblastoma cells expressing GAD, were used in combination with the GAD specific inhibitor AOAA (Sigma-Aldrich, Germany) to characterize the responsive contrast agent Gd-DOTAgad. Directed differentiation of CGR8 murine embryonic stem cells into inhibitory neurons was achieved with a 3 step protocol using chemically defined media for at least 10 days. Gd-DOTAgad was incorporated by electroporation (Bio-Rad Gene Pulser, USA). MR measurements were conducted on a ASPECT M2 system operating at 1T and on a Bruker Avance300 operating at 7.1T, using a saturation recovery spin echo sequence. MR phantoms consist of glass capillaries embedded in 1.5% agarose (Fluka, Switzerland). To determine the Gd(III) concentration, cell lysates were treated with HCl 37% (1:1) at 120°C over-night. Water proton R1 was measured at 20 MHz and compared to relaxation rates obtained with GdCl3 dilutions. MR signal enhancement was normalized to the content of internalized Gd(III).

Results: After activation of Gd-DOTAgad, the MR signal of labelled B35 cells increased about 30% in respect to unlabelled cells. The intracellular relaxivity of Gd-DOTAgad increased during an activation period of 6 hours and decreased again after 24 hours. The specificity of Gd-DOTAgad activation was confirmed by incubation of labelled cells in the presence of the GAD inhibitor AOAA (25-50 µM) resulting in no relaxivity change. Native and differentiated CGR8 cells were labelled by electroporation and incubated 5-8 hours for complete activation. Selectively in GAD expressing GABAergically differentiated cells, the observed R1 was about 30% and the normalized relaxivity r1 about 50% enhanced.

Conclusions: Gd-DOTAgad leads to MR signal enhancement in B35 neuroblastoma cells, due to the activity of the GAD enzyme. This effect was used to visualize the difference between native CGR8 cells and their neuronally differentiated derivatives by MRI and relaxometry. The implantation of these pre-labelled stem cells is in progress to evaluate the contrast in vivo. This approach holds potential to follow their differentiation fate in vivo with MRI.

Acknowledgement: This work was financially supported by grants from BMBF (0314104) and ENCITE EU-FP7 (HEALTH-F5-2008-201842) program.

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YIA applicant

SPECT IMAGING OF THROMBI USING FIBRIN-BINDING PEPTIDES

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Introduction**:** Thrombosis is the underlying pathology in a number of life-threatening cardiovascular diseases, such as heart attack, ischemic stroke, pulmonary embolism, and deep vein thrombosis. Furthermore, the presence of thrombi on atherosclerotic lesions is an indicator of plaque vulnerability [1]. The non-invasive localization and characterization of thrombi *in vivo* using diagnostic imaging modalities is therefore crucial for clinical decision making on possible therapeutic options. To this aim, we synthesized and evaluated a novel peptide-based, fibrin-targeted SPECT contrast agent.

Material and Methods: *Synthesis:* A fibrin-targeted peptide probe (RWQPCPAESWT-Cha-CWDPGGGK-DOTA) was prepared by conventional Fmoc solid-phase peptide synthesis. In addition, a scrambled peptide probe (WPTAD-Cha-RAW-PSQEWPAGGGK-DOTA) was synthesized as a negative control. Both probes were analyzed by liquid chromatography-mass spectroscopy (LC-MS). The peptides were labeled with ^{111}Indium prior to application. *In vitro:* Fibrin binding capacity was assessed using both a fibrin assay and a blood-clot assay. *In vivo:* Thrombosis was induced in the carotid artery of wild-type C57Bl6 mice by $FeCl_3$ injury. The *in vivo* biodistribution and thrombus-binding ability of these probes were evaluated by ?-counting. Furthermore, thrombi were visualized *in vivo* by means of SPECT-imaging of the radio-labeled peptides.

Results**:** *Synthesis:* The fibrin-binding peptide was successfully synthesized. The mass of the peptides found by LC-MS matched the theoretical expected mass (? mass < 0.2 Da). An ^{111}Indium labeling-protocol was established, yielding more than 95% radiochemical purity.*In vitro:* The fibrin-targeted probe showed high affinity for fibrin, as the fibrin-binding peptide bound 60-fold more to fibrin and 6-fold more to human blood-clots compared to the negative control peptide. *In vivo:* The fibrin-binding peptide showed increased uptake in the thrombus-containing carotid in comparison to the non-injured carotid, while the negative control peptide did not. The probes were excreted by the renal pathway and displayed short circulation half-lives (<20 min). Currently the ability of the fibrin-binding probe to visualize the induced thrombi *in vivo* using SPECT is under investigation.

Conclusions**:** The fibrin-binding peptide displayed both *in vitro* and *in vivo* high potency for binding to thrombi, whereas the negative control peptide did not. In addition, renal excretion and accompanying fast blood kinetics of the fibrin-binding probe show promise for translation towards clinical settings.

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COMPARISON OF TWO NEW EFFICIENT DUAL MRI/ FLUORESCENCE PROBES FOR PANCREATIC ISLET LABELING

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Introduction: Magnetic resonance imaging (MRI) is a powerful, noninvasive technique that allows monitoring the fate of the transplanted pancreatic islets (1,2). For islet visualization on MR images, contrast enhancement managed by iron oxide nanoparticles were used as the first, but lately, some other transition metals have come into the use (1,3). In our study we tested contrast agents based on the perovskite Mn/fluorescein and Gd chelate/rhodamine B. The aim of this study was to find out MR properties, cellular distribution and quality of the islets labeled with these dual MRI/fluorescence probes.

Methods: Islets were labeled for 24 hours with perovskit Mn or Gd contrast agent (Gd-R CA). MRI in gelatin phantoms was performed in vitro using a 4.7T Bruker Biospec spectrometer. Islet vitality was tested by differentiation staining of live/death cells. Islet function was tested by glucose stimulated insulin secretion. Anti-C-peptide and anti-CD68 antibodies were used for pancreatic beta cells and macrophages identification.

Results: Labeled islets were visible as hypointense (perovskit Mn) or hyperintense (Gd-R CA) spots on MR scans. With the use of specific staining with anti-C-peptide and anti-CD68 antibodies we could identify the contrast agent (green fluorescence in case of perovskit Mn and red fluorescence in case of Gd-R CA) inside the beta cells and macrophages. As compared to control islets, the function as well as vitality of the labeled islets was not impaired.

Conclusions: Labeled islets were clearly visible as hypointense spots (perovskit Mn) or hyperintense white spots (Gd-R CA) on MR images scanned in vitro. Cellular localization of the contrast agent was confirmed by fluorescence microscopy. These novel contrast agents based on the perovskite manganite or gadolinium chelates represent efficient dual MRI/fluorescence probe for islet labeling characterized by chemical stability, safety and excellent MRI properties. Especially for in vivo experiments these types of contrast agents are needed for proper detection of islets, so both bimodal contrast agents seem to be promising for islet imaging.

Acknowledgement: This work was supported by a grant from the ENCITE - Seventh EU Framework Program 201842.

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THE INFLUENCE OF THE CHELATE ON PET IMAGING USING GA-68 AND CU-64-LABELLED SOMATOSTATIN ANTAGONISTS

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Introduction: Positron Emission Tomography (PET) is becoming a dominating modality in molecular imaging with ^{68}Ga and ^{64}Cu being among the most attractive positron emitters. Many patients with somatostatin receptor (sst)-positive tumors were studied with ^{68}Ga-labelled somatostatin agonists. Recently, we showed that radiolabelled somatostatin antagonists may be preferable to agonists as they showed better pharmacokinetics, including higher tumor uptake (1, 2). The aim of this work was to study the influence of the chelator and the radiometal in the pharmacological properties, and as a consequence in the image contrast, of this new class of compounds. The main goal was to develop new PET radiopharmaceuticals for clinical application.

Methods: Three macrocyclic chelators, DOTA, NODAGA and CB-TE2A were coupled to a novel antagonist, named LM3. NODAGA-LM3 was labelled with ^{64}Cu and ^{68}Ga at room temperature. CB-TE2A-LM3 and DOTA-LM3 were labelled with ^{64}Cu and ^{68}Ga, respectively, at 95°C. All radiotracers were evaluated *in vivo* (biodistribution and microPET studies) in sst2-tumor xenografted mice, at 1 h (^{68}Ga and ^{64}Cu) and at 4 and 24 h (^{64}Cu) post-injection. Blocking studies were done with an excess of cold peptide.

Results: All radiotracers were prepared with labelling yield > 95% and specific activities 15-20 MBq/nmol (^{64}Cu-NODAGA/CB-TE2A-LM3) and 100-120 MBq/nmol (^{68}Ga-NODAGA/DOTA-LM3). Pharmacokinetics strongly depended on the chelators and the radiometals labelled to them. All radiotracers show high tumor uptake at 1 h, particularly ^{64}Cu-/^{68}Ga-NODAGA-LM3 (35.5±5.7 and 37.3±5.5 %IA/g, respectively). ^{64}Cu-NODAGA-LM3 exhibited fast background clearance, while about 40% remained in the tumor after 24 h. ^{64}Cu-CB-TE2A-LM3 had slower clearance and, surprisingly, almost no washout from the tumor within 24 h (e.g. 26.9±3.3 and 21.5±2.1 %IA/g at 4 and 24 h, respectively). Although tumor-to-normal tissue ratios were increasing over time for both radiotracers, the difference in the pharmacokinetics led to significantly higher values for ^{64}Cu-NODAGA-LM3 than for ^{64}Cu-CB-TE2A-LM3 (e.g. tumor-to-kidney: 12.8±3.6 and 1.7±0.3, respectively, tumor-to-muscles: 1342±115 and 75.2±8.5, respectively, at 24 h). MicroPET-images reflected the improved tumor-to-background contrast of ^{64}Cu-NODAGA-LM3. Improved image contrast was also achieved with ^{68}Ga-NODAGA-LM3, compared to ^{68}Ga-DOTA-LM3.

Conclusion: Four new PET radiotracers based on ^{68}Ga- and ^{64}Cu-labelled somatostatin antagonists were developed. Their pharmacokinetics is strongly influenced by the chelate. Among all radiotracers ^{64}Cu-/^{68}Ga-NODAGA-LM3 meet all necessary criteria for translation into a clinical PET-tracer.

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YIA applicant

FOLATE TARGETED MRI IMAGING PROBES

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Introduction. In respect to other molecular imaging modalities such as PET or SPECT, the low sensitivity is the main limitation of the Magnetic Resonance-Molecular Imaging approach. Therefore, the success of a MR-Molecular Imaging protocol strongly relies on the amplification effects associated to the accumulation of the agents at the pathological site. To this purpose the use of nanoparticles as carriers for MRI contrast agents (CA) has both the advantage to transport a high number of CA units at the site of interest and an improved efficiency of their contrast enhancement properties. Nanoparticles have to be functionalized with characteristic ligands that bind specifically to receptors that are expressed primarily on malignant cells. Folic acid is a vitamin essential for the proliferation and maintenance of cells. Normally, mammalian cells obtain their normal folate requirement via a low affinity reduced folate carrier or proton-coupled folate transporter, accessible folate receptors are expressed in significant numbers only on cancer cells, activated macrophages, and the proximal tubule cells of the kidney. Folate conjugates bind to folate receptors on these cell types with high affinity (Kd ‘ 10-9 M) and they are internalized through receptor-mediated endocytosis. In this study, liposomes containing lipophilic Gd-complexes, functionalized with folic acid have been considered to visualize ovarian cancer cells. (OVCAR, IGROV-1). Since CAs are administered intravenously, liposomes (100-200 nm diameter) extravasation is usually the ‘in vivo’ key step for tumor selective detection. For this reason a naturally occurring smaller size imaging probe based on the apoferritin cage (10-15 nm) loaded with Gd-HPDO3A (a commercially available CA) or manganese ions has been proposed in alternative to liposomes. Protein aminic groups were functionalized with folate units using standard conjugation protocols in order to give to the imaging probes tumor targeting capabilities.

Methods. Human ovarian carcinoma cells (OVCAR-3 and IGROV-1) have been incubated for 6h in the presence of increasing concentrations of folate-targeted liposomes and Gd/Mn loaded apoferritin. The amount of internalized paramagnetic ion was measured by ICP-MS. MRI of labeled cells was performed at 7T.

Results. The uptake by ovarian cancer cells of folate targeted liposomes was significantly higher with respect the non-targeted liposomes. This indicated that the non-specific binding of pegylated non-targeted liposomes was almost nil. The amount of internalized Gd-loaded liposome was sufficient to permit MRI cell visualization. Similar experiments were repeated using Gd or Mn loaded apoferritin. A lower paramagnetic ion concentration internalized by tumor cells was found by incubating apoferritin at the same concentration of paramagnetic ion used with liposomes. Therefore, the smaller size of apoferritin based imaging probes should allow a faster extravasation and therefore tumor accumulation. ‘In vivo’ studies on nude mice grafted with IGROV-1 are in progress.

Conclusions. Folate targeted liposomes and apoferritin display enough sensitivity to allow ovarian cancer cells MRI visualization. Furthermore, both imaging probes can also be loaded with antitumor drugs in order to perform imaging guided therapy.

A NEW 18F-LABELED IBT TRACER ALLOWS HIGH-CONTRAST IMAGING OF B-AMYLOID IN DIFFERENT AGE GROUPS OF AN APP/PS1 MOUSE MODEL OF ALZHEIMER'S DISEASE

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Aim: ß-Amyloid (Aß) pathological aggregation is discussed as an early and causal factor in the pathogenesis of Alzheimer's disease (AD). Aß has been depicted and quantified as an AD marker by means of positron emission tomography (PET), until now mostly by using ^{11}C-labelled tracers. The ^{18}F-Labeled tracers reported so far suffer from lower specific binding to Aß rich regions compared to their ^{11}C-analogues. We have developed a series of 2-phenyl imidazo[2,1-*b*]benzothiazoles (IBTs) as a class of PET tracers for Aß imaging in AD brain. The present work was focused on evaluating the performance of our most promising ^{18}F-labeled compound, [^{18}F]FIBT, in a preclinical evaluation protocol and its direct comparison with PiB in two age groups of APP/PS1 transgenic mice.

Methods: To evaluate the PET/CT imaging of [^{18}F]FIBT, this new tracer and PiB were investigated in two groups of (n= 8) tg mice of age 24 month old and 13 month old, and a group (n= 3) of 25 month old wt mice. The PET/CT evaluation was confirmed with ex vivo multi-modal evaluation, comprising small-animal PET, ex vivo dual tracer autoradiography, ex vivo regional brain distribution, and immunohistochemistry staining of Aß.

Results: By means of small-animal PET imaging in the APP/PS1 mouse model, we demonstrated a specific uptake of [^{18}F]FIBT in Aß-containing cortex brain regions. The binding pattern was conformed by regional brain biodistribution, ex vivo autoradiography and immunohistochemistry in the same animal. In the APP/PS1 model, [^{18}F]FIBT was identified to have a volume of interest (VOI) ratio cortex/cerebellum in old tg animals of 1.62 ± 0.12, in young tg animals 1.27 ± 0.15 whilst the corresponding value obtained in wild-type mice was 0.95 ± 0.01 ($p<0.05$). Immunohistochemical staining revealed localization of activity to individual Aß plaques on sections of APP/PS1 mice.

Conclusions: [^{18}F]FIBT, is easy to prepare and convert to production under conditions conform with GMP, with high binding affinity to Aß and allows high contrast in vivo PET imaging of Aß in Tg animals with different ages. [^{18}F]FIBT compared to PiB shows higher binding affinity, similar in vivo stability, higher signal strength and due to longer half life is much more suitable for imaging studies. The compound will be further evaluated and prepared for human studies and compared with other ^{18}F-labeled ß-amyloid tracers.

Acknowledgement: Financial support was received from the German Research Council, grant HE4560/1-2.

PARALLEL SESSION 4:

BIOMEDICAL APPLICATIONS OF PHOTONICS

(together with the Dutch IOP Photonics)

chaired by:

Vasilis Ntziachristos, Munich

Clemens W.G.M. Löwik, Leiden

YIA applicant

IN VIVO MULTI-PHOTON MOLECULAR IMAGING TECHNIQUE REVEALS PARENCHYMAL AND INTERSTITIAL CELL CROSS-TALKS IN CHRONIC INFLAMMATORY DISEASE: METABOLIC SYNDROME AND THROMBOSIS

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Introduction: To elucidate the underlying mechanisms of adult common diseases based on chronic inflammation, it is vital to examine the multi-cellular kinetics in living animals. Especially, metabolic syndrome is a major risk factor of cardiovascular events, and obese visceral adipose tissue remodeling and malfunctioning based on chronic inflammation and local immunological changes plays a central role

Methods: Therefore, we developed 'in vivo molecular imaging technique' based on single- and multi-photon microscope to assess the dynamic cellular interplay in diseased conditions. Fluorescent antibodies and dyes were directly injected into living animals, and images were obtained by spinning disk or resonance scanning microscopy from the abdominal window. To assess dynamic interplay between multiple cell-types associated with obesity, our visualization technique was applied to adipose tissue. In addition, we also examined the multicellular process of developing thrombosis in vivo.

Results & Discussion: In vivo imaging revealed close spatial and temporal interrelationships between angiogenesis and adipogenesis in obese adipose, and these inflammatory cell clusters included inflammatory macrophages (Ref 1). Increased leukocyte-platelet-endothelial cell interactions in the microcirculation of obese adipose were also observed, a hallmark of inflammation (Ref 2). The activation of platelet and increased adhesion molecule contribute to the inflammatory cellular dynamics, such as 'rolling and adhesion' of leukocyte and platelet. We also found that large numbers of CD8+ effector T cells infiltrated into obese adipose (Ref 3), and these CD8+ T cells contribute to the macrophage recruitment in obese adipose tissue, and adipose tissues inflammation in obesity.

We also applied this technique to another field, thrombosis, which is a common complication of metabolic conditions. To assess the dynamic cellular interplay in thrombosis and the underlying multi-cellular processes, 'in vivo imaging technique' which can identify single platelet in vivo can be a powerful tool. Thrombus formation was induced ROS production by laser irradiation, and we visualized single platelet kinetics in developing thrombus in living animals with much greater temporal and spatial resolution, to characterize the kinetics of platelet activity, cell shape changes, and contribution of coagulation system (Ref 4). Using this technique, we found that Lnk (adapter protein), which regulate integrin outside-in signaling, contribute to the stabilization of developing thrombus in vivo. In addition, we established human iPS-derived platelets and analyzed its function in vivo using this technique (Ref 5). We tracked the kinetics of injected artificial platelet in living mice, and confirmed that the injected platelets circulate, and contributed to the thrombus formation, indicating the clinical usefulness of this strategy for future.

Conclusions: Our results clearly demonstrated the power of our imaging technique to analyze complex cellular interplays in inflammatory diseases, especially parenchymal and stromal cell cross talks, and to evaluate new therapeutic interventions against them.

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NON-INVASIVE IMAGING OF CELL DEATH IN A MOUSE BRAIN CRYOLESION MODEL USING A TARGETED NEAR-INFRARED FLUORESCENT PROBE

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Introduction: Cell death imaging, as a method to diagnose disease states and treatment efficacies, is one of the most important unsolved problems in molecular imaging. There is currently a major international research effort to develop molecular imaging agents that target cell death biomarkers. PSVue® 794 (Molecular Targeting Technologies Inc., USA, www.mtarget.com) is a new near-infrared (NIR) fluorescent probe that targets phosphatidylserine exposed on the membrane surfaces of dead and dying cells. PSVue® 794 can identify the necrotic foci in tumors and acute muscle damage in living rodents.[1] Here, we report that PSVue® 794 can be used to monitor brain cell death by non-invasive NIR fluorescence imaging in a brain cryolesion mouse model.

Methods: 4T1-luc Mouse breast cancer cells were used for assessing PSVue® 794 in an in vitro cell-based cryo-death assay. 4T1-luc cells were grown to 80% confluency in wells and dry ice was applied for 10 s to the bottom center of each well. PSVue® 794 was then added to the wells and cells incubated at 37°C for 10 min. After incubation, the wells were washed, luciferin was added, and the wells were imaged using an LI-COR Odyssey™ and IVIS Spectrum. To initiate cryo induced cell death in vivo, a narrow metal cylinder was pre-cooled in liquid nitrogen and applied to the parietal region of each mouse`s head for 20 s or 60 s. The mice then received an intracardiac injection of either PSVue® 794 (3.0 mg/kg) or the untargeted control probe, 794-Control (3.0 mg/kg). Mice were subjected to non-invasive NIR fluorescent imaging at the indicated timepoints (0,1,3,6,24h post injection) in an IVIS Spectrum. After 24 h, mice were sacrificed, and ex vivo images were acquired with the skin above the parietal region and the skin and skull removed. Brain tissues were collected for further histological analyses.

Results: PSVue® 794 targeted cryo-induced 4t1-luc cell death with high specificity allowing for visualization by fluorescence microscopy. In vivo optical imaging showed that both PSVue® 794 and 794-Control accumulated at the brain cryolesion though PSVue® 794 had significantly higher target-to-non-target ratios and signal-to-noise ratios than the control in both the 20 s and 60 s cryolesion models. Histological data confirmed PSVue® 794 targeting to the brain cryolesion with 794-Control showing negligible accumulation in the brain.

Conclusion: PSVue® 794 non-invasively targeted and detected cell death in a brain cryolesion mouse model. This is the first demonstration of optical imaging of a brain cryolesion in a living mouse using a synthetic targeted cell death probe. The reliable and robust molecular probe, PSVue® 794, will likely be useful for monitoring the progression of brain injury. The brain cryolesion mouse model is easy to conduct and highly reproducible making it an ideal model for validating cell death imaging probes.

Acknowledgement: This study is supported by the Volkswagen grants, the ENCITE project, and NIH grants R01GM059078 (B.D.S.) and T32GM075762 (B.A.S.).

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DECOMPOSING PHYSIOLOGICAL AND MOLECULAR BIOMARKERS USING MULTISPECTRAL OPTOACOUSTIC TOMOGRAPHY

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Introduction: Disease detection and the overall study of therapeutic strategies are closely related with sensing and quantifying anatomical, physiological and molecular tissue biomarkers. Multi-Spectral Optoacoustic Tomography (MSOT) is a powerful novel imaging modality that decomposes the spectral responses of tissue chromophores in vivo, with high resolution and at depths of several millimeters to centimeters [1]. It can therefore offer the separation of absorbers of interest, due to intrinsic tissue molecules or extrinsically administered photo-absorbing agents and nanoparticles [2].Of particular focus in this study was to examine the separation and quantification of oxygenated and deoxygenated hemoglobin from background absorption and study the relative contributions of the two hemoglobin forms vs. other tissue molecules in vivo and post mortem as it associates with various pathologies, including malignant tumors [3,4]. We show MSOT as an accurate tool in separating the contributions of hemoglobin over background tissue (non-hemoglobin based) absorption and assessing disease states.

Methods:4T1 mouse mammary tumor cells (~10^6) were implanted by subcutaneous injection at the back and the side of nude mice (CD-1 Nude, Charles River Laboratories, Sulzfeld, Germany). For in-vivo imaging mice were kept under 1.5% isoflurane-anesthesia. Images were acquired at several time points: 5, 8, 10, 12, 14 and 16 days after implantation and at multiple wavelengths: 700nm, 730nm, 760nm, 780nm, 800nm, 860nm, 900nm using a whole mouse-body MSOT system. Multi-spectral decomposition techniques were applied to separate spectra representative of the hemoglobin contribution vs. other absorption contributions.

Results: By means of spectral unmixing it was possible to determine the distribution of oxygenated and deoxygenated hemoglobin in various mouse tissues in vivo: heart, liver, major blood vessels and correlate the hemoglobin (oxy- and deoxy-) quantification with post mortem states of tissues. Longitudinal studies of cancer xenographs further reveal the oxygenation heterogeneity from the periphery vs. the center of the growing tumor. Enabled by whole-mouse body, real-time imaging MSOT, the results allow insights into the previously undocumented ability to non-invasively study with high resolution the oxygenation distribution in various animal organs in vivo.

Conclusions: We show the superior performance of MSOT over other optical/opto-acoustic methods, in particular as to its ability to visualize with high resolution spectral signatures of multiple molecules through entire animals. We find that MSOT provides a unique possibility to detect hemodynamic related biomarkers indicative of disease inside the tissue. These findings can also help understand the evaluate treatment regimes.

Acknowledgements: We acknowledge support from the German Federal Ministry of Education and Research (BMBF) through the GO-Bio program.

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IMAGING THERMO-INDUCIBLE EXPRESSION OF REPORTER GENES IN TUMOR-INFILTRATRING MACROPHAGES.

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Introduction: Success of a gene therapy strategy depends on several parameters including the choice of the therapeutic gene, the efficiency of the delivery method and the reliability of the control of gene expression. Molecular imaging tools are of utmost importance to follow the time course of gene therapy at preclinical and clinical levels. Recently, we proposed an in vivo strategy to deliver a transgene to a tumor and to locally control its expression (Fortin et al. 2010). This strategy consisted of engraftment of single transgenic (luciferase) bone marrow cells (BMC) into a wild type mouse to create a chimera. When a subcutaneous tumor is induced, circulating leukocytes accumulate into and around the tumor as part of an inflammatory process. In the present study, using a double transgenic (luciferase + mPlum) mouse as BMC donor, we followed in vivo leukocyte accumulation using both bioluminescence imaging (BLI) and fluorescence imaging (FRI). In addition, we identified cell types expressing the transgene by fluorescent microscopy on tissue sections.

Methods: BMC were obtained from C57/BL6 (CD45.2) transgenic mice expressing firefly luciferase (LucF) and mPlum genes under transcriptional control of the heat shock protein 70 (Hspa1b) promoter. BMC were transplanted into CD45.1 mouse pre-treated with Busilvex® (25 mg/Kg) to induce medular aplasia. Engraftment efficiency was measured 2 months later by flux cytometry (CD45.1/CD45.2). Tumors (CMT-93) were implanted subcutaneously on the leg and heated by focused ultrasound (44°C, 8 min) 1 month later for transgene activation. LucF and mPlum expression were monitored in vivo by BLI and FRI. Chimeric mice were then fixed by intracardiac perfusion of 4% paraformaldehyde. Double immunohistochemistry was performed on 30 µm-thick tumor sections. mPlum was revealed using rabbit polyclonal DsRed primary antibody and anti-rabbit Alexa 546 secondary antibody. Macrophages and lymphocytes were revealed using CD68 and CD45-R antibodies, respectively, biotinylated secondary antibody and avidin-coupled Alexa 488. Slices were mounted with medium including DAPI coloration and visualized by epi-fluorescence and confocal microscopy.

Results: Local heating (44°C, 8 min) of the tumor allowed for detection of BLI and FRI signals in and around the tumor. Maximum BLI signal was found 6 hrs post-heating while FRI signal appeared later (around 30 hrs). In tissue sections, mPlum was mainly detected in CD68-positive cells and much more rarely in CD45-R-positive cells.

Conclusions: Local heating of tumors induced expression of transgenes placed under the Hspa1b promoter in bone marrow-derived cells. Most of these cells remained in the vicinity of or within the tumor as both luciferase activity 6 hrs and mPlum fluorescence 30 hrs post-heating were concentrated at the tumor site. Transgenic bone marrow derived cells were identified as macrophages in tumor sections. Bone marrow engraftment of genetically engineered cells may thus be used to address transgenes to a tumor.

Acknowledgement: We thank Dr. A. Planas and co-workers for providing the hsp/mplum mouse strain. This work was supported by: Conseil Régional d'Aquitaine, Ligue Nationale contre le Cancer and Diagnostic Molecular Imaging. References: Fortin P-Y et al. 2010. Proceedings of World Molecular Imaging Congress, Kyoto, Japan (Abstract 0192).

YIA applicant

COMBINED *IN VIVO* BIOLUMINESCENCE AND NEAR-INFRARED FLUORESCENCE IMAGING FOR THE DETECTION OF 4T1-LUC2 BREAST CANCER DEVELOPMENT

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Introduction: Both bioluminescence imaging (BLI) and fluorescence imaging (FLI) are widely used for non-invasive detection of tumor progression in small animals. Compared to FLI, BLI is far more sensitive and specific. It produces signals with hardly any background. However, since BLI utilizes native light emitted from genetically engineered tumor cells expressing reporter proteins, such as luciferase, this technique is only applicable in experimental animal models. Only FLI, in the form of optical image-guided surgery, is applicable clinically. For this purpose, most often near infrared fluorescence (NIRF) imaging is preferred because of its deep photon penetration accompanied by minimal tissue autofluorescence. Yet, the usefulness, in terms of sensitivity and selectivity, of various NIRF probes to detect tumor progression in animal models and in clinical situations remains to be assessed. To address this issue, we examined the ability of four commercially available NIRF probes to detect tumors and their metastases in a mouse 4T1-luc2 breast cancer model, and determined the relation between the obtained FLI and BLI signals. The examined tumor-specific probes were either activatable by matrix metalloproteinases, cathepsins (MMPSense680™, ProSense680™, PerkinElmer Inc.), or targeted to glucose transporter or the epidermal growth factor receptor (800CW 2-DG™, 800CW EGF™, LI-COR Inc.).

Methods: In vitro, methods included cell-based fluorescent assays by Odyssey (LI-COR Inc.), and the visualization of probe uptake by confocal microscopy. In vivo, 20.000 4T1-luc2 cells were implanted into the upper mammary fat pad of nude mice. After time-dependent(Day4,7,11,14,18) whole body BLI (IVIS-100™,Caliper Inc.) and FLI (Maestro™, CRI Inc.), thoracic cavities of mice bearing tumors were surgically opened and reimaged to reveal metastases of surrounding tissues. Tissues with positive signals were collected for histochemical analysis.

Results: In vitro, we showed that all four probes were either strongly activated by 4T1 luc2 cells, or bound with high affinity to the cells. In vivo, we showed that at Day4, there were already tumor signals detectable in intact mice, both by BLI and FLI. At Day18, clear lung and axial lymph node metastases could be detected by BLI and FLI after surgically opening up the mice. Furthermore, a strong correlation between the measured BLI and FLI signals was found for each of the four probes: Prosense680 ($r=0.7228$, $p<0.01$), 800CW 2-DG ($r=0.7041$, $p<0.01$), MMPSense680 ($r=0.9184$, $p<0.0001$) and 800CW EGF ($r=0.8752$, $p<0.0001$). In addition, tumor progression and metastases were confirmed histologically.

Conclusions: We showed strong specificity of four NIRF probes towards 4T1-luc2 breast cancer cells in vitro. These probes could also quantitatively detect 4T1-luc2 tumor progression in vivo. Using BLI as an internal control, we showed that tumors and their metastases can be detected by FLI with high precision, which is of great importance for the application with clinical purposes.

Acknowledgement: This study is supported by the Dutch CTMM Project MUSIS.

YIA applicant

SENSITIVE *IN VIVO* DUAL COLOR BIOLUMINESCENCE IMAGING USING MULTICOLOR LUCIFERASES

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Introduction: In the last decade bioengineering of luciferases has led to the generation of mutants with different characteristics in terms of emission wavelengths, kinetics, thermal stability, brightness and level of gene expression in mammalian cells [1,2].Here, we report the re-engineering of different luciferase genes for in vitro and in vivo dual color imaging applications to address the technical issues of using dual color luciferases for imaging.

Methods: A new red emitting codon-optimized luciferase reporter gene mutant of Photinus pyralis, Ppy RE8 [3] was used in combination with the green click beetle luciferase, CBG99. Human embryonic kidney cells (HEK293) were transfected with vectors that expressed red Ppy RE8 and green CBG99 luciferases. Populations of red and green emitting cells were mixed in different ratios. After addition of the shared single substrate, D-luciferin, bioluminescent (BL) signals were imaged with an ultrasensitive cooled CCD camera using a series of band pass filters (20nm). Spectral unmixing algorithms were applied to the images where good separation of signals was observed. Furthermore, HEK293 cells that expressed the two luciferases were injected in different organs at different depths in the animals.

Results: Good quantification and spectral separation of the dual BL signals in a mixed population of cells was achieved when cells were injected subcutaneously or directly into the prostate. When injected in the liver, the CBG99 spectrum was bimodal with a peak around 600nm and a shoulder at a lower wavelength. This is likely due to the presence of large amounts of hemoglobin, the principal absorber of green light, which prevents good spectral unmixing of the signals within this organ.

Conclusion: In respect to previously used dual assays, our study demonstrated enhanced sensitivity combined with spatially separated BL emission spectra using a suitable spectral unmixing algorithm. This new D-luciferin-dependent reporter gene couplet allows more accurate quantitative gene expression studies in vivo by simultaneously monitoring two events in real time.

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YIA applicant

LONGITUDINAL AND REAL-TIME IMAGING OF THE PHARMACOLOGICAL STIMULATION OF HIF ACTIVITY IN TUMOR ALLOGRAFTS

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Introduction: The oxygen-sensitive transcription factor Hypoxia Inducible Factor (HIF) plays a key role in regulating the adaptation of cells to decreased oxygen levels. In cancer, HIF is activated in response to tumor hypoxia and promotes the survival and proliferation of tumor cells by inducing genes involved in e.g. angiogenesis and anaerobic glycolysis. Moreover, HIF activity has been implied to reduce the effectiveness of chemo- and radiation therapy. HIF stability is controlled by prolyl hydroxylase domain proteins (PHD) which hydroxylate HIF-a subunits and thereby mark them for proteasomal degradation. Depending on molecular oxygen as a co-factor, PHD activity is inhibited during hypoxia which leads to the stabilization of HIF. Here we examined the effect of chronic pharmacological inhibition of PHDs on cancer progression in a mouse allograft tumor model using multimodal in vivo imaging approaches.

Methods: We investigated the impact of long-term application of the PHD inhibitor dimethyloxlalylglycine (DMOG) on hypoxic signaling events and cancer progression both in vitro, in C51 tumor cells, and in vivo, in s.c. C51 allograft tumors implanted into the neck of Balb/C nude mice.Using bioluminescence imaging of a reporter construct, driving the expression of luciferase from a HIF sensitive promoter (1), which was stably transfected into the C51 cells, it was possible to assess HIF activity. Furthermore, positron emission tomography (PET) in combination with the hypoxia sensitive radiotracer ^{18}F-misonidazole (FMISO) and magnetic resonance imaging (MRI) were employed to analyze the impact of chronic DMOG application on the level of tumor hypoxia and tumor vascularization in vivo.

Results: Paradoxically, long term DMOG application over a period of 10 days resulted in a decrease of HIF transcriptional activity in tumor allografts whilst there was no significant effect of the treatment on the tumor vascular network and the oxygenation level. In vitro experiments demonstrated that chronic application of DMOG to C51 cancer cells induces a negative feedback loop which acts to down-regulate HIF-1 protein levels and transcriptional activity, most likely through inhibition of PHDs.

Conclusion: Our results show that chronic stimulation of the HIF system in C51 cells in vitro and in allograft tumors in vivo causes the activation of a signaling loop which acts to decrease HIF-1 protein levels and activity. These findings imply a novel strategy to decrease HIF-1 levels in tumors which may potentially increase the efficacies of irradiation and chemotherapy based approaches in cancer treatment.

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ESMI PLENARY LECTURE 1 BY

TON G. VAN LEEUWEN

and

HONG ZHANG

chaired by

George Themelis, Munich

Eric Kaijzel, Leiden

CONSTRUCTION OF UPCONVERSION PHOTONIC NANOPLATFORM FOR BIOIMAGING AND PHOTODYNAMIC THERAPY OF CANCER

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In recent years, development of upconversion nanoparticles (UCNPs) capable of converting NIR- to visible photons under normal condition has been drawing considerable attention in biological and medical fields. In comparison to traditional fluorescent labels, such as organic dyes and quantum dots (QDs), UCNPs can be excited by NIR photons with an excellent signal-to-noise ratio owing to the absence of auto-fluorescence and reduction of light scattering. Besides UCNPs have also high photostability against photobleaching, and allow deep penetration in bio-tissue.

However, constructing such a qualified nanoplatform is a challenge bearing in mind that the inherent upconversion efficiency of a single emitter is extremely low under the biological/medical condition. Thus optimization of every step in energy transfer process and even more importantly, discovery of new mechanisms to enhance the upconversion efficiency, is becoming vital.

We have in recent years been focusing on the construction and optimization of the upconversion nanaoplatforms, typically rare earth ions doped NaYF4 and other nanohosts. In this presentation our efforts in increasing the upconversion luminescence and eventually in improving the singlet-oxygen generation will be introduced. Also preliminary results in realizing the photoswitchable dual functionality (photodiagnosis and photodynamic therapy) of these nanoplatforms will be demonstrated.

Acknowledgements: This work is under financial support from Dutch Innovation Research Program (IOP), KNAW-CAS joint research program and Natural Science Foundation of China.

PROMISES AND PITFALLS OF PHOTO-ACOUSTIC MOLECULAR IMAGING WITH GOLD NANOPARTICLES

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Photoacoustic imaging is a hybrid technique, based on detection of ultrasound transients generated by local absorption of ns pulses of light. Various studies have demonstrated the capability of photoacoustic imaging to visualize the increased vascularization around tumors, e.g. in tumor models (1) as well as in patients (2). In this presentation we will focus on the use of gold nanorods as a molecular contrast enhancer. Gold nanorods can be tailored to have an intense and narrow longitudinal plasmon absorption peak in the far-red to near-infrared wavelength region (3). Owing to the relatively transparency of tissue to light in that region, gold nanorods are not only excellent candidates as contrast agents for photoacoustic imaging, but also as photothermal therapeutic agents.

We investigated various aspects of the gold nanorods: the effect of size and shape on optical properties, their stability upon irradiation (4), their distribution after intravenous injection in rats (5), their conjugation and uptake by tumor cells (4) and the consequences of these effects on their ultimate optical properties. We will show drastic changes in optical properties during irradiation. PEGylation of gold nanorods resulted not only in a reduced toxicity (6), but also in a prolonged blood circulation in rats (half-life time of 19 h (5)). Transmission electron microscopy after incubation of cells with antibody-conjugated gold nanorods, showed that the particles are closely packed and clustered inside vesicles in the cells. Using dark-field microscopy we show that plasmonic interactions between nanorods in this situation causes blue-shifting of the LP absorption peak. As a consequence, no direct lethal damage to cells can be inflicted by laser irradiation at the (infra-)red absorption peak.

Thus, based on these results we conclude that owing to their long circulation time, PEGylated gold nanorods are promising candidates for therapeutic and diagnostic imaging purposes. However, care has to be taken to predict and interpret pulsed laser induced photoacoustic signals, because the absorption cross-section of these particles may change due to their clustering in the cells, as well as to their change in shape during irradiation.

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day two: monday 20 june 2011

ESMI PLENARY LECTURE 2 BY

ROBERT GILLIES

chaired by

Kevin Brindle, Cambridge

Arend Heerschap, Nijmegen

HETEROVALENT TARGETING

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Compared to other targets, cell-surface receptors are attractive in that there are many degrees of freedom in the design of targeting agents that do not have to cross the plasma membrane. Such approaches have been successfully applied in targeting cancers and metastases. Multivalent ligands, i.e. those containing multiple copies of pharmacophores, bind to their targets with higher affinity, compared to their monovalent counterparts and this has been employed to develop larger agents that bind with extremely high affinity. However, agents that contain a single type of pharmacophore (i.e. homo-multivalent) are limited to targets that are overexpressed.

Increases in the specificity and the numbers of possible targets can be achieved with hetero-multivalent ligands, htMVLs; i.e. those containing two or more heterologous pharmacophores that non-covalently crosslink receptor combinations. Such an approach can increase the number of potential targets by three orders of magnitude for each increase in valency. In previous work we have demonstrated the development and application of htMVLs in vitro. More recently, using a well-characterized proof-of-principle system, we have shown that a synthetic htMVL can discriminate cells containing two cognate receptors from cells that contain only one in vivo. In this system, a heterobivalent ligand (htBVL) was constructed containing truncated forms of melanocortin (MSH) and cholecystokinin (CCK) pharmacophores connected via a synthetic linker modified to carry a reporting group (Cy5 or DOTA:Eu). Target cells were expressed both MC1 and CCK2 receptors. Controls cells expressed only one receptor. Further controls used unlabeled ligands as blocking agents. In vitro, the bivalent ligand bound with higher affinity to the target cells compared to control cells or the monovalent ligands, and was internalized. Target and control cells were grown bilaterally as tumors in immuno-compromised (nu/nu) mice that were injected with either the Cy5 or the Eu-labeled htBVL. In vivo and ex vivo imaging showed specific binding to target tumors and internalization. Engineered hetero-multivalent ligands against target receptor combinations in vivo open the possibility that such platforms can be used for specific delivery of therapeutic payloads to any cell.

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PARALLEL SESSION 5:

IMAGING CANCER TREATMENT AND EVALUATION

(together with the ESR)

chaired by

Frauke Alves, Göttingen

Fabian Kiessling, Aachen

YIA applicant

MAPPING OF OXYGEN BY IMAGING LIPIDS RELAXATION ENHANCEMENT (MOBILE): APPLICATION TO CHANGES IN TUMOR OXYGENATION OF MAMMARY CANCER MODELS

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Introduction. Tumor hypoxia is a major factor of resistance to treatments. In order to individualize the treatments and select patients who can benefit from tumor reoxygenation treatment, there is a critical need for methods able to monitor dynamically and noninvasively tumor oxygenation. Variations in T_1 and T_2^* are potentially valuable MRI tools to changes in tumor oxygenation. T_2^* is sensitive to the relative Hb/HbO2 ratio in vessels (2), while T_1 change is sensitive to dissolved oxygen which acts as a T_1-shortening paramagnetic contrast agent. Recently, changes in tumor oxygen concentrations have been shown to produce changes in relaxation rate R_1 of water (3). This technique still lacks a good sensitivity. Here, we propose to exploit the higher solubility property of oxygen in lipids than in water (4) to monitor the changes in R_1 of the lipid peak and translate it into pO2 values. For this purpose, we developed a method to map variations in oxygenation based on the changes in the relaxation properties of the tissue lipids. This technique is called MOBILE for Mapping of Oxygen By Imaging Lipids relaxation Enhancement. We monitored the evolution of the R_1 of lipids in vivo in mammary cancer models before and during a carbogen breathing challenge.

Methods. NT-2 and MDA Mammary cancer models were implanted subcutaneously in the leg of NMRI and nude mice, respectively (n=5). Mice were anesthetized by isoflurane. An OxyLite MR compatible probe was introduced inside the tumor to monitor local changes in pO_2. Triplicates MR measurements of R_1 H_20, R_1 lipids, and R_2^* were acquired during air breathing. Then, breathing gas was switched to carbogen, and triplicates MR measurements were acquired at 10, 25, and 40 minutes after the gas switch. Finally, the animals were killed by switching the gas to nitrogen, and 3 measurements were acquired after the death of the animals. Experiments were performed on a 11.7T (Bruker, Biospec), and with a segmented IR FISP (Inversion-Recovery Fast Imaging with Steady state Precession) sequence.

Results. The higher solubility of oxygen led to a higher sensitivity when considering the evolution of R_1 of lipids as a function of oxygenation, compared to the R_1 of water: the change observed in R_1 measured in lipids was larger than the change in R_1 of water after the carbogen breathing challenge and after death. This correlated well with the evolution of the tumor pO_2 as measured by the OxyLite on the same tumor.

Conclusion. The measurement of R_1 in lipids offers an increased sensitivity when monitoring the changes in tumor oxygenation compared to previously described techniques that measure the variations of R_1 in the water component. MOBILE presents unique properties of sensitivity to variations in oxygenations as well as translational properties by using endogenous contrast.

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IN VIVO TRACKING OF ADOPTIVELY TRANSFERRED T CELLS ENGINEERED TO EXPRESS CHIMERIC ANTIGEN RECEPTORS (CAR) RECOGNIZING THE PROSTATE SPECIFIC MEMBRANE ANTIGEN (PSMA)

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Introduction: Immunology-based approaches have been proposed as a promising curative option to effectively attack postoperative minimal residual disease and distant metastatic localizations of prostate tumors. In this regard, however, results from clinical trials have shown that a single-approach immunotherapy (antibody-mediated or cell-mediated) might be insufficient to eradicate tumor cells and allows them to survive and adapt to the body's defence mechanisms or to passively administered cures. We exploited the concept that combination of two powerful tools, by endowing T cells with recognition capacity and high specificity of antibodies, can lead to results superior to those obtained by single-approach treatments. To this aim, we developed a CAR containing both the CD3zeta and CD28 signalling moieties fused to a scFv targeting the human Prostate Specific Membrane Antigen (hPSMA), to engineer human PBMC for the adoptive immunotherapy of prostate cancer. As a transfer method, we employed last-generation lentiviral vectors (LV) carrying a synthetic bidirectional promoter capable of robust and coordinated expression of two transgenes, thus allowing to co-express the CAR in conjunction with a reporter gene (luciferase). Using an in vivo fluorescence or bioluminescence imaging approach, we aimed at assessing and comparing survival, biodistribution and tumor homing properties of CAR-engineered T cells administered in mice bearing human PSMA-expressing prostate tumors.

Methods: Different fluorophores (Qdot, Cy5.5, VivoTag680, DiR) were used to label CAR-transduced T cells, and compared for in vitro toxicity and in vivo sensitivity of detection. In vivo experiments were performed using 6- to 8-wk-old male immunodeficient mice, which were inoculated subcutaneously or intravenously with PSMA-transfected PC3 prostate tumor cells. Upon intravenous inoculation of T cells, 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. Alternatively, mice were inoculated with luciferin and imaged with Lumina II (Caliper) in bioluminescence.

Results: CAR-expressing LV efficiently transduced short-term activated PBMC that, in turn, were readily stimulated to produce cytokines and exerted a relevant cytotoxic activity by engagement with PSMA+ prostate tumor cells. Among the different fluorophores tested for T cell labelling, DiR appeared the less toxic in vitro and the most brilliant for in vivo detection, and hence was selected for further studies. Upon in vivo transfer in tumor-bearing mice, T cells provided a different pattern of biodistribution according to the imaging technique employed. Indeed, bioluminescence showed an immediate and intense signal into lungs that rapidly disappeared during the first 24 hours post-injection. On the other hand, DiR-labelled T cells apparently accumulated in the liver but could be also visualized in lymph nodes and within the tumor mass.

Conclusions: Quantitative monitoring of genetically engineered human T lymphocytes by imaging provides spatial and temporal information on T cell trafficking, persistence and accumulation to the tumor site, thus providing critical clues for predicting tumor response and for choosing the 'right' T cell subset to employ to optimize the results.

MOLECULAR ULTRASOUND IMAGING IN DIFFERENTLY AGGRESSIVE BREAST CANCER XENOGRAFTS USING CLINICALLY TRANSLATABLE MICROBUBBLES (BR55)

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Introduction: Molecular ultrasound imaging has demonstrated its ability to detect disease-associated endothelial receptors reflecting the angiogenic status of a tumour [1]. The best characterised marker for angiogenesis is the vascular endothelial growth factor receptor type 2 (VEGFR2). Antibodies targeting the VEGFR2 have been conjugated to microbubbles usually via the Biotin-Streptavidin coupling method [2]. However, streptavidin is potentially immunogenic and should be avoided in humans. Thus, a clinically translatable contrast agent (BR55) [3] containing VEGFR2-specific ligands [4] that were directly inserted into the microbubble membrane have been recently introduced

The purpose of this study was to analyse the circulating characteristics of these novel VEGFR2 targeted BR55 microbubbles using a clinical ultrasound system (Siemens Acuson S2000) at a clinical frequency of 4 MHz (9L4 transducer). Further, their ability to discriminate differences in the angiogenesis in differently aggressive breast cancer xenografts was investigated. In addition the degree of tumour vascularisation was assessed with a nonspecific long-circulating contrast agent (BR38).

Methods In healthy mice the circulation characteristics of BR55 were investigated. Using BR38 the relative blood volume (rBV) of highly aggressive MDA-MB-231 (n=5) or less aggressive MCF-7 (n=6) tumours was determined. In the same tumours in-vivo binding specificity of BR55 was tested and VEGFR2 expression assessed. Data validation included quantitative immunohistological analysis.

Results BR55 had a blood half-life of 218s. BR38-enhanced ultrasound showed higher vascularisation in MDA-MB-231 tumours as compared to MCF-7 tumours (p=0.022), which was in line with immunohistology (p=0.033). Binding of BR55 was significantly higher in MDA-MB-231 than in MCF-7 tumours (p=0.049), which corresponded to the VEGFR2 levels found histologically (p=0.015). After the administration of a VEGFR2-blocking antibody (in-vivo competitive binding experiments) there was significantly less accumulation of BR55 within tumors. (p=0.027). Ultrasound measurements and histological quantification revealed the same ratio of VEGFR2 expression in MDA-MB-231 as in MCF-7 tumours.

Conclusions Clinically translatable VEGFR2-targeted ultrasound contrast agents are well capable to characterising and distinguishing breast cancers with different angiogenesis and aggressiveness. BR55 accumulation within the tumour endothelium faithfully reflects the VEGFR2 status and depicts even small differences in angiogenesis. In addition, our results show that the differences in the angiogenesis between the two tumour types could be better depicted with the molecular information compared to the vessel information.

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YIA applicant

PARAMETRIC CORRELATION OF FDG-PET AND DW-MRI TO MONITOR RESPONSE TO TUMOR THERAPY

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Introduction: Changes in Apparent Diffusion Coefficient (ADC) obtained from Diffusion Weighted Magnetic Resonance Imaging (DW-MRI) show early changes induced by chemotherapy in an oncologic setting with increases in ADC values. This increase suggests a decreased tumor cellular density, collapsed cell membrane barriers, and increased mobility of water molecules, allowing for the differentiation of necrosis and edema from viable tissue[1]. Concurrently, FDG is one of the most widely used PET tracers, and high uptake values are linked with hypoxic and proliferating cells, while low uptakes are correlated with necrosis and normoxic cells[2]. With the increasing prevalence of combined PET/MR imaging devices in Clinics around the world, the understanding of complementary information obtained from different biomarkers is important, and could lead to a clear delineation of the tumor microenvironment *in vivo*.

Methods: 8 week old NMRI Nu/Nu (n=8) mice obtained from Charles Rivers, Germany were injected with 1.2 million NCI-H460 Tumor cells, and were allowed to grow until tumor volume was around 300 mm^3. One baseline scan was made before initiation of treatment regiment of 2 mg/kg every three days with Cetuximab (Merck, Germany), an anti-EGFR monoclonal antibody (mAb). Up to three more FDG PET and ADC scans were made until tumors reached a critical size and had to be sacrificed. After the last MR imaging session a line parallel to the coronal plane was marked on the tumor in order to correlate histological slices (CD-31, Ki-67, and H&E) to *in vivo* MR and PET images.

Results: Significant linear correlations as determined by the Pearson's correlation coefficient were determined from the average values in tumors for imaging data obtained from the control and treatment groups respectively: FDG (SUV) vs. ADC (control: P =0.001 r = 0.8; treatment: P=0.03 r =-0.64), tumor volume vs. ADC (P = 0.0007 r = 0.834/ P= 0.03 r = 0.68), tumor volume vs. FDG (SUV) (P = 0.02 r = 0.62/ P= 0.001 r = 0.83), and tumor volume vs. the intratumoral pearson correlation of FDG and ADC voxels (P=0.008 r = -0.81/ P=0.03 r = -0.67). FDG and ADC voxel values showed a strong negative correlation in each tumor where treated tumors experiencing the largest negative correlation coefficient. Histology revealed high ADC values for necrotic and edemic regions which negatively correlated to FDG values in those same regions. Histology is in good agreement with images.

Conclusions: We have shown that FDG and ADC values are highly negatively correlated in xenografts tumors, and that this correlation changes in a significant in response to treatment. Furthermore, complementary information obtained from the tumor microenvironment reveals grouping in the scatter plots of ADC vs. FDG (SUV) that have the potential to be modeled in order to segment the tumor into distinct biologically relevant compartments.

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YIA applicant

TUMOUR REGRESSION AND APOPTOSIS AFTER ERUFOSINE (ERPC3) TREATMENT IN A PRECLINICAL MODEL OF MALIGNANT GLIOMA

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Introduction : Glioblastoma multiforme is the most malignant type of primary human brain tumour. There is a strong need of improved anti-glioblastoma treatments because the current median survival for patients is less than one year. Erucylphosphohomocholine (ErPC3, erufosine) has been shown to induce apoptosis in otherwise highly apoptosis-resistant glioma cell lines and its effect appears to depend on the 18 kDa Translocator Protein (TSPO) [1]. [^{18}F]DPA-714 is a recently developed TSPO radioligand that can be used in positron emission tomography (PET) to non-invasively image glioma. Objectives : To investigate 1) the effect of ErPC3 on 9L tumor cell growth *in vitro* and *in vivo* (orthotopic rat glioma model), and 2) the use of [^{18}F]DPA-714 as a radiotracer to monitor ErPC3 treatment.

Methods : Western Blot analysis was used to measure the TSPO expression level in 9L glioma cells. Cells were incubated with ErPC3 (0-100µM) and treatment efficacy was characterised by cell viability, cell death and apoptosis assays (DNA degradation and cleavage of caspase-3). 9L rat glioma cells ($2x10^5$ cells) with defined TSPO level and sensitive to ErPC3 treatment were then implanted into the striatum of Fischer rats (n=6 ErPC3-treated, n=5 sham-treated). 11 days after implantation of glioma cells, rats received either ErPC3 (40mg/kg body weight) or sham-treatment at 48h intervals and for a maximum of 2 weeks. Tumour growth as well as ErPC3-treatment efficacy was monitored by [^{18}F]DPA-714 PET before and after therapeutic intervention. Validation of imaging was done by histology (hematoxylin/eosin), TUNEL staining and immunohistochemistry (anti-TSPO, anti-CD11b, anti-GFAP).

Results : *In vitro*, 9L cells showed high TSPO expression (Western Blot). ErPC3 treatment (24 hours, 50 µM) yielded decreased cell viability (40.0 ± 7.6 % of controls), a 4-fold increase in apoptosis and a 5-fold augmentation of caspase-3 cleavage. *In vivo*, [^{18}F]DPA-714 PET-imaging showed a significant difference ($p < 0.001$) in tumour to contralateral brain signal ratios between treated (2.7 ± 0.6) and mock-treated (4.9 ± 1.0) animals after one-week of ErPC3 administration. However, the treatment resulted a) 7-14% loss of body weight whereas that of control animals was stable and b) did not improve survival. Histological analysis (hematoxylin/eosin) determined a mean tumor volume of 185 ± 134 mm3 for mock-treated animals in contrast to 107 ± 71 mm3 in ErPC3-treated animals. Furthermore, TUNEL staining showed an increased apoptotic effect after ErPC3 treatment with a ratio (TUNEL-positive cells/ Tumour area) of 21.6 ± 8.2 in the treated group compared to 0.5 ± 0.1 in sham-treated animals. Immunohistochemistry revealed enrichment in CD11b-positive microglia/macrophages and astrocytes (GFAP+) in the tumor area under ErPC3 medication.

Conclusion : This study confirms the apoptotic effect of ErPC3 in rat glioma, both *in vitro* and *in vivo* and demonstrates the feasibility of employing [^{18}F]DPA-714 PET to monitor ErPC3 treatment.

Acknowledgment : Supported by the Joint INCa / DAAD Translational Research Programme on Cancer; The authors are grateful to Genzyme for providing erufosine.

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YIA applicant

NEAR-INFRARED FLUORESCENCE SENTINEL LYMPH NODE DETECTION IN BREAST CANCER PATIENTS: THE GREEN LIGHT STUDIES

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Introduction: Detection of the sentinel lymph node (the first node to which tumor cells metastasize) is important in the diagnosis and treatment of breast cancer. Currently, blue dyes and radiotracers are used as standard of care. These modalities have disadvantages, including the use of ionizing radiation and staining the surgical field in an unnatural blue color. Near-infrared (NIR) fluorescence imaging is a technique that can be used to visualize lymph nodes during surgery, in real-time. This technique uses invisible NIR light (700-900 nm) that can penetrate several centimeters in living tissue. Currently, indocyanine green (ICG) is the only NIR fluorescent contrast agent that is approved for in vivo clinical applications. Premixing of ICG with human serum albumin (HSA, complex is ICG:HSA) improved the fluorescence quantum yield and sentinel lymph node retention in preclinical experiments. The current studies focus on optimizing the use of NIR fluorescence imaging for the sentinel lymph node procedure in 3 clinical trials with a total of 64 patients.

Methods: In all studies, the Mini-FLARE intraoperative imaging system (Frangioni Lab, Boston, USA) was used for intraoperative detection of the NIR fluorescence signal. First (study 1), the optimal dose of ICG:HSA was studied in a phase I study in which 24 consecutive breast cancer patients were included. These patients received the standard of care sentinel lymph node procedure (using blue dye and 99mTc-nanocoll) and were injected with 1.6 mL of ICG:HSA (dose groups ranging from 50 to 1000 µM). The potential advantage of premixing of ICG with HSA was then studied in a randomized, double-blind study (study 2), with 18 consecutive patients. Patients were injected with 1.6 mL of 500 µM ICG alone or ICG:HSA. Subsequently, sentinel lymph node mapping was performed using 500 µM ICG in 24 patients, with randomization between the use or omission of patent blue (study 3).

Results: The sentinel lymph node was successfully detected in all patients. In the dose finding study (study 1), a total of 35 lymph nodes were detected (average 1.45), all of which were radioactive, 30 nodes were blue. The optimal dose was between 400 and 800 µM. For following studies, a dose of 500 µM was chosen. No differences were observed when premixing ICG with HSA (study 2), in the number of sentinel lymph nodes identified (average of 1.4 per patient), nor in fluorescence intensity (P = 0.18). Therefore, no advantage was demonstrated of premixing ICG with HSA in a clinical setting. No difference in fluorescence was observed when patent blue dye was used or omitted (study 3). The sentinel lymph node was detected in all patients using NIR fluorescence before the blue dye could be observed.

Conclusion: These studies demonstrate the successful use of NIR fluorescence and ICG in sentinel lymph node mapping of breast cancer patients. The optimal paramaters are a dose of 500 µM ICG that is not premixed with HSA, and the use of patent blue can be omitted. These data can be used to perform larger validation studies to assess patient benefit.

CXCR4 IMAGING AND SURGICAL GUIDANCE BASED ON A SINGLE TARGETED HYBRID IMAGING AGENT

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Introduction: Accurate tumor staging via evaluation of specific biomarkers is key in the treatment of cancer and visualization of marker-positive tumor cells may aid radical excision of the lesions. The development of imaging agents for these specific biomarkers is emerging in both the pre-clinical and clinical setting. For example, several imaging agents have been developed that target the potentially interesting biomarker Chemokine receptor 4 (CXCR4).[1,2] In stead of using a different imaging agent for every step in the diagnostic trajectory, hybrid imaging agents with both radioactive and fluorescent properties can be used throughout the whole diagnostic process. We used a single CXCR4 targeting hybrid tracer to determine the expression level in biopsy tissue, plan the surgical intervention, guide the surgical excision and validate the targeting efficacy *ex vivo.*

Methods: Ac-TZ14011, an antagonistic peptide that targets the CXCR4 receptor was labeled with a multifunctional single attachment point (MSAP) reagent . Next to the targeting moiety MSAP-Ac-TZ14011 contains a DTPA chelate (enabling radioactive labeling with ^{111}In) and a fluorescent dye with Cy5.5 spectral properties.[3] The level of CXCR4 expression in tumor tissue was assessed by FACS analysis using the fluorescent beacon. SPECT/CT and fluorescence imaging were then used to provide surgical guidance. To validate the targeting efficacy, tracer distribution was evaluated ex vivo via fluorescence imaging (macro scale), confocal microscopy and FACS analysis (micro scale).

Results: FACS analysis of CXCR4-positive tumor cell suspensions incubated with MSAP-Ac-TZ14011 clearly revealed CXCR4 positive cell-populations and even enabled staging of lesions based on their differences in CXCR4 expression. After target validation in the mouse tumor model, ^{111}In-MSAP-AcTZ14011 enabled 3D SPECT/CT imaging revealing the location of the tumor and surgical fluorescence guidance towards the tumor. Ex vivo examination of the tumor tissue enabled evaluation of the tracer distribution and target specificity via fluorescence imaging.

Conclusions: The hybrid imaging agent ^{111}In-MSAP-AcTZ14011 allows for the integration of the different diagnostic processes involved in targeted imaging and surgical guidance. Hereby showing the added value multimodal imaging labels in this translational research field.

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PARALLEL SESSION 6:

EX VIVO AND *IN VIVO* MICROSCOPY AND CELL TRACKING

(together with the FP7 project ENCITE)

chaired by

Mathias Hoehn, Cologne

Boudewijn Lelieveldt, Leiden

Hans Tanke, Leiden

LIGHT SHEET-BASED FLUORESCENCE MICROSCOPY (LSFM) REDUCES PHOTOTOXIC EFFECTS AND PROVIDES NEW MEANS FOR THE MODERN LIFE SCIENCES

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Most optical technologies are applied to flat, basically two-dimensional cellular systems. However, physiological meaningful information relies on the morphology, the mechanical properties and the biochemistry of a cell's context (Pampaloni 2007). A cell requires the complex three-dimensional relationship to other cells (Pampaloni 2010). However, the observation of multi-cellular biological specimens remains a challenge. Specimens scatter and absorb light, thus, the delivery of the probing light and the collection of the signal light become inefficient; many endogenous biochemical compounds also absorb light and suffer degradation of some sort (phototoxicity), which induces malfunction of a specimen.

In conventional and confocal fluorescence microscopy, whenever a single plane is observed, the entire specimen is illuminated (Verveer 2007). Recording stacks of images along the optical z-axis thus illuminates the entire specimen once for each plane. Hence, cells are illuminated 10-20 and fish embryos 100-300 times more often than they are observed (Keller 2008). This can be avoided by changing the optical arrangement. The basic idea is to use light sheets, which are fed into the specimen from the side and overlap with the focal plane of a wide-field fluorescence microscope. In contrast to an epi-fluorescence arrangement, such an azimuthal fluorescence arrangement uses two independently operated lenses for illumination and detection (Stelzer 1994; Huisken 2004). Optical sectioning and no photo-toxic damage or photo-bleaching outside a small volume close to the focal plane are intrinsic properties. Light sheet-based fluorescence microscopy (LSFM) takes advantage of modern camera technologies. LSFM can be operated with laser cutters (e.g. Colombelli 2009) and for fluorescence correlation spectroscopy (FCS, Wohland 2010). During the last few years, LSFM was used to record zebrafish development from the early 32-cell stage until late neurulation with sub-cellular resolution and short sampling periods (60-90 sec/stack). The recording speed was five four Megapixel large frames/sec with a dynamic range of 12-14 bit.

We followed cell movements during gastrulation, revealed the development during cell migration processes and showed that an LSFM exposes an embryo to 200 times less energy than a conventional and 5,000 times less energy than a confocal fluorescence microscope (Keller 2008). Most recently, we implemented incoherent structured illumination in our DSLM (Keller 2010). The intensity modulated light sheets can be generated with dynamic frequencies and allow us to estimate the effect of the specimen on the image formation process at various depths in objects of different age.

IMAGING ORGANOGENESIS: MOUSE LIMB DEVELOPMENT

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Quantitative mapping of the normal tissue dynamics of an entire developing mammalian organ has not been achieved so far but is essential to understand developmental processes and to provide quantitative data for computational modeling. We have developed a four-dimensional (4D) imaging approach that can be used to quantitatively image tissue movements and dynamic GFP expression domains in a growing transgenic mouse limb by time-lapse optical projection tomography (OPT). I will describe our completed and ongoing work to tackle both the imaging and in-vitro culturing problems presented by this challenge.

MULTIMODAL/MULTI-SCALE IMAGING OF BONE MARROW STEM CELLS AND THEIR MICROENVIRONMENT (NICHES) IN HEALTH AND DISEASE

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Introduction: Haematopoietic stem cells (HSCs) reside in specialized bone marrow (BM) environments called stem cell niches (HSCNs). Two types of HSCNs have been reported, involving osteoblasts (osteoblastic niche) or blood sinusoids (vascular niche). However the bone marrow microenvironment has not yet been precisely characterised and the localization, composition and regulation of the niche(s) remain highly controversial. Leukaemic stem cells (LSCs) originate from HSC/progenitors and are responsible for the development of acute myeloid leukaemia. Because they can resist to treatments, they are believed to causing relapse. It is not clear yet whether LSC need a specialized niche to regulate their behaviour. This study aims at improving our knowledge on these different points.

Methods: Multi-parametric observation of live bone marrow within intact bones was implemented thanks to combined multiphoton / confocal intravital microscopy after in-vivo contrasting procedures. In order to precisely quantify bone remodelling activity and vascular density within different bone marrow compartments, we have developed a new strategy that we called 'In-vivo fluorescence trapping', taking advantage of different near-infrared probes. Comparative analysis of the systemic trafficking and quantification of proliferation capabilities of different types of murine HSCs or human leukaemia cells were realised using bioluminescence imaging. Flow cytometry and histology were used to sort specific populations of cells, analyse the frequency of stem cells or leukemic cells, quantify perfusion efficiency and hypoxia and cross-validate in-vivo imaging procedures.

Results: Unexpectedly we demonstrate a strong heterogeneity between different BM compartments in terms of blood vessels density and bone remodelling activity, which correlates with sites of development of normal HSCs but also LSC and bone metastasis from solid tumours. We also demonstrate for the first time that osteoblastic niches are in close proximity to vascular niches and we analyzed the hypoxic status of stem cells. Eventually we are exploiting these technologies to characterise the development of human leukaemia and its consequences on BM microenvironment using intravital microscopy of intact live BM.

Conclusions: This study confirms and extends our recent statement considering the critical need for multimodal imaging (Lassailly et al., 2010). Furthermore, it demonstrates that combination of different imaging modalities for in-vivo and ex-vivo analysis is a powerful strategy which allows us to bring a new light on the structure of the bone marrow microenvironment and further improve our understanding of dynamic stem cells / niches interactions in health and disease.

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YIA applicant

IN VIVO TRACKING OF HUMAN NEURAL STEM CELLS IN THE MOUSE BRAIN WITH 19F MRI

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Introduction: MRI is a promising tool for monitoring stem cell based therapy. Conventionally, cells are loaded *in vitro* with iron oxide particles, which appear hypointense on ^{1}H MR images. However, the cell signal is not specific due to ambiguous background and not quantitative. An emerging field to overcome these drawbacks is ^{19}F MRI of cells labeled with perfluorocarbon (PFC) emulsions [1]. Fluorine is virtually absent in the body, i.e. only labeled cells generate contrast, and quantification is possible as the signal is proportional to the concentration of ^{19}F. Here it is shown, for the first time, that human neural stem cells (hNSCs), which have shown high therapeutic potential for brain pathology [2], can be labeled with PFCs, detected and quantified in vitro and after brain implantation.

Methods: hNSCs were incubated with a PFC emulsion (CELSENSE 1000, Celsense, USA), viability was assessed with trypan blue exclusion, and immunocytochemistry was carried out to evaluate the influence of the label on cell phenotypes. For *in vitro* MRI, hNSCs were suspended in gelatin at varying densities. For *in vivo* experiments, 150.000-300.000 labeled hNSCs or non-labeled controls were implanted into the striatum of mice (n=4). Animals were scanned with ^{1}H MRI and ^{19}F MRI (total time<1.5 h, 400x400x1000 µm^3 ^{19}F image resolution) on an 11.7 T scanner (Bruker BioSpec, Ettlingen, Germany) and transcardially perfused after the last MRI session.

Results: hNSCs were labeled most efficiently at a concentration of 80 µl emulsion/ml medium. The labeling lead to a decrease of cell viability compared to controls directly after incubation (83±4 % vs. 93±7 % living cells, $p<0.05$), which renormalized after 7 days in culture of the replated cells. We did not detect label-related changes in the numbers of Ki67, nestin, GFAP, or ßIII-tubulin+ cells during both, proliferation and differentiation. The dilution series indicated that 1.000 hNSCs needed to accumulate in one image voxel to generate significant signal to noise ratio (SNR) in vitro. We detected grafts in vivo with ^{19}F MRI over several days but not the controls. From SNR analysis we estimate a detection limit of a few 10.000 cells/voxel. The location and density of human cells (hunu+) on histological sections correlated well with observations in the ^{19}F MR images.

Conclusion: Our results show that hNSCs can be labeled with ^{19}F with little effects on viability or proliferation and differentiation capacity. Our optimized settings allowed higher resolution and a lower detection limit than in other reports on MRI of ^{19}F labeled cells. This proves for the first time that ^{19}F MRI can be utilized for cell tracking in brain implantation studies, in which the graft may be initially small or diluted through cell migration induced by the damage.

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IMPROVED INFRARED MULTIPHOTON MICROSCOPY AND FLUORESCENCE LIFETIME IMAGING (FLIM) TO MONITOR TUMOR INVASION AND EXPERIMENTAL THERAPY *IN VITRO* AND *IN VIVO*

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Cancer invasion and metastasis are complex processes which depend upon both, the tumor cell and the tumor microenvironment. Thereby, local biophysical parameters such as tissue pressure and temperature as well as the architecture and composition of the extracellular matrix shape tumor invasion by either providing guidance structures or by acting as a physical barrier, restricting tumor cell motility. Using the dorsal skin-fold chamber model and infrared excited multiphoton microscopy, the progression of orthotopically grafted melanoma tumors was monitored, including the identification of anatomic structures guiding tumor invasion and the temperature sensitivity of tumor growth. Upon intradermal implantation, tumors developed zones of invasive growth consisting of multicellular collective invasion strands that retained cell-cell contact and vigorous proliferation, yielding velocities of up to 300 µm/day. Invasion-guiding tissue structures were identified by second harmonic generation (SHG; 1200/600 nm) detecting collagen fibers and striated muscle strands, third harmonic generation (THG, 1200/400 nm) visualizing nerve fibers, fat cells and tumor associated lipid microparticles, and by counterstaining blood vessels and phagocytes using fluorescent dextran. Both tumor growth and invasion dynamics in the window model strongly depended on the housing temperature of the animals. Increased mouse housing temperatures of 31°C significantly enhanced tumor growth rates and the formation of invasion strands, compared to normal housing at room temperature. Although at lower level, angiotropic invasion was still observed at lower temperatures, suggesting that processes directly adjacent to perfused areas benefit from the higher blood temperature. Thus, tumor metabolism and the extent and route of tumor invasion in window models are strongly energy dependent and affected by the housing temperature.

IR-excitation was further combined with fluorescence lifetime microscopy (FLIM) using a 16-channel FLIM detector, to monitor the uptake kinetics and therapeutic effect of chemotherapy on different tumor subregions in vitro. Fibrosarcoma cell spheroids were embedded in 3D collagen matrices and the local uptake of doxorubicin by different subregions of the tumor spheroid (main tumor mass versus collective and single cell invasion zones) was co-registered with fluorescence intensity to monitor cell invasion, morphology, proliferation and apoptosis induction. Thus, by combining FLIM with fluorescence intensity, second and third harmonic generation, infrared multiphoton microscopy represents a powerful approach to visualize the complex tumor microenvironment and its impact on local tumor invasion, metastasis and tumor response to therapy.

YIA applicant

QUANTITITATIVE, DYNAMIC AND LONG TERM *IN VIVO* IMAGING OF INTRAVASCULAR CIRCULATING TUMOR CELLS IN AWAKE ANIMALS, WITH A NOVEL MINIATURE FLUORESCENCE MICROSCOPE

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Introduction - Metastasis, the cause for 90% of cancer mortality[1], is a complex, poorly understood process. Circulating Tumor Cells (CTCs) provide a window into metastasis and are a biomarker bearing great potential prognostic value[2]. However, the rarity of these cells and a lack of reproducibility, specificity or sensitivity are rendering their interrogation by current techniques very challenging. Recent studies have shown that blood vessel invasion may happen at a very early stage, before the main tumor is detectable[3]. To provide an insight into this 'black box', we developed a novel intravital miniature microscopy setup capable of real-time long-term monitoring of CTCs in awake small animals. This system has the potential to uncover CTC dynamics over a tumor's lifetime - from primary tumor to metastasis.

Methods - As an alternative to conventional benchtop systems that are expensive, non-scalable and lack continuous imaging capabilities, we have engineered a miniature intravital microscopy system (IMM) for long term imaging in awake animals. The IMM can be mounted on the back of a mouse and is composed of a Dorsal Skinfold Window Chamber giving access to superficial vasculature, a custom-designed holder, and a novel lightweight Miniature Intravital Fluorescence Microscope with an excitation source at 488nm. Using a lentiviral construct encoding a fusion reporter gene, luciferase (Luc2) and enhanced Green-Flurescence Protein (eGFP), we have also developed an imageable orthotopic 4T1 model for breast cancer metastasis.

Results - In this model, following orthotopic implantation in mice in the mammary fat pad (n=20), primary tumor growth and metastatic burden can be monitored by whole-body bioluminescence imaging while CTCs can be followed continuously by IMM fluorescence imaging. We have demonstrated that we can observe lung metastasis as early as 6 days after primary tumor induction, with further metastatic sites in the liver and bone. This provides us with a window of 6 days where we propose to observe the continuous kinetics of CTCs using the IMM. We have monitored blood vessels of various sizes (40 - 90µm) for long periods of time (t=2h) in mice bearing a fluorescent model of metastatic breast cancer, based on systemic injection of labeled 4T1 cells (n=5). Using an in-house algorithm, we are able to detect, count and compute CTC trajectories, speed, and dynamics.

Conclusion - These data represent the first reported use of a miniature intravital microscopy setup for in vivo long-term imaging of CTCs in non-anesthetized animals and demonstrate its potential to uncover unexplored aspects of cancer metastasis.

Acknowledgements - This work has been supported by a Fulbright Science and Technology Fellowship Award, a Stanford Weiland Family Fellowship, a Graduate Student Fellowship from the Society for Nuclear Medicine and a Center for Cancer Nanotechnology Excellence grant.

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PARALLEL SESSION 7:

THERANOSTICS

(together with the EANM and

COST action BM0607)

chaired by

Marion de Jong, Rotterdam

Tony Lahoutte, Brussels (Jette)

THERAGNOSTIC APPLICATIONS OF NUCLEAR MEDICINE

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Nuclear medicine has been a theragnostic discipline for many years. Nuclear medicine started with radioiodine scans that were used for planning and monitoring of radioiodine therapy of benign and malignant disorders. Other examples include metaiodobenzylguanidine for diagnosis and therapy of pheochromocytoma/neuro- blastoma and more recently somatostatin receptor PET for selecting patients for treatment with Lutetium-177 or Yttrium-90 labeled somatostatin receptor ligands. With the rapid advances in molecular biology and genetics it is expected that novel targets such as bombesin receptors or integrins will be used clinically to diagnose and treat patients with cancer.

WHICH ROLE NANO- AND MICROPARTICLES CAN PLAY IN MOLECULAR CANCER DIAGNOSIS AND THERANOSTICS?

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Nanoparticles are frequently suggested as diagnostic molecular probes. However, their extravasation usually is low and delivery, e.g. to tumor cells, is even lower. This is complicated by the fact that unspecific extravasated nanoparticles generate a strong and long persisting background. Thus, for many molecular imaging applications small molecules appear preferable. The most promising applications for nanoparticles as diagnostics appear to be: i) blood pool imaging, ii) angiogenesis imaging (no extravasation needed), iii) cell tracking, and iv) theranostics. Theranostics combine diagnostic and therapeutic properties within the same molecule. In this context, the diagnostic label allows assessment of pharmacokinetic and pharmacodynamic behaviour and therapeutic efficacy.

In this talk, an overview will be given on nanoparticles that were successfully used for functional vascular characterisation, cell tracking, targeted imaging and as theranostics. Also important characteristics of USPIO are reported and it will be discussed how these can be modified by changing particle size and coating. Examples will be given for targeting transferrin-receptors, av3-integrins, and the riboflavin carrier protein (RCP). The use of nanoparticles as bi- or trimodal diagnostic labels of encapsulated cellular transplants ('bio-hybrid theranostics') will also be reported. Another example is about microbubbles with encapsulated USPIO and dyes. These microbubbles have excellent acoustic properties and exhibit a strong T2* contrast. By insonation microbubble destruction induces a transient vessel permeation, which is demonstrated for muscle and brain tissue. This effect may be used to better deliver therapeutic drugs. In addition, extravasation of USPIO and of dyes allows controlling the increase in vessel permeability by MRI and OI.

In conclusion, nanoparticles can be attractive diagnostic and theranostic tools but indications are different from those for small molecular weight diagnostics.

YIA applicant

SYNTHESIS AND FIRST IN VIVO EVALUATION OF 18F-ANTI-HER2-NANOBODIES: A NEW PROBE FOR PET IMAGING OF HER2 EXPRESSION IN BREAST CANCER.

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Introduction: Nanobodies are small (15 kDa) antibody fragments with beneficial pharmacokinetic properties (that are ideally suited) to be used as probes in molecular imaging [1, 2]. Nanobodies have already been radio-labeled with the SPECT isotopes ^{99m}Tc and ^{111}In and with the radiometal ^{68}Ga for PET imaging. Ontop of that, their pharmacokinetic profile is also optimal for imaging with ^{18}F. In this study, we present the development of ^{18}F-labeled nanobodies for molecular imaging of HER2 expression in breast cancer patients.

Methods: In this study, two anti-HER2 nanobodies differing only in the C terminal 6-histidine tag and one non-targeting Nanobody, were labeled with ^{18}F using N-succinimidyl 4-[^{18}F] fluorobenzoate ([^{18}F]SFB) chemistry [3]. [^{18}F]SFB was synthesized as described before and incubated with the nanobodies for 1h at room temperature. Assessment of radiochemical purity and stability was performed using High Performance Liquid Chromatography (HPLC). Binding potential of the ^{18}F-nanobodies was evaluated in vitro using HER2-expressing SKOV-3 cells. Biodistribution and tumor targeting of the ^{18}F-nanobodies was evaluated in nude mice bearing HER2 positive SKOV-3 xenografts. Additionally, blood clearance was analyzed in non-tumor bearing nu/nu mice.

Results: After labeling, HPLC analysis showed a radiochemical purity >95%. The ^{18}F-anti-HER2-nanobodies remained stable at least upto 2h in serum and PBS. In vitro studies showed that binding of both anti-HER2 nanobodies was specific. In vivo biodistribution analysis showed that the ^{18}F-anti-HER2-nanobodies were rapidly cleared from the blood via the kidneys with low retention in all other organs and tissues. The uptake of ^{18}F-nanobodies in HER-2 positive tumors was 3.09 ± 0.13 %IA/g for the His-tagged form and 2.62 ± 0.89 %IA/g for the non-tagged with a tumour-to-muscle ratio of 7.31 ± 0.21 and 12.06 ± 0.11 respectively, at 1 hour after injection. The tumor uptake of the non-targeting nanobody was negligible. The major difference between the two anti-HER2 nanobodies was the kidney retention, decreasing from 19.04 ± 1.84 to 6.70 ± 0.56 % IA/g for the non-tagged nanobody

Conclusion: We showed for the first time the successful labeling of HER2 targeting nanobodies with 18F. The labeled probe retains specificity for the HER2 receptor and avid tumor uptake was demonstrated already at 1h post injection. Kidney uptake was significantly lower in comparison to biodistribution studies of the same nanobodies labeled with other radioisotopes. The removal of the 6-histidine tag showed a significant impact on kidney uptake. 18F-anti-HER2-nanobody PET imaging of breast cancer should enable non-invasive assessment of HER2 receptor status in breast cancer patients.

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EVALUATION OF 67/68GA-DOTA-BZ-FOLATE FOR SPECT AND PET IMAGING OF FOLATE RECEPTOR POSITIVE CANCER

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Introduction: The folate receptor (FR) is overexpressed in a variety of tumor types (ovarian, endometrial, cervical, renal, colon cancer etc.) but highly restricted in normal tissues and organs. Only in the kidneys FRs are substantially expressed in the proximal tubule cells. Using folic acid radioconjugates for nuclear imaging of FR-positive cancer proved to be a promising concept. The aim of this study was to assess a novel folate radioconjugate for SPECT and PET imaging of cancer diseases.

Methods: All experiments were carried out with DOTA-Bz-folate (Endocyte Inc.) radiolabeled with $^{67/68}$Ga. Radiolabeling was performed under standard conditions at pH 4.5 at 95°C. Stability of the radiofolate was tested in human plasma and FR-specific binding was evaluated in cultured KB cells. Biodistribution and imaging studies were performed in KB-tumor bearing nude mice. Different application routes (i.v., s.c., i.p.) of the radiotracer were investigated. Combinations of the radiolabeled DOTA-folate with various drugs such as amino acids (e.g. lysine) or antifolates (e.g. pemetrexed) were also tested.

Results: In vitro the novel folate radioconjugate was stable in human plasma over several hours. High and FR-specific binding was observed in cultured KB cells. The uptake of Ga-DOTA-Bz-folate in KB-tumor xenografts of mice was high and specific (~ 6 % ID/g, 4h p.i.) and thus clearly visible on SPECT and PET images performed with the ^{67}Ga- and ^{68}Ga-radiolabeled DOTA-Bz-folate conjugate. Clearance from non-targeted organs and tissues was very fast. A significant accumulation of the radiofolate was, however, found in the kidneys (~ 80 % ID/g, 4h p.i.). Neither the application route of the radiofolate nor any of the drug combinations was able to increase the tumor-to-kidney ratios. Only the preinjection of the antifolate pemetrexed reduced kidney uptake to 25% of control values whereas the tumor uptake was retained.

Conclusions: The novel $^{67/68}$Ga-DOTA-Bz-folate conjugate proved to be a valuable imaging agent for visualization of FR-positive tumors via SPECT and PET. Due to the fast clearance from background organs, this tracer would be suitable for the detection of tumor metastases in the liver and abdomen. To reduce undesired radiofolate uptake in the kidneys, preinjection of pemetrexed would be the method of choice.

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YIA applicant

SPECT/CT IMAGING OF PROSTATE CANCER XENOGRAFTS USING A NEW BOMBESIN RECEPTOR ANTAGONIST

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Introduction: Bombesin receptor-targeted imaging and therapy of prostate tumours using bombesin analogues is a promising tool. Recent preclinical studies proved that radiolabelled bombesin receptor antagonists are superior to agonists, showing improved uptake in human prostate PC3 xenografts in mice and a faster clearance from the receptor-rich pancreas and background tissues. These properties in combination with their inherent biosafety and tumour growth inhibiting effects make bombesin antagonists attractive alternatives to agonists. Here, we explored the characteristics of a linear bombesin antagonist coupled with the chelator DOTA to the N-terminus, enabling stable binding of diagnostic (SPECT: In-111, Ga-67; PET: Ga-68) and therapeutic (Lu-177, Y-90, Bi-213) radionuclides.

Methods: The peptide H-ßAla-ßAla-JMV594 was assembled on solid support followed by coupling of the chelator DOTA. The purified peptide conjugate, JMV4168, was labeled with In-111 and Lu-177 in high purity (>95% radiochemical purity) and high specific activity (up to 100 MBq/nmol). In vitro assays were performed using receptor expressing PC3 cells to determine affinity (at room temperature) and internalization capacity (at 37C) of [In-111]JMV4168 in comparison to the bombesin agonist [In-111]AMBA. In vivo behaviour was tested in biodistribution studies and in high resolution SPECT/CT (dynamic) imaging studies with PC3 (androgen independent) and PC295 (androgen dependent) xenografted female SCID (BW 20g) or male nu/nu mice (BW 35g), 1-72 h post injection (p.i.) of 10 pmol (biodistribution) or 250 pmol (imaging) of [In-111] JMV4168 or [Lu-177]JMV4168. For in vivo blockade, a separate 4 h group was co-injected with excess JMV4168 (20 nmol).

Results: The GRPR affinity of JMV4168 was high with an IC50 value of 1.3 nM versus 0.7 nM for AMBA. Characteristically for antagonists, a low internalization rate was found in PC3 cells: 9% for [In-111]JMV4168 versus 78% for [In-111]AMBA during 1 h incubation. Biodistribution studies in vivo showed similar uptake for [In-111]JMV4168 and [In-111]AMBA over time in PC3 and PC295 xenografts. In female SCID mice, a higher tumor uptake was obtained than in male nu/nu mice (female SCID 17.0±2.8%ID/g - block 1.3±0.3%ID/g; male nu/nu 10.2±2.4 %ID/g) at 4 h p.i.. [Lu-177]JMV4168 kinetics resembled that of [In-111]JMV4168. High resolution dynamic SPECT/CT scans of [In-111]JMV4168 confirmed high uptake in the xenografts and fast wash-out from pancreas and kidneys in the first hour post injection. Scan quantification and post imaging tissue counting showed that uptake in PC295 and PC3 xenografts at 4 h p.i. of 10 or 250 pmol [In-111]JMV4168 was not significantly different.

Conclusions: We conclude that JMV4168 is a most promising bombesin receptor antagonist for future use in clinical imaging and therapy of bombesin receptor-positive human tumours, because of a high and selective uptake in human prostate cancer xenografts in mice in combination with a fast clearance from the receptor-positive pancreas and background tissues.

Acknowledgement: This study has been performed within the frameworks of the project 3-Binding and COST Action BM0607: 'Targeted Radionuclide Therapy'.

PARALLEL SESSION 8:

IMAGING IN DRUG DISCOVERY and DEVELOPMENT

chaired by

Uwe Haberkorn, Heidelberg

Adriaan Lammertsma, Amsterdam

NEUROIMAGING AS A CLINICAL PHARMACOLOGICAL TOOL IN EARLY DRUG DEVELOPMENT

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Clinical pharmacology plays an essential role in early drug development, by demonstrating what a new drug does to the body (pharmacodynamics), and what the body does to the drug (pharmacokinetics). An increasing armamentarium of neuroimaging techniques are being developed to investigate the complex processes that are involved in the pharmacokinetics and pharmacodynamics of new drugs. The two most important techniques are positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) or – spectroscopy (MRS). A major advantage of these imaging techniques is the direct spatial demonstration of the distribution and the effects of drugs, particularly in the central nervous system where this information can otherwise only be obtained indirectly at best.

Neuroimaging is also promising for translational research, since PET and MRI can be used in patients, healthy subjects and increasingly also in small laboratory animals. PET studies are often performed to investigate whether and to which extent a new drug penetrates into the central nervous system (CNS) and binds to its pharmacological target. Usually, a tracer dose of a radioactivally labeled PET-ligand is employed that has been validated for its binding to the specific target (usually a receptor, sometimes an enzyme or another biochemical target) to which the new drug also binds. Replacement of the ligand by competition with the drug allows the determination of the level of target occupancy and the duration of binding drug-receptor. This provides important information about the dosing regimens that are required for therapeutic efficacy trials.

Currently, PET-ligands are only available for a limited number of pharmacological targets, and although many pharmaceutical industries try to develop new ligands for each new potential mechanism of action, this is often not available by the time that the novel drug is ready for administration in humans. Moreover, PET-ligands provide information about drug-receptor binding, but not about intrinsic efficacy of the drug. Other methods should be used to examine the pharmacodynamic consequences of target inhibition or stimulation by the new compound. Traditionally (and still very important) functional methods are used to demonstrate how the drug affects the central nervous system (using subjective, neuropsychological, neurophysiological and neuroendocrine measures). Many of these functional methods can be used to show the extent and duration of brain penetration (as 'pharmacodynamic biomarkers'), and provide indications for desirable (therapeutically relevant) and undesirable (adverse) effects.

However, there is no accepted standardization of the multitude of available functional methodologies, and there is hardly any validated translational method that can be used in animals, healthy subjects and patients. Functional neuroimaging is still under development for drug research, but it could avoid some of the drawbacks and pitfalls of functional methods. Drug-induced changes of brain functions can be examined using PET, for instance with ligands that can demonstrate changes in metabolism (^{18}FDG) or regional flow ($^{15}O_2$). Increasingly however, magnetic response imaging (fMRI and MRS) is applied to show drug effects on neuronal systems. The sensitivity and spatial resolution of MRS is still insufficient to reflect most drug-induced metabolic changes related to local neuronal activity. Functional MRI relies on shifts of the paramagnetic characteristics of (de)oxygenated blood associated with neuronal energy expenditure, which cause changes of blood oxygen level dependent (BOLD) signals.

There are three MRI-applications in drug research, which are all still investigational. First, since most drugs are designed to affect only certain types of nerve cells (implicated in the disease), MRI can be used to directly examine the local and regional BOLD changes caused by drug-induced (de)activation of these target cells and their neuronal networks. However, the MRI signal is technically unstable and 'drifts' due to heating of the magnet *etc*, which leads to loss of quantitative information within an hour or so, basically restricting direct pharmacological MRI to acute (intravenous) administration of short acting drugs. The second and most widely used MRI application in early drug research is task-related fMRI, in which regional BOLD-changes are evoked by a specific task, chosen to (de)activate brain systems that are affected by the treatment or the disease. The problem of signal drift is avoided by averaging a large number of task-related scans ('on'), contrasted with alternated inactive periods ('off'). The usefulness of fMRI in drug development is determined by the relevance of the task, and this technique is particularly useful for medications affecting pain, memory, mood and similar well-defined and relatively basic but clinically important CNS-functions. The limited dimensions and high magnetic fields of the MRI machine itself impose further practical restrictions on the task selection for fMRI. A third new technique depends on the finding that the brain shows activity of about a dozen different networks, even when no specific task is performed. This activity can be examined repeatedly with MRI, showing that these networks are relatively stable under resting conditions. Recent studies have shown profound changes of these networks after single dose administrations of a range of different drugs. The effects on the networks appeared to be dose-dependent and to be highly specific, with little overlap between drugs that belonged to different pharmacological classes, but could all be considered to be 'CNS-depressants'. Resting state pharmaco-MRI circumvents many of the practical restrictions of PET (radioactivity, ligand production) and the other MRI-techniques. This new methodology also holds promise for translational drug research, and it is currently being examined in small laboratory animals, healthy volunteers and patients with different disorders.

QUANTITATIVE IMAGING STUDIES FOR IMPROVED CNS DRUG DEVELOPMENT

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The development of a successful new drug is an increasingly expensive process that suffers from high rates of attrition with estimated costs for each new drug that reaches the market in the order of $1 billion. One approach that has gained much interest in recent years in helping to improve this process is imaging. Non-invasive experimental medicine imaging studies in preclinical and clinical species can provide a range of different biological measurements across the drug discovery and development pipeline and they are already making a significant contribution. Almost 30% of new molecular entities approved for neuropsychiatric indications by the FDA between 1995-2004 were developed with contributions from imaging and there will be a growing need for information provided by imaging in drug development. Careful design of imaging studies and their appropriate introduction into development plans allows for decision making on whether to kill or progress an asset. The value and confidence in such decision making is increased when the studies are quantitative. Quantitative studies rely on the appropriate acquisition of necessary data and the application of quantitative bio-mathematical analysis techniques to provide well defined imaging endpoints that relate directly to the biological processes under investigation.

This presentation will explore how quantitative PET imaging can contribute to clinical drug development in addressing major questions such as

- Does the molecule reach the tissue of interest in potentially pharmacologically active concentrations?
- Does the molecule interact with the target of interest?
- What is the relationship between administered dose and interaction with the target?
- What are the effects of the drug and how long do they last?

YIA applicant

PRECLINICAL IN VIVO EVALUATION OF A ZR-89 LABELED ANTI-CD44 ANTIBODY AS PRELUDE TO CLINICAL IMMUNO-PET STUDIES

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Introduction: CD44 is a single-chain, single-pass, transmembrane glycoprotein, which is widely expressed in healthy tissues as well as in many hematological malignancies and solid tumor types. Interestingly, CD44 is associated with cancer stemcellness, and therefore an appealing target for antibody-based therapy. The investigational drug RO5429083 is a recombinant humanized mAb that specifically binds to the constant region of the extracellular domain of CD44. RO5429083 inhibits the binding of hyaluronic acid to CD44, and was shown to inhibit several $CD44^+$ xenografts in vivo, while toxicology studies with high dose RO5429083 in cynomolgous monkeys did not reveal adverse effects. Therefore, CD44 might have a more critical role in malignant growth than in normal tissue maintenance and we hypothesize that despite the broad expression of CD44 in normal tissues, therapy with RO5429083 might be feasible. However, for obtaining optimal efficacy the dosing of the antibody might be very critical, and immuno-PET is considered to be a powerful tool to get insight in dose-response relationships. Past years we have developed GMP-compliant radiolabelling procedures for clinical immuno-PET studies with ^{89}Zr and ^{124}I-labeled monoclonal antibodies. As prelude to clinical trials ^{89}Zr-RO5429083 was GMP produced and evaluated for specific and measurable uptake in nude mice bearing CD44-expressing tumor.

Methods: ^{89}Zr-RO5429083 was prepared according to Verel et al [1]. 1 mg/kg ^{89}Zr-RO5429083 was evaluated in MDA-MB231 ($CD44^+$, responder), PL45 ($CD44^+$, non-responder) and HepG2 ($CD44^-$)-tumor bearing mice. In addition, a dose escalation study was performed in MDA-MB231 tumor bearing mice at 2, 8, 20 and 40 mg/kg (predose) ^{89}Zr-RO5429083.

Results: ^{89}Zr-RO5429083 was prepared with >99% radiochemical purity and optimal immunoreactivity. Tumor uptake in MDA-MB231 tumor bearing mice was high (32.4 ± 6.8 %ID/g) and uptake in HepG2 tumor bearing mice low (5.7 ± 1.2 %ID/g) at 6 days post injection (p.i.). In addition, decreased blood levels were observed for $CD44^+$ tumor bearing mice (5.8 ± 3.6 %ID/g vs 14.7 ± 2.6 %ID/g). PL45 tumor bearing mice showed comparable tumor uptake and blood levels as MDA-MB231 tumor bearing mice. A dose escalation study revealed that tumor uptake and tumor-to-blood ratios at 48, 72 and 144 p.i. decreased at higher antibody dose, indicating saturation of the CD44 target.

Conclusions: An efficient method for the labeling of RO5429083 with ^{89}Zr has been developed, resulting in an optimal quality conjugate. ^{89}Zr-RO5429083 at a low dose of 1 mg/kg showed high and specific uptake in $CD44^+$ xenograft models, while uptake became less at higher antibody dose. With this method selective targeting and target saturation can be confirmed in patients with $CD44^+$ tumors. However, as shown herein with xenograft PL45, efficient targeting does not guarantee antitumor effects, and therefore early response monitoring with e.g. ^{18}FDG is recommendable in clinical studies.

References: [1] Verel I. et al. (2003), J Nucl Med 44, 1271-1281.

YIA applicant

APOTENTIALIMAGINGMODEL'PREDICTIVEOFSCHIZOPHRENIA', USING NMDA ANTAGONIST AS KEY PSYCHOACTIVE LEAD-COMPOUND: A MULTI-MODAL MR NEUROIMAGING REPORT

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Accumulating experimental and clinical evidences suggest that N-methyl-D-aspartate (NMDA) receptors hold potential clues in determining the biological substrate of schizophrenia. This observation has prompted considerable research effort to target these receptors, with compounds which act as NMDA antagonists.

To pursue this rational we used pharmacological, resting state and diffusion MRI in rats, sub-chronically treated with NMDA antagonist, memantine. Pharmacological MRI (phMRI) was used to map the neuroanatomical target sites of memantine following acute and sub-chronic treatment. Resting state fMRI (rs-fMRI) and diffusion MRI was used to study the strengths of the functional connectivity and the ultra-structural changes before and after sub-chronic memantine treatment, respectively.

Dose-dependent phMRI activation was observed following acute doses of memantine in the prelimbic cortex. Following sub-chronic treatment localized effects in the hippocampus (HC), cingulate (Cg), prelimbic and retrosplenial cortex were observed. Decreases in functional connectivity amongst the hippocampal and frontal cortical structures were apparent through rs-fMRI investigation, potentially reflecting a loss of connectivity in the neuronal networks. Further, diffusion kurtosis MRI showed decreases in fractional anisotropy and mean diffusivity changes, potentially suggesting ultra-structural changes in the HC and Cg. Moreover, corroborating evidences were also documented using electrophysiology (in key ROIs) and pharmacokinetic studies. Key studies (in literature) report the implications of frontal cortex in psychosis and the key role of hippocampus in the aetiology of schizophrenia.

Our observations add further weightage to the rationale of using NMDA antagonist, memantine and the utility of functional, resting state and diffusion MRI in the development of imaging model predictive of schizophrenia.

We acknowledge the financial support of J&JPRD for the Postdoctoral Fellowship.

IN VIVO 1H MRS PROFILING AS TRANSLATIONAL BIOMARKER FOR DRUG EFFICACY: REGION-SPECIFIC EFFECTS OF MODEL COMPOUND VIGABATRIN ON GABA AND GLUTAMATE IN THE RAT BRAIN

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Introduction: *In vivo* 1H Magnetic Resonance Spectroscopy (MRS) is an emerging modality in translational neuroscience with a wealth of putative applications in drug discovery & development as it provides non-invasive access to major metabolite and neurotransmitter pools in the brains of living organisms. Yet, the face value of MRS for routine application in pharmacological research remains to be established in terms of reproducibility, reliability and sensitivity to pick up region-specific changes in the neurochemical profile upon drug treatment. In the present study, we set out to qualify preclinical 1H MRS in this regard and used vigabatrin, an anti-epileptic drug known to elevate GABA levels through irreversible GABA-transaminase inhibition, as a model compound for pharmacologic intervention. We determine the variability and the smallest changes in the neurotransmitters GABA and glutamate that are reliably and region-specifically detectable in rat striatum and prefrontal cortex (PFC), i.e. two regions implicated in psychiatric disorders.

Methods: 32 male Sprague Dawley rats were included in the study and randomly assigned to four treatment groups receiving vehicle (control group), 30mg/kg, 100mg/kg or 300mg/kg vigabatrin, respectively, in two applications 26h and 2h prior to MRS. Throughout the MRS experiment animals were maintained under anesthesia (1.8-2.4% isoflurane in 1:5 oxygen:air mixture supplied by a face mask); rectal temperature, respiration rate and end-tidal CO2 were tightly monitored. Data were collected on a Bruker BioSpec 9.4T/20 cm MR scanner equipped with a 72 mm bird-cage resonator for excitation and a circularly polarized surface coil for reception. 1H MR spectra were acquired using PRESS single voxel spectroscopy (TR 2s, TE 10ms, spectral width 4kHz collected in 2048 complex points, 512 averages, 17min acquisition time) from two 16uL voxels encompassing the striatum and medial PFC, respectively. Metabolite quantification from MR spectra was carried out using LCModel (http://s-provencher.com).

Results: Consistent, highly reproducible spectra were obtained in all animals in striatum and PFC at an average signal-to-noise-ratio of 12.5±2 and 16.3±2.3, respectively (mean±SD across animals). GABA and glutamate concentrations were estimated from the spectra with Cramer-Rao lower bounds <12% and <3%, respectively, indicating that changes of >24% and >6% in these neurotransmitters could be detected in individual spectra at 95% confidence. Significant dose-dependent changes were observed for GABA in striatum and PFC, and for glutamate in PFC only, confirming that drug-induced changes in the neurochemical profile can be detected reliably using MRS. Furthermore, changes in GABA and glutamate levels differed significantly between anatomical regions. A prospective power analysis estimates that minimal changes of 4% in glutamate and 14% in GABA can be assessed with a power of 80%, at an alpha level of 5% and a group size of n=8 animals (unpaired t-test). These estimates were corroborated by the minimal drug-induced dose- and region-specific effects on neurotransmitter levels observed experimentally.

Conclusion: In sum, we have demonstrated that MRS neurochemical profiling qualifies for providing quantitative readouts of GABA and glutamate as a putative biomarker to assess drug-induced metabolic changes and to monitor treatment effects localized to key brain areas targeted by pharmacological intervention.

Notes

PARALLEL SESSION 9:

IMAGING IN CARDIOVASCULAR DISEASE

(together with the ESR)

chaired by

Nicolas Grenier, Bordeaux

Markus Schwaiger, Munich

YIA applicant

MOLECULAR MR IMAGING OF ATHEROSCLEROTIC PLAQUES BY A VASCULAR CELL ADHESION PROTEIN-1 TARGETED CONTRAST AGENT

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Introduction: Several MRI studies have shown atherosclerosis can be detected in vivo. Non-targeted contrast agents (CA) are able to generate good plaque enhancement yet without discrimination of the different plaque components. Inflammation drives atherosclerotic plaque instability and acute thromboembolic events. Vascular Cell Adhesion Molecule-1 (VCAM-1), a protein that mediates both rolling-type adhesion and firm adhesion, is weakly expressed under baseline conditions but is rapidly induced in activated vascular endothelium. There is currently no clinical imaging technique available to assess the degree of inflammation associated with plaques. This study aims at visualising and characterising atherosclerosis using VCAM-1 targeted USPIOs as an MRI probe for detecting inflamed atherosclerotic lesions.

Methods: A binding peptide for VCAM-1 was identified by phage displayed peptide library screening. Our synthetic peptide or a scrambled variant were covalently conjugated to the carboxyl groups exposed by the bisphosphonate coating of USPIO through their amino-terminal groups. The in vivo imaging potential of VCAM-1 targeted USPIOs was investigated both in aged ApoE$^{-/-}$ mice and young ApoE$^{-/-}$ mice exposed to a Western diet. In the latter mice atherosclerosis was induced in a timed manner by placement of a constrictive collar around the left carotid artery. MRI of the aortic arch was performed on a 9.4 T vertical Bruker system, a 24 hours kinetics being obtained after intravenous injection of USPIO using a cine MRI FLASH sequence.

Results: We successfully identified a highly specific peptide with nanomolar affinity for human VCAM-1. High resolution MRI performed 1.5 hours after i.v. injection of VCAM-1 targeted USPIOs in aged ApoE$^{-/-}$ mice with advanced plaques showed enhanced uptake of the contrast agent compared to the passive uptake of the scrambled variant. 24 Hours after injection, passive uptake was comparable in both groups. In mice with a collar around the carotid artery, uptake of VCAM-1 targeted USPIOs (corrected over the plaque area) was significantly increased in mice 3 weeks post collar placement. Histology revealed colocalisation of VCAM-1 positive endothelial cells and iron deposits in the vessel wall.

Conclusions: Our data indicate that VCAM-1 targeted USPIO holds great promise for diagnosis and staging of inflammatory plaques in atherosclerosis.

Acknowledgement: Fons Lefeber and Kees Erkelens are gratefully acknowledged for technical support.

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DIRECT VISUALIZATION OF NITRIC OXIDE IN CELLS AND ARTERIES WITH A COPPER (II) FLUORESCENT PROBE AND TWO-PHOTON MICROSCOPY

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Introduction: Imaging and visualization of Nitric Oxide (NO), a well established messenger that facilitates vasomotor function within the vascular system, is essential to better define its role in (patho)physiology. We study a novel NO-sensitive copper-fluorescein complex (CuFL) with two-photon laser scanning microscopy (TPLSM).

Methods: To study cellular NO production both in vitro and ex vivo CuFL fluorescence was determined using TPLSM. CuFL was characterized and compared with DAF-2-DA. In endothelial cell (EC) cultures, hydrogen peroxide (H2O2), acetylcholine and shear stress were used as stimulant. Isolated murine carotid arteries were incubated ex vivo with H2O2 and acetylcholine to stimulate NO production. Isolated arteries were mounted in a home-built perfusion chamber and transmural pressure was applied to mimic physiological condition. Endothelium functional capacity of artery, that depends on the amount of NO produced and the vasodilatation effect was evaluated by measuring luminal diameter of artery prior and post stimulation with inducers of NO.

Results: CuFL was internalized by the cell immediately after addition. NO synthesis was apparent in cultured endothelial cells (ECs) under conditions of stimulation within one hour after adding CuFL. CuFL demonstrated higher specificity and sensitivity for NO compared with DAF. CuFL is NO-specific, as this fluorescence signal is abolished by NOS-inhibitor (L-NAME). The sensitivity of CuFL was considerably higher when compared with the traditional Griess assay. Ex vivo, the cellular NO content could be visualized with TPLSM in both ECs and smooth muscle cells (SMCs) under influence of stimulus like acetylcholine and H2O2 that increase NO production. Moreover we show the possibility of imaging NO-production in the vasculature by visualization of NO and its effect on vascular tone in explanted murine carotid arteries.

Conclusions: TPLSM of CuFL allows direct detection of endogenously formed NO production in cells and arteries with high spatial and temporal resolution. The described method opens avenue to assess structure-function relationships in healthy and diseased vessels in terms of NO dynamics. Importantly, this study will enable to investigate regulatory pathways involved in the complex interplay between NO and vascular diseases.

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VISUALIZING ENHANCED ARTERIAL AGING IN ANEURYSMAL FIBULIN-4 MUTANT MICE

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Introduction: Fibulin-4 is a secreted glycoprotein expressed in medial layers of blood vessels. All reported fibulin-4 patients suffer from cardiovascular complications including aortic aneurysms, arterial tortuosity and elastin abnormalities. We used fibulin-4 mouse models (Fibulin-$4^{+/R}$ and Fibulin-$4^{R/R}$ animals) that express reduced levels of fibulin-4 to identify differences in global aortic protein expression patterns that lead to the development of these aortic abnormalities. While heterozygous Fibulin-$4^{+/R}$ mice show only mild arterial abnormalities, homozygous Fibulin-$4^{R/R}$ mice display elongated and 2-3 fold dilated ascending aortas. A full unbiased qualitative MS/MS proteomic screen of the aorta protein extracts identified an increase in mitochondrial oxidative phosphorylation as the major dysregulated pathway in both the Fibulin-$4^{+/R}$ and Fibulin-$4^{R/R}$ animals

Methods: Altered production of reactive oxygen species (ROS) in the aortas of fibulin-4 mutant mice was indirectly imaged using a chemiluminescence probe (L-012), a modified luminol derivative and a sensitive marker of ROS. Aortic wall stiffness was assessed by small animal ultrasound imaging using a VisualSonics Vevo 2100 at 25MHz. Systolic and diastolic ascending aortic diameters were recorded in M-mode

Results: An overlay of the aortic proteome and transcriptome yielded a limited set of biomarkers. In the Fibulin-$4^{R/R}$ mice the biomarkers pointed towards altered regulation of 17ß-estradiol and the inflammation associated TNF-a pathway. Interestingly, deregulation of the 17ß-estradiol pathway was also found in the Fibulin-$4^{+/R}$ mice. The signaling molecule 17ß-estradiol is a metabolite that deregulates the production of ROS which is, similar to mitochondrial dysfunction, a hallmark of aging and age-related cardiovascular diseases. Increased arterial aging was subsequently demonstrated by functional analyses that revealed a gradual increase in ROS production, endothelial dysfunction and reduced aortic distensibility in Fibulin-$4^{+/R}$ and Fibulin-$4^{R/R}$ mice.

Conclusions: These results uncover new regulatory pathways likely to be associated with enhanced arterial aging in aneurysmal fibulin-4 mice. The magnitude of the aortic decay appears to be fibulin-4 dose dependent, indicating that our Fibulin-$4^{+/R}$ and Fibulin-$4^{R/R}$ mouse models are sensitive and might be helpful to identify underlying molecular changes preceding and accompanying aneurysm formation.

YIA applicant

IN VIVO MOLECULAR MRI OF ICAM-1 IN MOUSE CARDIAC ISCHEMIA/REPERFUSION INJURY USING A LIPOSOMAL NANOPARTICLE

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Introduction: Intercellular adhesion molecule-1 (ICAM-1) expression on vascular endothelium is of major importance for the inflammatory response after myocardial infarction, because of its involvement in leukocyte-endothelium transmigration. To monitor cardiac inflammation, targeted contrast agents for *in vivo* molecular imaging of ICAM-1 expression are highly desired. The aim of this work was to develop paramagnetic liposomes for MR-imaging of ICAM-1 and characterize their interaction with vascular endothelium: (a) *in vitro* in the competing presence of leukocytes and under physiological flow conditions and (b) *in vivo* after cardiac ischemia/reperfusion injury.
Materials & methods: *Contrast agents* - Paramagnetic, fluorescent liposomes were prepared and conjugated with anti-ICAM or IgG antibodies (aICAM-L and IgG-L, respectively). Liposome size, morphology, antibody coupling efficacy and relaxivity were determined. *In vitro* - Mouse endothelial cells with either low or high ICAM-1 expression levels were incubated with liposomes (2h, 37°C), after which cellular fluorescence, cellular MR-relaxation rates ($R_{1,2}$ at 9.4T) and liposome distribution were determined. The relation between liposome binding to endothelial cells and liposome concentration was studied at 4°C. Furthermore, affinity of liposomes for endothelial cells was evaluated during coincubation with leukocytes. Liposome-ICAM interaction kinetics were assessed at a shear stress of 0Pa, 0.25Pa and 0.5Pa. *In vivo* - Liposomal blood circulation half-lives were determined in mice (n=6) by evaluating the blood R_1 up to 24h after intravenous administration (0.05mmol Gd/kg). The biodistribution over various organs and association with blood leukocytes were determined by confocal laser scanning microscopy (CLSM). Myocardial infarction was induced in mice (n=4) by 30min coronary artery ligation. *In vivo* T_1w short-axis multi-slice FLASH images were acquired at 9.4T after 8h of reperfusion, before and up to 1.5h after liposome administration. Cardiac signal enhancement was normalized with respect to intercostal muscle (NSE). Cardiac contrast agent distribution was determined by CLSM.
Results: *In vitro* - Low and high levels of ICAM-1 expression on endothelial cells could be distinguished upon incubation with aICAM-L, using fluorescent and MRI assays ($p<0.05$ vs. IgG-L). Most aICAM-L colocalized with the cell membrane. Cellular fluorescence was linearly related to the concentration of aICAM-L ($R^2=0.99$). aICAM-L showed significant ($p<0.05$ vs. IgG-L), but reduced adherence to endothelial cells in the presence of leukocytes. Under physiological flow conditions, ICAM-1 specific association of aICAM-L with endothelium was significant ($p<0.05$), though binding was reduced 3.3-fold (0.25Pa) and 5.2-fold (0.5Pa). *In vivo* - The circulation half-life of aICAM-L (8.3±1.2min) was shortened compared to IgG-L (29.3±3.9min). Accumulation of aICAM-L was observed within lung vasculature. aICAM-L and IgG-L were cleared through the reticuloendothelial system. Little association with blood leukocytes was observed. Specific binding of aICAM-L in cardiac ischemia/reperfusion injury was detected with *in vivo* MRI (NSE=1.06 (aICAM-L) vs. NSE=0.85 (IgG-L)). CLSM visualized aICAM-L in the infarct and infarct borders, in accordance with ICAM-1 expression patterns, whereas IgG-L were confined to the infarction.
Conclusion: ICAM-1 specific paramagnetic liposomes were developed that associated with ICAM-1 in the competing presence of leukocytes and under physiological shear stress. *In vivo*, ICAM-1 related liposome accumulation was observed in cardiac ischemia-reperfusion injury and in the lung vasculature.

YIA applicant

PASSIVE TARGETING OF PARAMAGNETIC LIPID-BASED CONTRAST AGENTS TO ACUTE MOUSE CARDIAC ISCHEMIA/REPERFUSION INJURY

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Introduction: Paramagnetic MRI contrast agents of low molecular weight (MW) are widely used to determine myocardial infarct size and for myocardial first-pass perfusion imaging. MRI contrast agents of higher MW also have been used for visualization of perfusion defects. In addition, liposomes have been shown to accumulate in the infarcted myocardium in targeted drug delivery studies. The aim of this study was to assess the circulation and extravasation kinetics of paramagnetic micelles and liposomes with MRI in a mouse model of acute myocardial ischemia/reperfusion.
Methods: *Contrast agent* - Paramagnetic micelles and liposomes, containing a Gd-DOTA-carrying lipid and a NIR-labeled lipid, were prepared. Hydrodynamic diameters were determined by dynamic light scattering. Relaxometry was performed at 9.4T. *Blood circulation half-lives* - Blood samples were taken from Swiss mice (n=3/group) before and up to 48h after injection of Gd-DTPA (0.3mmol Gd/kg), micelles (0.05mmol Gd/kg) or liposomes (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 mono-exponential decay function. *In vivo MRI* - Cardiac ischemia/reperfusion was induced in Swiss mice (n=3/group) by transient ligation (30min) of the left coronary artery. Contrast agents were injected at the start of reperfusion or 24h thereafter (doses equal to blood circulation half-life study). One day after surgery cardiac T_1-w short-axis multi-slice FLASH images were acquired at 9.4T, using ECG and respiratory triggering. Regions of interest were drawn in infarct, remote and noise-only areas and contrast-to-noise ratios (CNRs) were calculated. After MRI, mice were sacrificed and macrophages and vessels were labeled fluorescently for confocal laser scanning microscopy (CLSM).
Results: Gd-DTPA, micelles and liposomes resulted in r_1 relaxivities of 3.9$mM^{-1}s^{-1}$, 6.3$mM^{-1}s^{-1}$ and 3.2$mM^{-1}s^{-1}$ respectively. The diameter of micelles was 21.8±1.0nm and of liposomes 122.8±2.1nm. Blood circulation half-lives were 0.30±0.05h, 3.90±0.44h and 2.31±0.40h for Gd-DTPA, micelles and liposomes. As expected, administration of Gd-DTPA 24h after ischemia/reperfusion resulted in immediate hyperenhancement of the infarcted area and a high CNR (40.9±4.5%), followed by a fast wash-out. Conversely, injection of micelles or liposomes initially resulted in negative CNRs (-10.2±2.1% and -11.6±4.6% respectively) revealing reduced perfusion in the infarcted myocardium. Micelles accumulated within 1.4h in the infarct area, and consequently the CNR increased to 4.4±7.9%. After 24h circulation, more accumulation was observed (CNR 18.7±5.4%). CLSM revealed massive accumulation of micelles in the infarcted myocardium only. Liposomes did not accumulate in the infarct area within 1.4h and a persistent negative CNR was observed (-7.8±2.7%). CLSM showed the presence of liposomes in the border zone of the infarction. This is likely caused by the larger size of liposomes, leading to slower extravasation kinetics. After 24h, liposomes also extravasated in the infarct area, resulting in a positive CNR (15.2±2.1%). CLSM confirmed accumulation of liposomes in the infarcted myocardium, as well as association with vessels and macrophages.
Conclusion: Paramagnetic micelles and liposomes exhibit interesting circulation and extravasation kinetics in myocardial infarctions and could aid in assessment of infarct size and perfusion status. Additionally, these lipid-based nanoparticles are suitable for targeted drug delivery enabled by the specific accumulation in the infarcted myocardium.

EVALUATION OF ANDROGEN DEPRIVATION THERAPY ON MYOCARDIAL AND TUMOR [18F]FDG UPTAKE IN SMALL RODENTS

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Introduction: Low testosterone level has been linked to metabolic syndrome and cardiovascular disease in clinical research [1, 2]. However, androgen deprivation therapy (ADT) is one of the widely used treatments in patients with hormone-dependent prostate cancer. [^{18}F]FDG is the glucose analogue which can reflect the glucose metabolism by PET imaging. The aim of this study was to investigate the effect of surgical castration on glucose uptake changes in tumors and hearts in the xenograft models with hormone-dependent prostate tumors CWR22 and PAC120 by [^{18}F]FDG-PET imaging.

Methods: Tumor bearing BALB/c-nude male mice aging 8-10 weeks were imaged with [^{18}F]FDG-PET twice (pre and post ADT) and overnight fasted before imaging. After baseline imaging (PAC120: n=6; CWR22: n=16) mice were surgical castrated, simulating ADT, and imaged again 2 (PAC120: n=7) or 3 (CWR22: n=8) weeks later. 20 min static (1h after the tracer administration) and 90 min dynamic PET data acquisitions were performed with 10±2 MBq of [^{18}F]FDG. The ROIs (region of interest) were delineated on the tumor and the myocardium (myo). A 2-tissue compartment model was applied to estimate the influx constant (Ki) in dynamic analysis. The input function was derived from the ROIs on the left ventricle and corrected for partial volume, spillover effect and metabolites. [^{18}F]FDG uptake in standard uptake value (SUV) was measured in both dynamic and static studies.

Results: In CWR22 model, the tumor SUV at 3 weeks post castration (*p.c.*) was significantly lower than baseline (tumor-baseline: 0.87± 0.27, 3 weeks *p.c.*: 0.55±0.30; $p<0.05$), while the myo SUV and Ki (n=4) of dynamic studies were significantly higher *p.c.* (SUV: myo-baseline: 1.43± 0.81, 3 weeks *p.c.*: 5.26±0.99; Ki: myo-baseline: 0.07±0.03, 3 weeks *p.c.*: 0.29±0.10; all $p<0.05$). In PAC120 model, the myo SUV was significantly higher *p.c.* than baseline (myo-baseline: 2.99±0.83, 2 weeks *p.c.*: 4.64±1.55; $p<0.05$).

Conclusion: A reduced [^{18}F]FDG uptake in the hormone-dependent prostate tumors, especially in CWR22 model, was observed after ADT which was simulated by surgical castration. A higher myo [^{18}F]FDG uptake in both models was observed after ADT. The glucose metabolic changes in myocardium induced by ADT will be further studied in healthy, non-tumor bearing animals.

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YIA applicant

IN VIVO MOLECULAR IMAGING OF ACUTE THROMBOSIS AND THROMBOLYSIS WITH NOVEL PLATELET-TARGETED MICROBUBBLES VIA CONTRAST ENHANCED ULTRASOUND.

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Introduction: Molecular imaging is a rapidly emerging enabling technology allowing non-invasive detection of vascular pathologies. However, imaging technologies offering a high resolution are currently not inherently real-time applications. We hypothesized that contrast enhanced ultrasound (CEU) with microbubbles selectively targeted to activated platelets would offer real-time molecular imaging of evolving arterial thrombosis.

Methods and Results: Lipid-shell based air-filled microbubbles were conjugated to either a single-chain antibody (scFv) specific for activated GPIIb/IIIa (LIBS-MB), or a non-specific scFv (control-MB). Flow-chamber experiments demonstrated strong adhesion of LIBS-MB to immobilized activated platelets at $50s^{-1}$ compared with control-MB (84 ± 10 vs 15 ± 2; $p<0.001$). Increasing the shear rate to $1000s^{-1}$ and $6000s^{-1}$ dislodged most control-MB while LIBS-MB remained strongly attached ($p<0.001$). Platelet-rich thrombi were induced in carotid arteries of C57Bl6-mice in vivo by ferric chloride injury. Thrombi were then assessed with CEU-imaging before and 20 min after microbubble injection. Thrombosis was detected via the greyscale area, which was strongly increased after LIBS-MB but not after control-MB injection (214.25 ± 33.5 vs 9.96 ± 10.38; $p<0.001$). After thrombolysis with urokinase, CEU-imaging showed a significant reduction in thrombus size ($p<0.001$).

Conclusions: We are able to demonstrate that our targeted microbubbles specifically bind to activated platelets in vitro and allow real-time molecular imaging of acute arterial thrombosis as well as monitoring pharmacological thrombolysis in vivo. This non-invasive and cost effective imaging modality provides a unique opportunity to detect arterial (micro)thrombi at an early stage allowing for early diagnosis and therapy.

PARALLEL SESSION 10:

REPORTER SYSTEMS and REPORTER ANIMALS for MOLECULAR IMAGING

chaired by

Chiara Roncoroni, Milan

Michal Neeman, Rehovot

YIA applicant

IN VIVO STEM CELL TRACKING USING MULTIMODALITY MOLECULAR IMAGING

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Introduction: Determining the fate and function of stem cells after implantation plays a pivotal role in the development of new therapeutic applications in regenerative medicine. In the current study we used lentiviral vectors encoding for various reporter genes and micron-sized iron oxide particles (MPIOs) to label mesenchymal stem cells (MSCs). Labeled cells were implanted in rodents and monitored for survival and osteogenic differentiation using bioluminescence imaging (BLI), micro single photon emission computed tomography/CT (SPECT/CT), or micromagnetic resonance imaging (MRI). We demonstrate that we are able to track tissue-specific differentiation of MSCs using optical, nuclear, and MR-based imaging modalities in various implantation models.

Methods: MSCs were labeled with MPIOs and injected into rat degenerated coccygeal intervertebral discs (IVD). In addition, MSCs were transduced with a lentivector encoding for GFP and Luciferase. The cells were injected into NOD/SCID mice via the tail vein and BLI was used to track the cells. MSCs were transduced as well with a lentivector encoding for the human sodium iodide symporter (hNIS) gene and implanted subcutaneously in mice. We used SPECT/CT to detect uptake of 99mTc-pertechnetate by the implanted cells. MSCs were also transduced with lentivectors encoding for either Luc or hNIS genes driven by the human osteocalcin (hOc) promoter. Expression of the reporter gene was evaluated in vitro in conditions of osteogenic induction. MSCs transduced with lenti-hOC-Luc were further infected with adeno- BMP2 vector and implanted in mice. BLI was performed to quantify osteogenic activity in this model.

Results: MPIO-labeled cells were tracked in the rat IVD up to 25 days post-implantation. In mice injected systemically with Lenti-GFP-Luc labeled cells, the bioluminescent image was seen in the lungs 1 hour after injection and lasted for 48 hours. Lenti-hNIS labeling was verified using immunostaining. The graft of lenti-hNIS labeled cells demonstrated radioisotope uptake in vivo. In vitro, cells labeled with lenti-hOC-hNIS stained positively. Luciferase expression was detected only in lenti-hOC-Luc labeled cells that also expressed BMP2.

Conclusions: We were able to monitor implanted stem cells in a minimally invasive, longitudinal, and quantifiable manner. The use of functional reporter vectors allows us to monitor cell function as well as cell fate using different imaging modalities, at various implantation sites.

Acknowledgments: NIH R01AR056694, CIRM RT1-01027, USAMRMC N08217008 and EU FP7 ENCITE grants supported this study.

YIA applicant

BIOLUMINESCENCE IMAGING OF ENDOGENOUS STEM CELLS IN A MOUSE MODEL OF STROKE

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Introduction: In recent years it has been shown that neurogenesis in the adult mammalian brain can be altered after brain injuries such as stroke. The modulation of endogenous neurogenesis, migration and survival of stem cells has considerable therapeutic potential. Research into new therapies based on endogenous neurogenesis would definitely benefit from efficient imaging tools that allow non-invasive monitoring of a single animal over a longer time period. In this study we aimed to monitor endogenous neural stem cells in a photothrombotic stroke model using bioluminescence imaging.

Methods: We injected conditional FLExSwitch><eGFP-T2A-Fluc lentiviral vectors in the SVZ of white female Nestin-Cre transgenic mice that express Cre recombinase under the control of the rat nestin promoter. This allows specific labeling of neural stem cells with both Fluc (bioluminescence imaging) and eGFP (immunohistochemistry). Animals were scanned at regular time points before and after stroke until 3 months after surgery with both bioluminescence and magnetic resonance imaging for additional anatomical information. We further characterized the stem cells in the peri-infarct area by immunohistochemistry.

Results: In contrast to sham-operated animals, stroke animals showed a 4-fold increase in photon flux at 2 days after surgery and even a 6-fold increase at 1-2 weeks post surgery. This increase was also confirmed by immunohistochemistry for eGFP. Moreover, a clear relocation of the BLI signal was detected in some animals 2 weeks after surgery in comparison to previous time points.

Conclusions: Although this study still needs to be finalized, the results support the ability to follow the fate of endogenous stem cells after stroke via bioluminescence imaging.

Acknowledgements: We gratefully acknowledge the financial support by the European Commission for EC-FP6-STREP-STROKEMAP and DiMI LSHB-CT-2005-512146, BRAINSTIM SBO-IWT-060838, the FWO Vlaanderen project G.0484.08 and the K.U. Leuven Center of Excellence 'MoSAIC' (Molecular Small Animal Imaging Center).

IN VIVO TRACKING OF HUMAN NEURAL PROGENITOR CELLS IN RODENT CNS VIA MRI AND OPTICAL IMAGING

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Introduction Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease manifested through the deterioration and loss of function of motor neurons in the spinal cord and brain stem. While drug treatments have shown moderate inhibition of the disorder, stem cell therapy, such as using human neural progenitor cells (hNPC), has become increasingly attractive for treatment of this disease. Still, one of the major roadblocks in these efforts arises from the dynamic nature of hNPC proving it hard to predict their migration and homing *in vivo*. Therefore, having the ability to track hNPC in the central nervous system would allow researchers to better monitor cell behavior once transplanted. So far, superparamagnetic iron oxide (SPIO) has been the gold standard for tracking cells *in vivo* using magnetic resonance imaging (MRI). While this method provides high signal strength, it decays significantly within a few months, making long term tracking difficult (Watson, *et al*.). For this reason, we have explored over-expression of ferritin, an intracellular iron-storage protein, which by binding endogenous iron molecules allows for detection of hNPC using MRI. Furthermore, since MRI provides high resolution but relatively low sensitivity, we are also exploring the sensitivity benefits of optical imaging by over-expressing luciferase in hNPC.

Methods We have established two hNPC lines stably expressing either ferritin or luciferase ($hNPC^{Fer}$, $hNPC^{Luc2}$) through lentiviral infection. We have used these cells to repeat our proof of concept work done with hNPC transiently expressing the proteins. We scanned $1x10^6$ $hNPC^{Fer}$ and $1x10^6$ $hNPC^{Luc2}$ using MRI and optical imaging, respectively *in vitro*. We then imaged these cells *in vivo* following injection of $7x10^5$ hNPC into the rat striatum using appropriate imaging modalities. To test whether the protein expression decreases following transient transfection and lentiviral infection, we performed immunocytochemistry for ferritin and luciferase, respectively.

Results We have shown that hNPC expressing either ferritin or luciferase can be visualized via T2* gradient echo MRI sequence and bioluminescene protocol in the optical imager, respectively, *in vitro* and *in vivo* in rat striatum following transient and stable expression. In confirmation of the *in vivo* data, *in vitro* protein expression was found to decrease for both proteins from approximately 90% on the day following transfection to less than 20% ten days later due to its transient expression. Protein expression did not change for either protein following lentiviral infection, suggesting stable expression in hNPC.

Conclusions The ability to detect $hNPC^{Fer}$ and $hNPC^{Luc2}$ *in vivo* permits evaluation of long-term tracking of transplanted cells. It also allows us to test the effects of continual production of ferritin and luciferase on hNPC and surrounding tissues *in vivo*. Altogether, these studies may be expanded by using different cell lines and may have important clinical applications for ALS and other neurodegenerative disorders.

Acknowledgements This work was funded by: Promega Corp., Celsense Inc., and NIH (R01 CA118365)

Reference Watson et al. Structure-specific patterns of neural stem cell engraftment after transplantation in the adult mouse brain. 2006. Human gene therapy 17:693-704

YIA applicant

ASSESSING GENE EXPRESSION THROUGH A B-GALACTOSIDASE MR-RESPONSIVE SYSTEM

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Introduction The development of reliable means for following gene expression in vitro and in vivo aroused great interest in recent years. Gene expression is commonly monitored by introducing a marker gene to follow the regulation of the gene of interest. For example beta-galactosidase (ß-gal) is a commonly used marker that can be detected by colorimetric assays. Magnetic Resonance Imaging offers an alternative to light microscopy, allowing the interrogation of intact tissues at cellular resolution. Recently, Bogdanov et al showed that melanin-like polymers can form when hydroxo-functionalized Gd-chelates are in the presence of the suitable enzymes (e.g. tyrosinase or myeloperoxidase).[1] Our goal is to exploit this approach in order to set up a MRI method to assess the expression of ß-galactosidase (ß-gal).

Methods Relaxometric methods have been used for the in vitro physico-chemical characterization of the Gd-based probe either through the measure of NMRD profiles over a frequency range from 0.01 to 70 MHz and at a fixed frequency value of 20 MHz on Stelar Relaxometers (Mede - Italy). The efficiency of responsiveness to ß-gal and tyrosinase enzymes has been assessed through relaxometric and spectrophotometric measures either in enzyme containing buffer solutions and in cellular systems (ß-gal negative B16F10 and positive B16F10lacZ - murine melanoma cell lines). MR Imaging in vitro (on capillaries containing the cell pellets) and in vivo (on C57B16 mice bearing B16F10 and B16F10lacZ tumors) , were recorded on Aspect M2 spectrometer operating at 1T using a standard T1 weighted multislice multiecho sequence.

Results The probe consists of a Gd-DOTA based complex containing the tyrosine -OH functionality protected by the galactose moiety (Gd-DOTAgal). Upon cleavage of the galactose moiety the tyrosine group becomes available for the tyrosinase activated melanin-like polymerization. It is well established that the relaxivity of Gd-complexes increases, in the field range 0.5-1.5T, if the paramagnetic centers are part of macromolecular systems. [2] The in vitro relaxivity of the probe has been investigated by 1H NMRD before and after the addition of Tyrosinase and ß-gal. The relaxivity of the activated probe increased by 100% after 50 min of incubation in the solution containing the two enzymes. The enzymatic responsiveness has been assessed to be very good either in vitro (by using B16F10 melanoma cells transfected or not for ß-gal expression) and in vivo (on C57B16 mice bearing B16F10 and B16F10lacZ tumors). DCE MRI investigations conduced at 1.0T allowed to assess an increase of signal enhancement (45%) after 4-5 hours post intratumor injection of the probe in the ß-gal expressing tumor that was not observed in the control WT tumor.

Conclusions The Gd-DOTAgal derivative has been proven to be a good ß-galactosidase expression reporter, either in vitro and in vivo, in the case of melanogenic cells/tissues. Besides melanoma cells, Gd-DOTAgal is expected to be a good gene-reporter for doubly (ß-gal and Tyr) expression cells.

Acknowledgement This work was financially supported by grants from Meditrans EU-FP6 (NMP4-CT-2006-026668) and ENCITE EU-FP7 (HEALTH-F5-2008-201842) programs.

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YIA applicant

TARGETED MAGNETOLIPOSOMES FOR VISUALIZATION OF HEPATOCYTES

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Introduction: Cell labeling strategies can be classified in (a) unspecific uptake of contrast agents and (b) those that aim for specific uptake or binding of targeted contrast agents. It was the aim of this study to evaluate the specificity of functionalized magnetoliposomes (MLs) to target hepatocytes in vitro and in vivo. In case of liver cirrhosis or viral hepatitis where hepatocytes are damaged and healing is difficult, differentiation of stem cells into hepatocyte like cells is a potential therapeutic approach (1). In order to make use of these hepatocytes for cell based therapies, it is necessary to isolate hepatocytes from cell mixtures (containing undifferentiated stem cells, cells from meso-, endoderm and hepatocytes) and potentially follow their location in vivo. On the other hand, systemic administration of functionalized contrast agents to visualize hepatocytes rather than Kupffer cells is of importance to assess liver function non-invasively. Thus in this work, we have used galactose-terminal entities which are recognized by asialoglycoprotein receptors (ASGPR), which are hepatic receptors (2).

Methods: ML synthesis: Cationic (DSTAP)), anionic (DMPG)) in a dimyristoylphosphatidylcholine (DMPC) matrix were synthesized as described before. Lactosyl-bearing MLs were produced similarly to the anionic MLs using 95% DMPC and 1% 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-lactosyl (DOPE-lac) (3, 4). In vitro experiments: Targeted uptake with HepG2 in vitro has been evaluated (5). Differentiation of mESCs was performed according to (1). Differentiated cells were labeled with anionic and lactose MLs (100µg Fe/ml). Labeled cell samples were collected for RT-PCR, electron microscopy, immunostaining, MRI, flow cytometry and magnetic cell separation to confirm and quantify the uptake in hepatocyte like cells. In vivo experiments: Initial experiments were done by injecting MLs (200µg Fe/ml) in black 6 mice intravenously. Animals were scanned immediately and Day 1, 3, 5 and 10 post injections. At different time points livers were isolated for immunostaining. MRI: MR images were acquired using a Bruker Biospec 9.4 T small animal scanner. 3D T2*-weighted MRI (FLASH, TE=12ms, TR=150ms) and T2-maps (with 8 echoes) were acquired with respiration gating.

Results: Differentiated hepatocytes did not show any toxic effects on total cell count and marker expression post labeling. Lac MLs were specifically taken up by hepatoblasts, which was confirmed with TEM. Magnetic separation confirmed that Lac MLs target only hepatocytes from the culture which are 25-30% of the cells, which coincides with the positive fraction. In vivo experiments showed that all particles accumulate in the liver 24hrs post injection. Clearance of anionic MLs was rapid. However, Lac MLs remained detectable by day 10.

Conclusions: Initial in vitro experiments revealed high specificity in uptake of lactose-functionalized MLs by hepatocyte like cells. This can be used for MR imaging as well as for cell separation. The potential of Lac MLs for the evaluation of liver function was also confirmed by initial in vivo experiments.

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IMAGING RHOA ACTIVATION DURING INVASION OF MUTANT P53-DRIVEN PANCREATIC CANCER CELLS IN LIVE ANIMALS

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The study of cells in culture has enabled dissection of the biochemical logic circuits which drive many pathological processes, including cancer. Recently, however, it has become clear that the development of targeted therapies requires study at the molecular level of cell signalling events in their naturally occurring complex 3D environments. Interrogation of molecular dynamics within living tissue requires development of new experimental approaches to the study of cell migration. We have previously used FRAP to compare the dynamics of eCadherin in A431 squamous carcinoma cells grown in culture and in sub-cutaneous murine tumors.

Our results demonstrated significant differences in the fraction of eCadherin immobilised in cell:cell junctions, especially in response to treatment with the Src inhibitor Dasatinib. Now we have used FLIM-FRET to investigate the activity of Rho during invasion of pancreatic ductal adenocarcinoma cells (PDAC) driven by mutant p53 (R172H). Rho was specifically activated at the poles of invasive cells in both organotypic cultures and murine subcut tumors, but not in 2D cell culture. Rho activation was also not observed in non-invasive p53 knockout PDAC cells. Interestingly, differences in invasiveness between mutant and knockout p53 cells correlate with the mobile fraction of eCadherin determined by FRAP experiments: more mobile cells have a higher mobile fraction of eCadherin. Dasatinib, which has been shown to inhibit the metastasis of PDAC cells from primary tumors to the liver, specifically inhibited the activation of Rho at the cell poles but did not reduce the level of basal activation within the cell body.

Our results show for the first time the utility of FLIM-FRET in the analysis of dynamic biomarkers during drug treatment in living animals.

A NEW TYPE MULTI-MODALITY IRON-BASED NANOPARTICLE PLATFORM, SYNTHESIS AND CHARACTERISATION

Dr Szigeti[1], Ms Hegedus[1], Mr Rácz[1], Mr Bozó[1], Ms Horváth[1], Ms Fekete[1], Mr Benyó[1], Mr Máthé[2]

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Introduction: We aimed at developing an stable, inert particle family of reproducible size, form and quality with a simple radiolabelling process for SPECT and PET alike and with a simultaneous contrast in MRI and/or X-ray CT.

Methods: Prussian Blue {Fe4[Fe(CN)6]3, PB} is capable of irreversibly intercal ating metal isotopes and paramagnetic ions. We examined the changes in size, dispersion and shape of the PB nanoparticles (PBNP): the effect of proportion of basic components and different coatings, incubation time and pH was studied. Size and shape were determined using dynamic light scattering (DLS) and atomic force microscopy. We radiolabelled PBNPs with 201Tl and used thin layer chromatography and AFM as quality control. We performed T2 and T1-weighted in vitro MRI measurements using a Mediso nanoScan® PET/MR system with various PBNP concentrations. In vivo wholebody imaging of the radiolabelled particle biodistribution was performed in mice (n=8) with a neurotrauma model injecting cca. 10 MBq of radioactivity iv.

Results: We were able to reproducibly change the size of PBNPs between 20 and 200 nm and to synthetize them in non-coated form or coated with citrate or polyvinil-pirrolidone. Ageing did not affect geometric parameters of the PBNPs (6 weeks at room temperature). AFM has demonstrated the PBNPs being cuboidal with the proportions of e.g.153x150x50 nm. Radiolabelling yield was >98% in all experiments. A contrast increase was observed in T2-weighted MR measurements. Test animals showed no acute nor chronic in vivo toxicity signs. Using 201Tl-labelled non-coated PBNPs of 60 nm longest side, we could detect a focal uptake in brain due to blood-brain barrier (BBB) leakage 3 hours post neurotrauma. Citrate-coated 90 nm 201Tl-PBNPs were imaged in mice for 24 hours and were detecting increased perfusion around the trauma region 3 h post trauma while apparently not accumulating in trauma site.

Conclusions: Controlled size and shape PBNP production was achieved. PBNPs were successfully labelled with 201Tl. The synthetized cuboidal PBNPs were capable of giving a signal in SPECT and MRI. The developed synthesis process and coating variations enable for further functionalizing of the NPs. Given the well proven capabilities of PB for chelating, a large inventory of PET and SPECT metal isotopes is available for PB-like multimodal NP synthesis.

Research Support: CROmed Ltd.

References: Máthé and Szigeti PPA US 61/299357 (2010) and PCT US Pat. Pending (2011)

ESMI PLENARY LECTURE 3 BY

GERO MIESENBÖCK and MARK LYTHGOE

chaired by

Veerle Baekelandt, Leuven

Jorge Ripoll, Heraklion

LIGHTING UP THE BRAIN

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An emerging set of methods enables an experimental dialogue with biological systems composed of many interacting cell types - in particular, with neural circuits in the brain. These methods are sometimes called 'optogenetic' because they employ light-responsive proteins ('opto-') encoded in DNA ('-genetic'). Optogenetic devices can be introduced into tissues or whole organisms by genetic manipulation and be expressed in anatomically or functionally defined groups of cells. Two kinds of devices perform complementary functions: light-driven actuators control electrochemical signals; light-emitting sensors report them. Actuators pose questions by delivering targeted perturbations; sensors (and other measurements) signal answers. Optogenetic approaches are beginning to yield previously unattainable insight into the organization of neural circuits, the regulation of their collective dynamics, and the causal relationships between cellular activity patterns and behavior.

OPTOGENETICS AND MRI: SHEDDING LIGHT ON THE BRAIN

M. Lythgoe
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Since the discovery of X-rays, Magnetic Resonance Imaging (MRI) has been one of the most important technical advances in clinical and experimental imaging. MRI was introduced into the clinic only 30 years ago and has become one of the key technologies in diagnostic medicine. Although MRI is traditionally used to provide structural images of tissues and organs, it was the emergence of functional MRI (fMRI) that had the most profound impact on neuroscience and basic cognitive research. Yet despite the influence of this technique, little is known about the cellular basis of regional hemodynamic responses underlying the blood oxygenation level-dependent (BOLD) fMRI signals. While numerous studies use fMRI as a tool, only a small number have addressed the cellular basis of the BOLD response. A recent review in the journal Nature posed the searching question, 'What does functional imaging measure?' (1), which refers to the elusive cellular basis of fMRI (2). Recently studies have now combined MRI and optogenetics to shed light on the BOLD response (3,4) and investigate cortical plasticity (5). The term optogenetics is applied to the experiments in which particular brain cells are engineered to express light-sensitive proteins (reporters and actuators/effectors) (6). Voted the "Method of the Year 2010", optogenetics allows minimally invasive and temporally precise control of the activities of specific cellular populations (7-9) in vitro and in vivo. In this seminar I will review recent contributions to the field of combined MRI and optogenetics and role in preclinical brain imaging.

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day three: Tuesday 21 june 2011

ESMI PLENARY LECTURE 4 BY

DANIEL ANTHONY

chaired by

Klaas Nicolay, Eindhoven

Hervé Boutin, Manchester

IMAGING INFLAMMATION WITH TARGETED CONTRAST AGENTS

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At a time when pathology is otherwise undetectable, we have reported *in vivo* detection of vascular cell adhesion molecules and platelets in acute brain inflammation, using magnetic resonance imaging (MRI) in rodent models. However, it was important to establish whether our novel MR-imaging agents would address the clinico-radiological paradox by revealing the presence of chronic active non-enhancing lesions in clinically relevant models of disease.

Such lesions present particular difficulties for the neuroradiologist to accurately assess lesion load and activity by MRI. The MOG-EAE (myelin oligodendrocyte glycoprotein-experimental autoimmune encephalomyelitis) model provides a stringent test of MS detection; twenty-eight days after the injection of recombinant cytokines into the corpus callosum, which initiated a MS-like MOG-EAE lesion, sLex-MPIO (microparticles of iron oxide) revealed the clear presence of the chronically activated brain endothelium in the region of and immediately adjacent to the site of the focal MS-like lesion. This lesion is invisible on conventional MRI scans at this time point. The asymmetric detection observed with sLex-MPIO particles was not seen when the control-MPIO were injected into other focal MOG-EAE animals.

Another important goal of our molecular imaging programme was to discover whether our novel contrast agents could be used to improve the preclinical testing of new medicines. Prophylactic neutralisation of IL17A is effective in preventing ABH-EAE disease establishment, abolishing subsequent relapse. VCAM-MPIO MRI revealed that VCAM-1 expression was significantly higher in IgG-treated mice than in mice treated with anti-IL17A and there were more Gd-DTPA-enhancing lesions on T1-weighted images in the IgG-treated mice. However, during relapse at day 42, no significant differences were observed among the treatment groups in the level of VCAM-1 expression or in the number of Gd-DTPA-enhancing lesions.

Thus our serial imaging studies reveal that while anti-IL17A improved clinical signs in the relapse phase it did not improve our MRI-based indices of disease. This highlights the potential for clinical scores to misrepresent the extent of disease activity at a cellular level and the need for targeted imaging agent that reveal the true extent of disease burden.

FATE AND FUNCTION OF MICROGLIA IN ALZHEIMER`S DISEASE

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Generation of neurotoxic amyloid-ß peptides and their deposition along with neurofibrillary tangle formation represent key pathological hallmarks in Alzheimer's disease (AD). Recent evidence suggests that inflammation may be a third important component which, once initiated in response to neurodegeneration or dysfunction, may actively contribute to disease progression and chronicity. Various neuroinflammatory mediators including complement activators and inhibitors, chemokines, cytokines, radical oxygen species and inflammatory enzyme systems are expressed and released by microglia in the AD brain. Degeneration of aminergic brain stem nuclei including the locus ceruleus and the nucleus basalis of Meynert may facilitate the occurrence of inflammation in their projection areas given the antiinflammatory and neuroprotective action of their key transmitters norepinephrine and acetylcholine. While inflammation has been thought to arise secondary to degeneration, recent experiments demonstrated that inflammatory mediators may stimulate amyloid precursor protein processing by various means and therefore can establish a vicious cycle. Despite the fact that some aspects of inflammation may even be protective for bystander neurons, antiinflammatory treatment strategies should therefore be considered. Nonsteroidal antiinflammatory drugs have been shown to reduce the risk and delay the onset to develop AD. While, the precise molecular mechanism underlying this effect is still unknown, a number of possible mechanisms including cyclooxygenase 2 or c-secretase inhibition and activation of the peroxisome proliferator activated receptor c may alone or, more likely, in concert account for the epidemiologically observed protection. Data on microglial activation in AD along with suggestions to modify and alter the pro- into an antiinflammatory phenotype will be reviewed and discussed.

PARALLEL SESSION 11:

NEUROINFLAMMATION and NEURODEGENERATION

chaired by:

Andreas Jacobs, Muenster

Sabina Pappatá, Naples

Bertrand Tavitian, Orsay

PRESENCE AND LACK OF MICROGLIAL ACTIVATION: DIFFERENCES IN EARLY NEURODEGENERATIVE DISEASES AND PRION DISEASE

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Microglia activation has a key role in brain's immune response to neurodegeneration. Significant microglia activation was shown in vivo with 11C-PK11195 PET in patients with various neurodegenerative diseases However, its relevance in very early Dementia with Lewy Bodies (DLB) and Parkinson's disease (PD), as well as the association with the oxidative status of CSF, are unknown.Neuroinflammation has been reported in prion disease, but only in post mortem pathological assessment. In vivo mmicroglia activation has never been investigated in human prion diseases, such as in Fatal Familial Insomnia (FFI). It is possible that neuroinflammation has different role and effects in the two conditions.

We included 6 drug-naive PD patients (age = 70.22.9), 6 LBD (age = 728.1) and 12 matched healthy controls (HC). The expression of the PBR/TSPO protein, linked to microglia activation was also assessed in 9 unaffected carriers and one symptomatic case of FFI, an autosomal dominant prion disease. We obtained parametric images of 11C-PK binding potential (BP) using simplified reference tissue model and cluster analysis to obtain the input function. Proteomic study analyzed CSF proteins in DLB and PD by 2D-electrophoresis according to isoelectric point and molecular weight. UPDRS I-III and Hoen-Yahr scores in PD patients were respectively 26.78.5 and 1.70.6, in DLB 57.316.2 and 3.30.6. The neuropsychological performance was impaired only in DLB. Healthy subjects showed [11C]-PK11195 BP values similar to these previously reported (Cagnin et al. 2001). In comparison to the healthy controls, both groups showed significant [11C]-PK11195 BP increases (thus activated microglia) in the basal ganglia, thalamus and in the substantia nigra. DLB patients showed in addition extensive significant increases in all associative cortices (including the occipital cortex) and in the cerebellum. Thus, PD displayed a profile of increased microglia activation limited to the subcortical structures.

Proteomic analysis showed markedly increased protein carbonylation both in PD and LBD that was correlated with the [11C]-PK11195 increases (p=0.002). There were no significant changes in 11C-PK11195 uptake in the FFI patient and mutation carriers, when compared with healthy controls. Noteworthy, there were no significant [11C]-PK11195 BP increases in regions showing the major pathological changes in FFI (anterior and mediodorsal thalamus, anterior cingulate cortex). No correlation between BP values and age was observed in any of the region examined.

Microglia activation in PD and DLB patients in early phase and untreated reflects different pathologic phenotypes. In both patient groups, alterations of CSF proteins related to oxidative stress correlated with the amount of activated microglia as measured with PET, both measures representing early markers of neuroinflammation, associated with neurodegenerative process. The 11C-PK PET findings in FFI differ since there is no activated microglia either in the carriers and noteworthy in the affected patient. We suggest that microglia activation shown by post mortem studies and correlated with the site of neuronal loss might be a late event in the FFI pathological course. The possibility that neuronal loss in prion diseases occurs through an apoptotic process has been postulated, and is consistent with the lack of inflammation in these disorders.

QUANTIFICATION OF [18F]DPA714 BINDING IN THE HUMAN BRAIN: INITIAL STUDIES IN HEALTHY CONTROLS AND ALZHEIMER'S DISEASE PATIENTS

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Introduction: [18F]DPA714 is a novel tracer of the 18-kDa translocator protein (TSPO). This protein is highly expressed in microglia that are activated during chronic neuroinflammation, e.g. in case of Alzheimer's disease (AD). The aim of this study was to quantify [18F]DPA714 binding in the brain of both healthy controls (HC) and (AD) patients.

Methods: After intravenous bolus injection of 250±10 MBq [18F]DPA714, dynamic PET scans with a total duration of 150 min including online arterial sampling and with metabolite analysis were acquired in 9 AD and 7 HC subjects. Based on earlier kinetic model evaluations, all scans in this study are analysed using the first 90 min of data with a metabolite corrected plasma input two tissue reversible compartment model and with the simplified reference tissue model (STRM) using cerebellum as reference region. In addition, performance of parametric Logan and reference Logan plot analysis was evaluated against non linear regression based kinetic analysis. Finally, differences in tracer binding between subject groups were explored both at a regional level (n=56 volumes of interest) as well as using statistical parametric mapping (SPM5) based on parametric pharmacokinetic images.

Results: Average whole cerebrum grey matter V_T was 2.5±0.7 and 2.2±0.7 for HC and AD subjects, respectively. Corresponding BP was 1.00±0 and 0.86±0.50, respectively. SRTM based BP_{ND} values equaled -0.05±0.15 and -0.09±0.04 for HC and AD subjects, respectively. Parametric Logan V_T and reference Logan BP_{ND} data corresponded well (R^2 > 0.9 and 0.75, resp) with results obtained with full kinetic analysis. At a regional level no significant differences in V_T, BP and BP_{ND} between subject groups were found. Only SPM analysis based on parametric BP_{ND} image (i.e. reference Logan) showed a trend towards increased [18F]DPA714 binding in AD subjects in a few AD specific areas.

Conclusions: Various types of regional and parametric analyses indicated that differences in [18F]DPA714 binding throughout the brain between AD and HC subject groups are extremely small. [18F]DPA714 is therefore not suitable as a diagnostic imaging agent for AD.

PET IMAGING OF THE 18-KDA TRANSLOCATOR PROTEIN WITH THE RADIOLIGAND [18F]FEDAA1106 DOES NOT REVEAL INCREASED BINDING IN ALZHEIMER´S DISEASE PATIENTS

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Introduction. Imaging of the 18-kDa translocator protein (TSPO) is considered as a potential tool for the in vivo evaluation of microglia activation and neuroinflammation in the early stages of Alzheimer´s disease (AD). [^{11}C]PK11195 has been widely used for PET imaging of the TSPO and despite its limitations due to low brain uptake and high nonspecific binding, it has successfully shown increased TSPO binding in AD patients (1). The high-affinity radioligand [^{18}F]FEDAA1106 has been developed as a potential imaging tool for better quantification of in vivo TSPO binding (2). Aim. The aim of this study was to assess the quantification of the in vivo binding of [^{18}F]FEDAA1106 to TSPO in AD patients and healthy controls (HC).

Methods. Nine AD patients (6M/3F, age 69±4 y, MMSE 25±3) and seven HC (5M/2F, age 68±3 y) were studied with [^{18}F]FEDAA1106 (AD: 256±8 MBq, specific radioactivity: 271±127 GBq/µmol, HC: 251±15 MBq, specific radioactivity: 211±161 GBq/µmol) at Karolinska Institutet. PET measurements were acquired with the ECAT EXACT HR system (Siemens Medical Solutions) with two 60-min dynamic PET sessions and a 30-min interval between sessions, with a total duration of 150 min. Arterial blood radioactivity was measured with an automated blood sampling system for the first 5 min and with manual samples thereafter. Samples for metabolite analysis were taken at 2.5, 10, 20, 30, 40, 60, 90, 120, and 150 min. T1-weighted MR images were segmented into grey matter (GM), white matter, and CSF and coregistered to the PET images using SPM5. Regions of interest - including areas known to be involved in pathology - were manually delineated on several cortical and sub-cortical regions including the posterior cingulate (PC) and hippocampus (HIP). Quantification was performed using the Logan graphical analysis (GA). The outcome measure was the total distribution volume (V_T). An estimate of non-displaceable distribution volume was obtained with the Logan GA using the first 15 min of PET measurements ($V_{ND\ 1\text{-}15min}$). Binding potential (BP_{ND}) was calculated as: V_T / $V_{ND\ 1\text{-}15min}$ -1.

Results. No significant difference of V_T (GM: 10.40±4.97; PC: 10.73±5.11; HIP: 8.63±3.67 in HC vs. GM: 11.81±5.20; PC: 10.36±5.14; HIP: 9.01±3.93 in AD) or BP_{ND} (GM: 2.44±0.94; PC: 1.84±0.46; HIP: 2.47±0.66 in HC vs. GM: 2.94±1.45; PC: 2.05±0.48; HIP: 2.69±0.95 in AD) was observed between HC and AD patients.

Conclusions. No difference in TSPO binding between AD patients and HC was observed using [^{18}F]FEDAA1106. Possible reasons for this finding could be: 1) large variability of V_T among subjects due to biological heterogeneity of HC and AD patients; 2) the lack of a suitable reference region in the brain for TSPO quantification; 3) possible presence of subjects with a lower affinity binding site, that has been shown for other TSPO radioligands, including DAA1106 (3)

Acknowledgement. The study was supported by Bayer Schering Pharma. References 1. Cagnin A, et al. Lancet 2001; 358: 461-67. 2. Fujimura Y, et al. J Nucl Med 2006;47:43-50 3. Owen DRJ, et al. J Nucl Med 2011;52:24-32

YIA applicant

SMALL ANIMAL PET IMAGING OF THE TYPE 1 AND TYPE 2 CANNABINOID RECEPTOR IN PHOTOTHROMBOTIC STROKE MODEL

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Introduction: Recent evidence suggests involvement of the endocannabinoid system (ECS) in the pathophysiology of stroke and is therefore a potential novel target for therapy. No *in vivo* imaging data in stroke are available at present. The purpose of this study was to characterize type 1 (CB1) and type 2 cannabinoid receptor (CB2) binding alterations in a phototrombotic stroke model for the first time *in vivo* using small animal PET.

Methods: In total, 34 Wistar rats (male; 24 phototrombotic stroke, 10 sham-operated) were investigated at baseline, 24h, 72h, 1 week and 2 weeks after lesioning. MicroPET acquisitions were conducted on a Focus 220 system after isoflurane anesthesia and using approximately 18 MBq of [^{18}F]MK-9470 (CB1; n=16; 20min, 1h post) and 37 MBq of [^{11}C]NE40 (CB2; n=10; 60min dynamic). Parametric maps were generated, spatially normalized to Paxinos space and analyzed using SPM2. Structural 9.4T microMRI imaging was additionally done to assess stroke lesion size. *Ex vivo* validation (n=8) was carried via immunohistochemical staining for both CB1 and CB2. Blocking experiments with the CB1 receptor antagonist SR141716A were included to asses specific binding of [^{18}F]MK-9470.

Results: Relative [18F]MK-9470 binding strongly increased at 24h and 72h after stroke in the cortex surrounding the lesion (+ 28 ± 9%, p=1.3 10^{-7} and + 26 ± 8%, p=6.4 $10^{-6,}$ respectively). Relative [^{18}F]MK-9470 binding also increased at the insular cortex at 24h after surgery (+ 17 ± 8 %, p=5.0 10^{-5}). CB2 receptor imaging with [11C]NE40 did not significantly differ beween stroke and sham-operated rats.

Staining for CB1 confirmed our *in vivo* findings, while CB2 immunohistochemistry detected minor reactivity at 1 and 2 weeks after stroke in this model. Preblocking with SR141716A (10 mg/kg) 24 h after stroke uniformly decreased [^{18}F]MK-9470 binding with ~30% across all brain regions.

Conclusions: Time-dependent and strong regionally increased CB1 receptor availability, but not CB2 receptor alterations, are early consequences of photothrombotic stroke. This opens perspectives to study pharmacological interventions aimed at targeting endocannabinoid pathways involving CB1 receptor signaling.

Acknowledgement: Financial support of the Research Council of the Katholieke Universiteit Leuven (OT/05/58 - EF/05/008) is gratefully acknowledged.

TIME-DEPENDANT RECRUITMENT, ACTIVATION AND INACTIVATION OF MICROGLIA AND ASTROCYTES FOLLOWING AUTOLOGOUS MESENCHYMAL STEM CELL GRAFTING IN BRAIN TISSUE

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Introduction: The use of stem cell transplantation as a therapeutic tool to treat neurodegenerative disorders has gained increasing interest over the last decade. However, a profound knowledge of cell implant behaviour, survival and differentiation will be necessary to understand potential therapeutic effects of stem cell transplantation.

Methods: In this study we aimed to follow up the survival of grafted bone marrow-derived mesenchymal stem cells (MSC) in the central nervous system (CNS) of mice by non-invasive bioluminescence imaging (BLI) combined with a post-mortem histological study of cell differentiation and recruitment of inflammatory cells towards the implant site.

Results: BLI analysis shows stable survival of MSC-Luc/eGFP in vivo. These results were further validated by histology demonstrating the presence of Sca1+ and eGFP+ cells at every time point investigated (day 1 to day 14). At a very early time point (day 1), histological analysis did not show recruitment of microglia and/or astrocytes. However, starting from day 3, MSC grafts are invaded by Iba1+/CD11b+ microglia (activated microglia) and surrounded by a glial scar of astrocytes. From day 10 on, activated Iba1+/CD11b+ microglia were found in the surrounding of the implant, while Iba1+/CD11b- microglia remain within the MSC graft, which suggests that MSC might have certain immune-suppressive characteristics to modulate the activation status of microglia. More quantitative analyses of the actual numbers of GFP+, Iba1+ and CD11b+ cells within and/or surrounding the graft site are ongoing and will be presented.

Conclusions: Although the CNS has historically been considered to be immune-privileged, our data demonstrate that the CNS is not immune-ignorant to autologous cellular implants. Further research should be undertaken to understand the in vivo interaction between MSC, microglia and astrocytes.

YIA applicant

IMAGING INFLAMMATION FOLLOWING STATUS EPILEPTICUS USING A TARGETED IRON OXIDE CONTRAST AGENT

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Background: Status epilepticus (SE) is the most common medical neurological emergency. It is defined as a seizure persisting for longer than 30 minutes. It is associated with brain injury and the subsequent development of epilepsy. Recently it has been has been shown that neuroinflammation can contribute towards seizure generation[1]. Possible mechanisms include a direct effect of inflammatory cytokines on neurons and/or via blood brain barrier permeability alterations[2]. Based on these studies, it has been hypothesised that inflammation could provide a mechanism for the development of some partial epilepsies. Thus longitudinal imaging of inflammation could provide insight into the processes involved in epileptogenesis. VCAM-1 is important in the inflammatory cascade. At sites of inflammation it is expressed on endothelial cells where it mediates leukocyte rolling. It has recently been demonstrated that it is possible to image VCAM-1 using a targeted contrast agent[3]. For this study, we have synthesised an iron oxide based contrast agent specific to VCAM-1 to monitor expression in-vivo in the lithium-pilocarpine model of epilepsy.

Methods: Animal Model: (n=5) Sprague-Dawley rats were separated into three groups, post-status epilepticus (n=3), saline control (n=1) and positive control (n=1) in which TNF-a was injected into the right cerebral hemisphere to create a localised inflammatory insult. In the post-SE group animals were treated with lithium chloride and pilocarpine to induce SE. Diazepam was administered 90 min after SE onset. Contrast Agent: VCAM-MPIO was synthesised as described previously[3]. MRI: MRI was performed using a 9.4T horizontal bore system before and after injection of the contrast agent. VCAM-MPIO was injected via the jugular vein 22h post-SE or 22h post-saline administration. Animals were imaged 1h post-injection to allow time for clearance. Animals were perfusion-fixed with saline followed by PFA. The fixed-brains were imaged in ex-vivo MRI. Contrast Volume: Volumes of hypointensity were quantified on in-vivo scans by 3D object detection based on thresholding and voxel connectivity. Objects less than 4 voxels or greater than 80 voxels in size were not counted.

Results: Intracerebral injections of TNF-a caused VCAM-MPIO to bind preferentially to the affected hemisphere (contrast volume 2.04 µl verses 0.56 µl). In-vivo and ex-vivo scans show clear differences between the control and post-SE groups. Marked hypointensities were observed in the hippocampus and the cortex in the post-SE group indicating regional binding of MPIO. These regions correspond to the most vulnerable regions following SE. T2 was not elevated in the hippocampus in the post-SE group (52.5±2.4 ms) compared to controls (53.0±0.4 ms) in pre-contrast scans. We propose that VCAM-1 could be an early marker of neuronal injury following status epilepticus.

Conclusions: This is the first demonstration that it is possible to image VCAM-1 expression in-vivo in a rat model of epilepsy. This biomarker opens up the possibility to monitor the effect of anti-inflammatory therapies on VCAM-1 expression and subsequent outcome.

References: [1] M. Maroso et al., Nature Medicine 16, 413 (2010). [2] A. Vezzani et al., Nat Rev Neurol 7, 31 (2011). [3] M. A. McAteer et al., Nature Medicine 13, 1253 (2007).

INDUSTRY ROUND TABLE SESSION

organised and chaired by the respective companies

GENERAL SET-UP OF INDUSTRY ROUND TABLES

The Industry Round Tables are organised as a special break-out session (21 June 2011 starting at 9am)

Companies who were interested in hosting a round table had to submit a topic to the ESMI beforehand. The topics that will be discussed are related to a scientific theme or an application issue in molecular/preclinical imaging

Set-up: 4 round tables (one table per company) for 1up to 12 people each in one room (Breezaal and Kleine Foyer - see next page)

The company provides a host responsible for the round table meeting. The conference attendees can sign-in for the round tables at the respective booth or the companies invite people to join "their" table.

Important: the organisation and the content of the Industry Round Tables are in the responsibility of the respective company, but it will not be a product presentation and sales event.

THESE INDUSTRY ROUND TABLES WILL TAKE PLACE IN THE "BREEZAAL"

Organised by ART:
Can fluorescent *in vivo* lifetime imaging be used to unravel specific biomolecular interactions?
Moderator: Alan Chan

Organised by BIOSCAN and PHILIPS:
Exploring user requirements and technical specifications for a preclinical PET/MRI system
Moderator: Hein Haas

Organised by BRUKER:
Cost-effective low-field MRI as an alternative to Micro-CT
Moderator: Sarah-Rebecca Snyder

Organised by PERKINELMER:
Translational fluorescent biomarkers from cells to clinical practice
Moderator: William Vaccani

THESE INDUSTRY ROUND TABLES WILL TAKE PLACE IN THE "KLEINE FOYER"

Organised by VISUALSONICS:
New *in vivo* molecular imaging techniques
Moderator: Dave Bates

Organised by CALIPER LIFE SCIENCE:
Advances in multimodal imaging technologies for longitudinal and quantitative uCT/Optical preclinical modeling
Moderator: Jeff Meganck

Organised by LI-COR:
Near-infrared small animal imaging and applications
Moderator: Jeff Harford

Organised by GE HEALTHCARE / GAMMA MEDICA:
Solid-state SPECT / MR: let's shape the future together
Moderator: Dirk Meier and Richard Tabassi

Please contact the respective company at their booth for any further information.

PARALLEL SESSION 12:

IMAGE GUIDED SURGERY

chaired by:

George Themelis, Neuherberg

Alexander Vahrmeijer, Leiden

INTRAOPERATIVE FLUORESCENCE IMAGING: ONE STEP CLOSER TO CLINICAL USE

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Objective: To improve the surgical procedure and outcome by means of real-time molecular imaging feedback of tumor spread and margin delineation using targeted near-infrared fluorescent probes with specificity to tumor biomarkers.

Summary background data: Surgical excision of cancer is often confronted with difficulties in the identification of cancer spread and the accurate delineation of tumor margins. Currently, the assessment of tumor borders is afforded by post-operative pathology or, and less reliable, intraoperative frozen sectioning. Fluorescence imaging is a natural modality for intra-operative use, since it relates directly to the surgeon's vision and offers highly attractive characteristics such as high-resolution, sensitivity and portability. Via the use of targeted probes it also becomes highly tumor specific and can lead to significant improvements in surgical procedures and outcome.

Methods: The overexpression of folate receptor-a (FR-a) in 90-95% of (serous) epithelial ovarian cancers prompted the clinical investigation of intraoperative tumor-specific fluorescence imaging and intervention in ovarian cancer surgery using a FR-a targeted fluorescent agent. A novel real-time multi-spectral fluorescence imaging system was used for in-vivo detection and visualization of lesions. Ten patients with a suspected ovarian malignancy scheduled for an explorative laparotomy were included in this study. The number of tumor spots detected by visual inspection alone was compared to the number detected by fluorescence imaging. In vivo images were correlated with histopathological analyses.

Results: Four patients were diagnosed with malignant epithelial ovarian cancer and one patient with a borderline tumor. Five patients were diagnosed with a benign ovarian tumor, as verified by histopathology: three fibrothecoma, one cystic teratoma and one inflammatory process in the ovary. Detection of the number of tumor spots by fluorescence was significantly increased compared to visual observation alone. Fluorescent regions showed excellent correlation with histopathological findings.

Conclusion: In patients with ovarian cancer, intraoperative tumor-specific fluorescence imaging with a FR-a targeted fluorescent agent showcased improved sensitivity in detecting tumor lesions compared to visual inspection.

INTRODUCTION TO OPTICAL IMAGE-GUIDED SURGERY

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Despite many improvements in pre- and postoperative imaging modalities (e.g. CT, MRI, PET, SPECT), during oncologic surgeries, the surgeon mainly has to rely on visual inspection and palpation to determine what structures should be resected (tumors, lymph nodes) and what structures should be spared (e.g. nerves, bile ducts, ureters). The goal for the next years is to provide surgical oncologists with a real-time fluorescence-based tumor imaging technique to guide surgery for the complete and safe resection of tumor tissue. Moreover, this technology is already used for the real-time identification of sentinel lymph nodes in several types of cancer patients. Real time tumor identification will tailor surgical treatment and will hopefully improve survival and quality of life.

Image-guided surgery is based on the use of specific contrast agents (fluorescent probes). These probes consist of a tumor targeting protein (tumor-associated antibody, receptor ligand or enzyme substrate) conjugated to a fluorescent label. For fluorescence image-guided surgery, the near-infrared (NIR) region of the spectrum offers advantages such as high tissue penetration, low autofluorescence and the availability of NIR fluorescence labels for chemical conjugation to target molecules. Moreover, the NIR spectrum is invisible to the human eye and therefore NIR light will not interfere with the surgical field. The fluorescent signal can be detected with special camera systems and shown on a monitor to the surgeon. An overview of already performed clinical studies for the detection of the sentinel lymph node will be presented and requirements for real time tumor detection will be discussed as a short introduction to the Image Guided Surgery Session.

YIA applicant

PRECLINICAL OPTIMIZATION AND CLINICAL TRANSLATION OF NEAR-INFRARED FLUORESCENCE IMAGING OF COLORECTAL LIVER METASTASES USING INDOCYANINE GREEN

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Introduction: Near-infrared (NIR) fluorescence imaging using indocyanine green (ICG) is a promising technique to obtain real-time assessment of the extent and number of colorectal liver metastases during liver surgery. The current study aims to optimize dosage and timing of ICG administration.

Methods: The Mini-FLARE imaging system was used for real-time identification of liver tumors in 18 rats. Liver tumors were measured at 24, 48, 72 or 96 hours after administration of 0.04, 0.08, or 0.16 mg ICG (0.13, 0.26 or 0.53 mg / kg, respectively). Guided by these results, intraoperative identification of liver metastases was performed in 22 patients undergoing liver resection. NIR fluorescence imaging was performed 24 or 48 hours after administration of 10 or 20 mg ICG (approximately or 0.26 mg / kg, respectively). After intraoperative imaging, resection specimens were sliced to examine internal fluorescent patterns using the Mini-FLARE imaging system. Subsequently, frozen tissue sections were measured for fluorescence using the Nuance multispectral imager.

Results: Using NIR fluorescence imaging and ICG, all colorectal liver metastases (N = 34), could be identified in all rats. Average tumor-to-liver (TLR) ratio over all groups was 3.0 ± 1.2. Liver signal was lower in the 72 h time group compared to other time points, resulting in a significantly higher TLR. ICG dose did not significantly influence TLR, but a trend was found favoring the 0.08 mg dose group. Clinically, during intraoperative NIR fluorescence imaging, all superficially located metastases (< 1 cm beneath liver capsule) were identified (N = 44). Median TLR was 7.4 (range: 1.9 - 18.7) and no significant differences between time-points or doses were found. Liver signal was comparable to pre-injection signal at 24 to 48 hours post-injection, eliminating the need to test other time-points. In all patients, a fluorescent rim around the tumor was found, as described in earlier studies. Using fluorescence microscopy, this clear fluorescent rim was localized in stromal tissue in the transition area between tumor and normal liver tissue in all liver metastases. In this area, multiple cell types that are involved in tissue inflammation (e.g. granulocytes, lymphocytes) were found. In four patients, additional small (2 - 8 mm) metastases were identified using NIR fluorescence that were otherwise missed preoperatively and intraoperatively using only visual inspection and ultrasound

Conclusions: This study demonstrates that colorectal cancer liver metastases can be clearly identified during surgery using ICG and the Mini-FLARE imaging system. NIR fluorescence imaging has the potential to improve intraoperative detection of in particular small and superficially located liver metastases and can therefore be seen as an addition to the conventional imaging modalities.

CLINICAL PROOF OF CONCEPT OF A HYBRID SURGICAL GUIDANCE APPROACH DURING ROBOT ASSISTED LAPAROSCOPIC PROCEDURES

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Introduction: Integration of molecular imaging and in particular intraoperative image guidance is expected to improve surgical accuracy of robot assisted laparoscopic procedures. In this setting we set-out to show the clinical value of combined pre- intra-, and post-operative sentinel node imaging using an integrated diagnostic approach based on a hybrid tracer that is both radioactive and fluorescent.1,2

Methods: Prior to surgery the hybrid tracer ICG-99mTc-Nanocoll3 was injected into the prostate. Subsequent lymphoscintigraphy and SPECT/CT imaging of the pelvic region was performed to determine the location of the sentinel lymph nodes (SLNs). During the surgical procedure a fluorescence laparoscope, optimized for detection in the NIR-range, and a portable gamma-probe were used to localize the nodes identified on SPECT/CT. This study was performed in eleven prostate cancer patients with an increased risk of nodal metastasis that were scheduled for robot-assisted laparoscopic prostatectomy (RALP) with lymph node dissection.

Results: Preoperatively, SLNs could be accurately identified by SPECT/CT in ten patients. Intraperatively the combination of 'rough' gamma-tracing and optical detection via a near infra red optimized laparoscope enabled identification of 27 out of 28 SLNs. Fluorescence imaging particularly improved surgical guidance in areas with a high radioactive background signal such as the injection site (6 of 27 SLNs could not be detected). Fluorescence detection of the SLN, however, was in some cases restricted by the severe tissue attenuation of the signal (4 of 27 SLNs could not be detected). Therefore, guidance based on a combination of radioactivity and fluorescence detection is crucial. Ex vivo analysis of all the 112 excised LNs revealed a strong correlation between the radioactive and fluorescent content and the fluorescent signal could still be accurately detected in embedded tissue specimens.

Conclusions: Initial data indicate that multimodal ICG-99mTc-Nanocolloid, in combination with a fluorescence laparoscope, can be used to facilitate and optimize dissection of SLNs during (robot assisted) laparoscopic-procedures. Moreover the findings indicate that intraoperative multimodal tracing of the SLNs is superior to using a single modality.

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YIA applicant

INITIAL CLINICAL VALIDATION OF A MULTIMODAL RADIOACTIVE / FLUORESCENCE IMAGING AGENT FOR SIMULTANEOUS RADIOGUIDED AND OPTICAL SENTINEL NODE DETECTION

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Introduction:Functional (optical) imaging holds great promise to supplement surgeons' eyes. However, fluorescence detection is limited by tissue attenuation of the signal. Therefore, radio guidance to the areas of interest is still desirable. Recently, a hybrid imaging agent that is both radioactive and fluorescent was introduced [1]. This study aims to clinically validate a hybrid imaging agent obtained by adding indocyanine green (ICG) to 99mTc-nanocolloid in order to optimise intraoperative sentinel node identification.

Methods: So far, 18 patients (10 head/neck melanoma, 1 melanoma on the trunk, 7 penile carcinoma) scheduled for sentinel node (SN) biopsy were prospectively included in the analysis. After peritumoral injection of 99mTc-Nanocoll, lymphoscintigraphy was performed on the basis of a 10 minute dynamic study and static images at 10 minutes and 2 hours post injection, followed by SPECT/CT. The following day the procedure was repeated with injection of the hybrid imaging agent (ICG-99mTc-Nanocoll) in an identical fashion. The images of both procedures were compared. Intraoperative imaging was performed using a portable gamma camera and a dedicated fluorescence camera. After excision, radioactive and fluorescent signal intensities were quantified and correlated ex vivo.

Results: Lymphatic drainage was visualized in all (18/18) patients. A total of 49 sentinel nodes were preoperatively identified after the first scintigraphic study. The second scintigraphic study revealed the same number of sentinel nodes at the same locations in all patients (100% reproducibility). In total, 62 sentinel nodes were surgically removed. Intraoperatively, 60 sentinel nodes (96%) could be visualized by fluorescence imaging, of which 4 contained metastases. Ex vivo analyses showed a strong intensity correlation of the radioactive and fluorescent content in all excised SNs.

Conclusions: ICG-99mTc-Nanocoll has an identical lymphatic distribution pattern compared to 99mTc-Nanocoll, whilst simultaneously enabling intraoperative fluorescence imaging of radioactive SNs.

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YIA applicant

SENTINEL LYMPH NODE DETECTION WITH INTRAOPERATIVE FLUORESCENCE IMAGING IN GYNECOLOGIC CANCER

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Introduction The sentinel lymph node (SLN) procedure helps to identify those patients who need a full lymphadenectomy after histopathological examination, and has been proven safe in various tumor types like breast cancer, melanoma and vulva cancer. The current standard of practice is to use a combination of radioactive colloid and a blue dye. Recently, SLN detection with fluorescence imaging has been successfully applied in breast cancer. In the current studies we have assessed the technical feasibility of this concept in gynecologic cancer, by using indocyanin green (ICG) and an intraoperative multispectral camera system.1,2

Methods Ten patients with cervical cancer and ten patients with vulvar cancer were included in these technical feasibility studies. ICG and patent blue were injected peritumorally during surgery, after which the camera system was used to visualize real-time fluorescence through lymph vessels and, correspondingly, detection of the SLN. Histopathological examination was performed to reveal the possible presence of tumor cells in the fluorescent lymph nodes.

Results In patients with cervical cancer, real-time fluorescence was observed in six out of ten patients. A tumor >2 cm and a BMI >25 hampered detection of the fluorescent signal. In the vulvar cancer patients, transcutaneous detection of fluorescence was seen in five out of ten patients. All but one SLNs were fluorescent. Detection was again more difficult in patients with a BMI >25.

Conclusion In cervical and vulvar cancer, SLN detection with ICG is technically feasible. However, adipose tissue hampers detection. For future studies, we propose the combination of radiocolloid with ICG (van Leeuwen et al.) as a multimodality tracer in order to improve the detection rate of the SLN in patients with cervical and vulvar cancer.

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Notes

YIA applicant

COMPACT HYBRID IMAGING SYSTEM ALLOWS FOR CONCURRENT FMT AND MR IMAGING IN MOUSE BRAIN

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Introduction: Complementary information should enhance the understanding of biological processes. Therefore multimodality/hybrid imaging approaches have become an attractive concept. In particular, the combination of magnetic resonance imaging (MRI) providing structural information due to the high soft tissue contrast with the more functional and/or molecular readout of fluorescence molecular tomography (FMT) bears high potential. As simultaneous measurements are required for reliable results under identical physiological conditions a fully integrated non-contact FMT/MRI setup was designed and tested in phantom and animal experiments.

Methods: A compact FMT system fitting in the bore of a small animal MRI system based on a recently reported setup [1] was designed. The free beam illumination for the non-contact optical reflection measurements is located outside the magnet bore. It comprises a fiber coupled continuous wave laser source, a collimation lens with a pinhole, which is mapped by a 2f image to obtain a focal spot on the sample in the MRI bore and a scanning device to scan the point grid on the sample surface. This illumination geometry allows the flexibility to scan any kind of source pattern on the sample surface. The incident light beam is deflected onto the sample by two small mirrors mounted on the right side in the back plate of the sample platform. The deflected beam is passing through the rectangular window of the MRI transceiver surface coil. The fluorescence light is detected by a custom made CMOS detector working at 9.4T. A fixed focus objective lens was used to map the diffusive light pattern on the sample surface onto the 256x256 detector array. The appropriate fluorescence wavelengths were selected using band pass filters mounted on a filter wheel between the objective lens and the detector. The sample platform is mounted on a rail system which is rigidly fixed with the MR scanner.

Results: Experiments on tissue mimicking phantoms demonstrated that the chosen reflection geometry allows high resolution imaging of superficial structures in depth up to 3mm whereas a degradation was observed when the source is placed deeper. A spatial resolution <1mm could be achieved in phantom experiments where the separation distance of two fluorophores in depths of 2mm was analyzed. Sensitivity analysis with different dye concentrations in a phantom showed a lower detection limit of 2picomoles. For hybrid FMT/MR imaging the setup was tested using a transgenic mouse model for Alzheimer's disease: a plaque specific fluorescence dye AOI987 [2] was injected as an optical imaging probe. Differences in the fluorescence signal between the measured time points were recorded and revealed dye accumulation in the top layer of the animal's brain. Further verification with simultaneously acquired MR datasets showed that the fluorescence signal originates from cortical brain regions.

Conclusion: We demonstrated the feasibility of simultaneous MRI and fluorescence tomography measurements using a fully integrated non-contact FMT/MR setup in phantom experiments and simultaneous in-vivo experiments in mouse brain.

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PARALLEL SESSION 13:

(HYBRID) IMAGING TECHNOLOGIES

chaired by

Klaas Nicolay, Eindhoven

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SUPER-RESOLUTION RECONSTRUCTION IN MOLECULAR MRI - A FEASIBILITY STUDY

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Introduction**:** With its excellent contrast capabilities MRI is a prominent modality in the field of molecular imaging [1]. In practice, however, the spatial and temporal resolutions of an MR image are severely limited by noise and timing considerations. Therefore, quantification and characterization of events at the molecular level, using MRI, will generally require careful balancing of the trade-off space spanned by resolution, signal-to-noise ratio (SNR) and image acquisition time. High resolution, high SNR and low acquisition time are desirable properties of an experiment, and for a given imaging protocol improvement of one parameter is only achievable at the expense of one or two of the others. Super-resolution reconstruction (SRR) is the process of producing a high-resolution (HR) image from a sequence of low-resolution (LR) images, where each LR image transforms and samples the HR scene in a distinct fashion [2]. In this study, we investigate whether SRR, employed as a post-processing step, can advantageously modify the MRI trade-off space.

Methods: Experiments were performed using an FSE sequence on a 3T GE clinical scanner. A data set was acquired, containing 24 LR images of anisotropic resolution ((1x1x4) mm) of a water-filled plastic phantom. The field of view's (FOV) of these images were rotated around the phase encoding axis in increments of 180/24 degrees. Isotropic HR images were reconstructed from various numbers of LR images on a (1x1x1) mm grid using SRR. Effective resolution and SNR were measured on the reconstructed images and plotted against the number of LR images used for the reconstruction, in effect denoting the acquisition time. These plots reveal the relations between resolution, SNR and acquisition time. To compare reconstructed HR images with direct HR acquisitions, a set of six HR images ((1x1x1) mm), all with identical FOV, was acquired. Averaging one to six of these, the resolution and SNR of direct HR acquisitions could be measured versus the number of averages, in effect the imaging time, enabling comparison with the reconstructed images. Furthermore, direct acquisitions of isotropic resolutions (1.2 - 1.8 mm) were made. The SNR and effective resolution measured in these were extrapolated in the time dimension according to well-known theoretical relationships.

Results: Contrary to previous results [2], our experiments show that SRR cannot surpass the isotropic resolution of direct acquisition. However, we find that the SRR images achieve a resolution that is only slightly worse than that of the direct acquisition, while having significantly higher SNR.

Conclusions: Using SRR, the trade-off between resolution, SNR and acquisition time can be manipulated, and better trade-offs can be achieved than by direct acquisition. The potential of SRR to improve actual quantitative analyses in in-vivo molecular imaging experiments is yet to be explored.

Acknowledgements: This work was supported by the Medical Delta (HST-klein project) and by the European Commission in the Seventh-Framework Programme (ENCITE project).

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YIA applicant

DETECTION OF SINGLE MIGRATING DENDRITIC CELLS *IN VIVO* BY MRI

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Introduction:Dendritic cells initiate adaptive immune responses by presenting antigens to naive T cells in the T cell area of lymph nodes and the spleen. In certain cancer vaccines, autologous dendritic cells are used to to immunize patients against their own tumor cells, to generate therapeutic tumor immunity; tracking of these cells would be of great interest. Magnetic nanoparticles are often used as MR contrast agents; they provide strong T2* contrast, visible as hypointensities. Negative contrast is inherently problematic, as a relatively homogenous, strong background signal is required in order to visualize negative signal. When cells sufficiently labeled with magnetic nanoparticles are introduced into a magnetic field, their magnetic moment distorts the field into which they were introduced. The dimensions of this distortion markedly exceeds the dimensions of the cell - at 7T it extends up to 400 µm, well over 30 cell diameters. We show here a novel method using these distortions to overcome the lack of specificity of single-cell detection by MRI based on negative contrast.

Methods: For phantom experiments, HeLa cells were labeled with iron nanoparticles and suspended in low-melting point agarose in NMR tubes; NMR tubes were placed in a falcon tube filled with agarose. All agarose was degassed by sonication and vacuum. Dendritic cells were purified from mouse spleens by immunomagnetic selection, labeled with iron nanoparticles, and injected into the footpads of C57/Bl6 mice with LPS. Popliteal lymph nodes were imaged by MRI at time points from 12 to 48 hours post-injection. After MR imaging, mice were sacrificed and lymph nodes were fixed in paraformaldehyde, cryopreserved, embedded in OCT and frozen for histological sectioning. Frozen sections were cut and stained for iron content with prussian blue staining. MRI was performed on a 7T Bruker Pharmascan, using a volume coil for phantom experiments and a surface brain coil for in vivo experiments.

Results: Initial experiments with cell phantoms showed that individual spots at the expected density of single cells can be detected, with both HeLa cells and primary mouse dendritic cells. Post-processing of the MR images yields characteristic figures; cells are recognizable in the unprocessed images, but their visibility is dramatically enhanced by post-processing. An equal concentration of positively selected, unlabeled cells yielded no detectable signal. We purified dendritic cells from C57/Bl6 mice, labeled them with iron nanoparticles, and injected labeled cells into the footpads of littermates; contralateral footpads received LPS. Characteristic figures were observed in processed images, with no signal detected in uninjected or LPS-injected lymph nodes. Our post-processing is thereby more specific for labeled cells as compared to the unprocessed images, which necessarily rely on negative contrast.

Conclusions: By imaging the magnetic field distortion induced by iron oxide nanoparticles, it is possible to detect single dendritic cells in vitro and in vivo, even though the cells themselves are markedly smaller than the individual voxels. Additionally, these distortions are more specific for labeled cells than hypointensity-based negative contrast.

Acknowledgements: Thanks to Susanne Mueller for technical assistance with MRI.

YIA applicant

A BIMODAL TIME DOMAIN FLUORESCENCE AND ULTRASOUND SYSTEM FOR PROSTATE CANCER DIAGNOSIS

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Introduction The protocol for prostate cancer diagnosis is currently based on PSA determination followed by ultrasound guided biopsies. Their lack of specificity may lead to an increase of biopsies collection. To make this protocol more efficient and less invasive, a bi-modal [1] approach was recently proposed by our team, combining time resolved optical and ultrasound measurements. To bring such a bimodal system, and especially the combination of a time resolved imaging chain with ultrasounds, to the clinic is challenging. Therefore, a dedicated NIR pulsed laser source, a bimodal endorectal probe and a fast acquisition chain were designed to meet compactness, laser safety, sterilization requirements while providing accurate time-resolved measurements.

Methods The laser (775 nm, 10 ps, 50 MHz) was based on the telecom technology of doped fiber amplification. Six excitation fibers and four detection fibers located on the head of an ultrasonic probe constitute the optical module. This module collects the diffusion and fluorescence signals and drives them to a fast parallel TCSPC detection system. The fluorescence yield was reconstructed by processing intensity and the mean time of flight of each signal computed from the acquired set of time-resolved signal of source-detector combinations. To deal with the 3000 vertices and 15000 tetrahedrons required to mesh the prostate, adapted prior must be added to obtain a unique solution. This was done by using a matching pursuit algorithm which implements sparsity conditions, and provides spatial localization in less than one minute.

Results To evaluate the overall performance of the system in dealing with a realistic background signal, measurements and reconstructions were conducted on a combination of phantoms and small animals with ovarian tumors. High accuracy was shown on localization of a 45 µL fluorescent inclusion of ICG in a prostate mimicking phantom. We obtained a spatial accuracy better than 0.15 cm in all directions and a precision around 0.1 cm. The optical components are capable of withstanding the sterilization procedures thanks to the addition of a protection layer covering the probe. The design of this layer has a limited impact on the signal-to-noise ratio. The performance of the laser source has been shown comparable to that of a standard laboratory Ti:Sa laser. Finally, combined with the TCSPC acquisition system, the overall system was capable of acquiring fluorescence data in less than one minute.

Conclusions A novel device for time-resolved fluorescence imaging of the prostate was presented. The millimetric resolution of the reconstructed fluorescence map is compatible with the size of early stage tumors. Phantom and preclinical results showed the capability of the system to localize a fluorescing inclusion in vivo and up to a depth of 2.5 cm. Currently several solutions are explored to push further the depth of investigation.

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YIA applicant

PHOTOACOUSTIC IMAGING OF TUMOR VASCULATURE IN SMALL ANIMALS

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Photoacoustic tomography (PAT) is a relatively new high resolution imaging modality in the field of biomedical research. It relies on irradiating the biological tissue with a short laser pulse that is absorbed by hemoglobin and other chromophores in tissue causing thermoelastic expansion, which produces broadband pulses (MHz) of acoustic energy. These pulses propagate to the tissue surface and are detected by an array of ultrasound detectors. By applying various reconstruction schemes with prior knowledge of the tissue ultrasound-transmission parameters, the acoustic signals can be back-projected to a 2D tomographic image of internally distributed photoacoustic sources. Photoacoustic imaging does not suffer from limited resolution faced by optical imaging, while still using light as the probing energy. This allows combining the investigation of optical absorption contrast with the high resolution of ultrasound imaging.

In this work we present the design and development of computed tomography photoacoustic imaging system optimized for fast, and high resolution imaging of small animals. In addition to the conventional photoacoustic imaging system, we carefully positioned a 'passive element', a strong absorber of light with a small cross section (250 micro meter in diameter). Due to the photoacoustic effect ultrasound will be generated from the passive element. The generated ultrasound interacts with the sample and can be measured using the same ultrasound detector as used for photoacoustic measurements. Such measurements are made at various angles around the sample in a computerized tomography approach. Images of the ultrasound propagation parameters, attenuation and speed of sound, can be reconstructed by inversion of a measurement model. Simultaneously conventional photoacoustic imaging is also available in the instrument, since a majority of light goes on further to illuminate the object. We will present our latest measurements of a living mouse which has tumor implanted on the lower abdomen . A DEC (Dierexperimentencommissie) protocol was approved at the Erasmus Medical Center for the use of immunodeficient naked mice (NMRI nu/nu) in the studies. Tumor material from BLM non-pigmented melanoma tumors was transplanted to the donor animal in the flank. Photoacoustic imaging was performed in the imaging tank with water maintained at 37oC. The animal was kept under gaseous isoflurane anaesthesia (Rhodia Organique Fine Ltd., Avonmouth Bristol, UK) during the imaging experiments. The obtained results show that hybrid imaging of optical absorption (conventional photoacoustics) and ultrasound transmission parameters is valuable in visualization of the tumor location, size and shape.

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SIMULTANEOUS SUB-MILLIMETRE PET AND SPECT WITH A DEDICATED MULTI-PINHOLE GEOMETRY

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Introduction Non-invasive imaging at the molecular level is an emerging field in biomedical research. Here we introduce a new technology synergizing two leading molecular imaging methodologies: Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT). Both enable highly quantitative in vivo imaging of different sets of radio-labeled molecules providing complementary information about biological function.

Methods Simultaneous PET-SPECT was accomplished by a relatively simple hardware modification; a novel collimator containing clusters of narrow pinholes was incorporated in a stationary high-resolution animal SPECT device (U-SPECT-II, MILabs, The Netherlands [1]). The use of clusters of pinholes with narrow opening angles instead of traditional pinholes reduces resolution loss due to the strong pinhole edge penetration of 511 keV annihilation photons [2]. The novel collimator contains 48 clusters that consist of four pinholes each with diameters of 0.7 mm. Image resolution was determined with a Jaszczak hot capillary resolution phantom. In addition, we simultaneously acquired multiple functional data from mice that so far could only be obtained sequentially and with separate devices. Images were reconstructed using pixel-based OS-EM that corrects blurring due to limited system resolution and positron range.

Results For F-18 and Tc-99m, the smallest rods that could be resolved in the reconstructed resolution phantom images have a diameter of 0.8 mm and 0.5 mm respectively. Perfectly aligned images of PET and SPECT tracer molecule distributions in mice were acquired in a single scan. Resolution of PET mouse images is very competitive with coincidence PET while for SPECT tracers 0.5 mm resolution is still obtained.

Conclusion The high image resolution, the possibility of obtaining perfectly spatially and temporary aligned dual-tracer images, and the cost savings enabled by the fact that only a single scanner is needed for PET and SPECT may open unique research opportunities. Additional capabilities of this new technology include high resolution imaging of other high-energy single-photon emitters such as I-131.

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ZERO ECHO TIME (ZTE) MRI FOR EFFICIENT 19F-DETECTION IN MOLECULAR AND CELLULAR IMAGING

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Introduction: 19F MRI allows unambiguous and virtually background-free detection of contrast agents. It can be combined with the acquisition of high-resolution morphologic 1H images for coregistration using one imaging device. The critical point is the limited amount of 19F that can be administered. Therefore 19F MRI has mostly been used for labeling cells. Targeted molecular imaging has been demonstrated [1, 2], but is suffering from low signal.

Methods: The signal-to-noise (SNR) efficiency is limited by T2/T1. Gd-based contrast agents to shorten relaxation times are well established. Enhancing paramagnetic relaxation of fluorine to increase T2/T1 could considerably improve SNR efficiency. As an example, 19F relaxation times were investigated in solutions of trifluoroethanol (TFE) in water or agar gels containing different concentrations of Magnevist (Schering). The achievable SNR efficiency was determined. Practically, this approach is limited, since e.g. for perfluorocarbon nanoparticles, adding Gd showed only small effects [3]. To use this approach for molecular imaging, paramagnetic ions need to be combined with 19F. Accordingly, molecules that feature paramagnetic ions in close proximity to the nucleus of interest have been suggested in a range of fluorinated lanthanide complexes [4]. When the T2/T1 ratio is favourable, but the relaxation times are very short (in the range of 1ms), ultrashort (UTE) or zero echo time (ZTE) sequences can be used to efficiently sample the rapidly decaying signal. For a fast-relaxing compound (Cu(II)-hexafluoroacetylacetonate), SNR-efficiency using UTE/ZTE was determined and compared to measurements on TFE using UTE/ZTE and fast gradient echo sequences (FID-FISP). Each scan was optimized for SNR efficiency. To make these measurements directly comparable, same amounts of 19F and same acquisition times were used.

Results: For aqueous TFE solution, the T2/T1 ratio increased from 0.3 to 0.8 when adding up to 35 mM Gd, and agar gel from 0.15 to 0.74. SNR increased by a factor of approx. 5 in both cases, confirming the feasibility of our approach. 19F in the Cu complex with T1 ~ 1.7 ms was not detectable by conventional MR sequences due to very fast T2 relaxation, but could be visualized using ultrafast imaging methods. An increase in SNR by a factor of 10 was achieved with ZTE compared to both ultrafast and conventional imaging on TFE.

Conclusion: UTE/ZTE sequences are used to visualize materials when relaxation times are too short for conventional imaging. Applied to 19F MRI, a considerable advantage in SNR-efficiency can be achieved on compounds with a high T2/T1 ratio. For a constant 19F amount, a tenfold increase in SNR has been observed when a paramagnetic ion was in close proximity to the 19F nucleus. This seems to be a promising way to considerably lower the amount of 19F necessary for detection and to make targeted 19F imaging feasible in vivo.

Acknowledgement: Financial support from the DFG (SFB656, Z2) and the EU FP7 project ENCITE is gratefully acknowledged.

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