abstracts

Hot Topics in Molecular Imaging – TOPIM 2011

Emerging Imaging Methods in Medicine

January 16 – 21, 2011 in the
Ecole de Physique des Houches, France

THE „ECOLE DE PHYSIQUE DES HOUCHES“ IN LES HOUCHES...

...is a resort village in the Chamonix valley in the French Alps. Established in 1951, the Physics School is located in a group of chalets surrounded by meadows and woods, at an altitude of 1150 m facing the Mont-Blanc range - a very favourable environment for intellectual activity in ideal surroundings for skiing, hiking or mountaineering. The Mont Blanc is the highest mountain in the Alps, Western Europe and the European Union. It rises 4,810.45 m above sea level and is ranked 11th in the world in topographic prominence.

Ecole de Physique des Houches

La Côte des Chavants

74310 Les Houches, France

+33 4 50 54 40 69

HOW TO GET TO LES HOUCHES

By plane: Geneva Airport is 1 hour drive from Les Houches. The simplest way is to use a shuttle service (approximately 25€ up to the school, book at least three days in advance). Please find here an overview of the different providers:
www.chamonix.net/english/transport/transfers.htm

There is also a regular bus service between Geneva and Les Houches (max. twice a day). You should then take a taxi for the last 5 km from the Les Houches village to the school. You can also travel from Geneva to Les Houches by train (+ taxi from the train station to the School), but it is rather complicated and long (go through Annemasse on the French side or through Martigny on the Swiss side).

By train: arrival at the Les Houches station, with one change at Saint-Gervais (from France), or at Martigny (from Switzerland). There are about 10 trains per day between St Gervais and Les Houches. Then we strongly recommend to take a taxi and to pre-reserve by calling one of the following providers: taxi Garny: +33 6 12 35 30 72, taxi Persault : +33 4 50 54 41 09 and taxi Servoz : +33 6 84 66 86 73 to go up to the school ~5km.

By car: Les Houches are easily accessible from France (A41 highway), from Switzerland (Martigny and Col des Montets) and from Italy through the Mont Blanc Tunnel.

From Geneva and Le Fayet: 8km before Chamonix, 300 m after passing under the tunnel, bear right by the first road out for “Les Houches Bellevue”. When arriving at the cable car station “Bellevue”, turn right and continue upwards (roughly 2 km starting from the teleferic). 500m after the cable car station “Prarion”, turn left and follow small arrows at crossroads. Continue up to the end of Route de la Côte des Chavants. Here you are! From Chamonix: bear right for “Les Houches-Chef-Lieu”, turn right in Les Houches, and go ahead at the cable car station “Bellevue”. Then proceed as above.
Winter equipment for the car is recommended (snow chains). Please park your car at the parking place above the Ecole de Physique.

AT THE ECOLE DE PHYSIQUE DES HOUCHES

Arriving is possible on **Sunday January 16, 2011 from 3:00 pm** onwards. Please note that the school is closed before 3:00pm. Within the foyer of the main building you will find a plan of the allocation of rooms as well as the instructions how to get in the chalets. Participants are housed in individual “chalets” (electricity: AC, 50Hz, 220V). Someone from the school will be on-site on Sunday January 16 from 6:00pm to 7:30 pm but arrival is possible from 3:00pm onwards. The first joint meal will be the dinner at 7:30pm.

Please ensure that you are well equipped…it is winter in LesHouches: please bring your boots and warm clothes. Do not forget your flashlight; the school chalets are scattered on the mountainside and outside lights may be poor.

Breakfast is from 8:00 am to 8:45 am; **Lunch**: at 12:30 pm; and **Dinner**: 7:30 pm
Meals are taken at the school dining room. Drinks are not included. Coffee, tea (free!) and other drinks are available at the cafeteria.

WINTER SPORTS IN LES HOUCHES

“You will find it all in Les Houches: Alpine skiing, cross country, snowboarding, freestyle, telemark, speed-riding...”

For any enquiries in regards to the rental of ski equipment and/or an instructor, prices for a ski pass, ski maps, or the opening of skilifts please visit: www.leshouches.com/en/winter_ski-2-en-7.aspx

ÉCOLE DE PHYSIQUE
des HOUCHES

TOPIM 2011 – THE FIFTH HOT TOPICS IN MOLECULAR IMAGING WINTER CONFERENCE OF THE ESMI

Emerging Imaging methods in Medicine

Dear Participant,

"*The history of the living world is the elaboration of ever more perfect eyes within a cosmos in which there is always more to be seen*". Today, Teilhard de Chardin's prophecy is a truism for biologists' and physicians' ears. Images are so essential to the daily practice of medicine that it is difficult to imagine a blind practitioner; who would recognize a biology lab without a microscope or a screen display? Who remembers what neurology was like before MRI, or cell biology before the invention of the confocal microscope? Imaging fosters new methods at an ever increasing rate and has revolutionized biomedical sciences. Two words typify this revolution: *in vivo*. From high resolution opto-acoustics, low cost portable imaging magnets, intra-operative fluorescence molecular imaging approaches, to the development of hybrid systems such as MR-PET and MR-SPECT, the availability of new techniques, tools and instruments that can image life in real time and non invasively is more abundant than ever before. In parallel the utility and clinical propagation of such new methods go, well beyond their diagnostic utility, into improving surgical outcome, monitoring infection and inflammation and providing a new set of end points for therapeutic decisions. TOPIM 2011 aims in bringing together leaders in emerging imaging developments to present these techniques together with outlooks of their potential in improving the outcome and reducing the cost of healthcare.

The European Society for Molecular Imaging (ESMI) was created to sustain durably the actions initiated by the European Networks of Excellence beyond the end of their funding. ESMI (http://e-smi.eu) was created in July 2005 by prominent European actors from Academia, Biotech and Industry as a non-profit and apolitical society to promote the development of molecular imaging within Europe, fosters co-operation and provide durable integration. ESMI is committed to scientific and technical excellence, to innovation for better healthcare, to promotion of knowledge and to interdisciplinary co-operation.

TOPIM (for "hot TOPics in IMaging") is one of the major actions initiated by the ESMI.. Rather than proposing a catalogue of recent releases attempting to cover all aspects of the burgeoning imaging field, TOPIM concentrates on one aspect at the forefront of the discipline and selects a different "hot topic" every year, chosen according to pertinence and timeliness. The objective is to concentrate on the newest achievements of in vivo imaging research presented by key actors invited together with students. In its unique format, TOPIM fills the gap between classical meetings, open to all but with scarce discussion timeslots, and prospective meetings that concern small circles of experts out of which stem general ideas or recommendations.

TOPIM'07, the first conference of this kind, held February 19-23, 2007, on the hot topic: Imaging in Neuroinflammation was a great success.

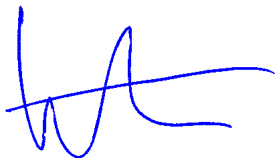
So were **TOPIM'08**: Imaging of nano-objects, held February 4-8, 2008,

TOPIM'09: Dual and Innovative Imaging Modalities (January 26-30, 2009) and

TOPIM'10: "Imaging and Systems Biology" (February 7-12, 2010).

The upcoming session, **TOPIM'11** will launch the debate on **Emerging Imaging Methods in Medicine**.

In the second decade of this millennium, time has come to clarify how and what imaging actually delivers to medicine. What role do images play in diagnosis? What do they tell us about the extension of a cancer or the severity of a neurological syndrome? How do they affect treatment planning? Is imaging capable to assess treatment efficiency? Can one rely on images to establish, modify or adapt the therapeutic strategy? What exactly are those "new eyes" that now guide the hands of surgeons? Can we identify vulnerable atheroma plaques at risk? How are the new imaging contrast agents and tracers contributing to the understanding "*in terms of physics and chemistry,* [of] *those processes by which we live, by which we become ill, by which we are healed and by which we die*" (Claude Bernard 1865)? How far do we stand from the sacred Graal of medicine, the so-called "theragnostics" bridging therapy and diagnostics simultaneously? How can the electromagnetic and mechanical energies forming the basis of image detection be used to selectively attack and destroy lesions while sparing normal surrounding tissues? Will "imagenetics" succeed in using these energies to force expression of specific genes in specifically targeted regions of a living organism? Will medicines find a way to take advantage of the clocks that rhythm our lives? A myriad of questions burn our lips and we want to hear what the specialists that have begun tackling those issues have to tell us.



Bertrand Tavitian on behalf of the
TOPIM'11 Committee

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	Monday, 17th January	Tuesday, 18th January
08:00 - 08:30	**BREAKFAST**	
08:30 - 09:10	Imaging is changing medicine: the TOPIM conferences **Bertrand Tavitian (Orsay, France)**	Radiopeptides in oncology **Marion de Jong (Rotterdam, The Netherlands)**
09:10 - 09:55	PET for treatment planning and monitoring in radiation oncology, **Nicole Wiedenmann (Freiburg, Germany)**	New Imaging Probes for PET and SPECT Based on Radiometals, **Helmut Maecke (Freiburg, Germany)**
09:55 - 10:15	**coffee break**	
10:15 - 11: 00 oral abstract presentations	[18F]fallypride binding in the mouse brain: test-retest and effects of registration, **Alain Seret (Liège, Belgium)**	Radiolabelled cholecystokinin-2 receptor binding analogues; preclinical and clinical evaluation, **Marleen Melis (Rotterdam, The Netherlands)**
	Molecular Imaging of MR-HIFU Thermally Ablated Tissue, **Nicole Hijnen (Eindhoven, The Netherlands)**	Oligoprolines as multivalent scaffolds for tumor targeting vectors, **Carsten Kroll (Basel, Switzerland)**
	In vivo study of cerbral ischemia using multimodal ultrafast ultrasound imaging, **Emilie Macé (Paris, France)**	Real-Time Monitoring of Tumor Vascular Occlusion by Vascular Targeted Photodynamic Therapy, **Noa Madar-Balakirski (Rehovot, Israel)**
11:00 - 11:40	Focused Ultrasound mediated Targeted Drug Delivery, **Chrit Moonen (Bordeaux, France)**	Light-activated focal vascular occlusion of tumor blood supply: A novel cancer therapy approach, **Yoram Salomon (Rehovot, Israel)**
11:40 - 12:20	New Perspectives in Biomedical Ultrasound, **Mikael Tanter (Paris, France)**	Advanced Imaging techniques for guiding Radiotherapy and follow-up in Gliomas, **Frederic Dhermain (Paris, France)**
12:20 - 13:20	**LUNCH**	
13:20 - 16:45	FREE TIME SLOT	
16:45 - 17:30 oral abstract presentations	Ultrasound-inducible fluorescent particles for internal tattooing, **Olivier Couture (Paris, France)**	In vivo imaging of Akt1 signaling in angiogenesis. **Katrien Vandoorne (Rehovot, Israel)**
	Carotid artery plaque characterization by contrast-enhanced ultrasound, **Filippo Molinari (Torino, Italy)**	Annexin-based apoptosis imaging: pitfall in the assessment of anti-angiogenic therapy? **Wiltrud Lederle (Aachen, Germany)**
	Simultaneous estimation of input functions: The B-SIME method, **Camille Jouvie (Orsay, France)**	Molecular CT imaging of cancer using targeted gold nanoparticles, **Rachela Popovtzer (Ramat Gan, Israel)**
17:30 - 18:10	Circadian timing of cancer treatments, **Francis Levi (Vilejuif, France)**	Multi-modal (MRI/PET/ALA-OI) imaging-based neurosurgery of gliomas, **Christian Ewelt (Münster, Germany)**
18:10 - 18:50	Molecular imaging of in vitro interactions between circadian clocks, metabolism and cell cycle, **Sandrine Dulong (Vilejuif, France)**	Imaging guided transcranial magnetic stimulation, **Berthold Langguth (Regensburg, Germany)**
	Image quality evaluation for 124I in the microPET focus 120 scanner using the NEMA NU4-2008 phantom, **Alain Seret (Liège, Belgium)**	
18:50 - 19:30	**19:00 Pot d'accueil**	Ultrasound induced Drug Delivery, **Holger Gruell (Eindhoven, The Netherlands)**
19:30 - 20:30	**DINNER**	

Wednesday, 19th January	Thursday, 20th January	Friday, 21th January
BREAKFAST		
Functionalized nanoparticles for biomolecular imaging and sensing, **Raphael Levy (Liverpool, United Kingdom)**	Imaging tumours with hyperpolarized MRI, **Kevin Brindle (Cambridge, United Kingdom)**	Application of imaging in the drug development process: advantages and limitations from an industria, **Werner Scheuer (Penzberg, Germany)**
Molecular imaging of atherosclerotic plaques, **Fabien Hyafil (Paris, France)**	Imaging intra and inter-cellular signaling in vascular remodeling, **Michal Neeman (Rehovot, Israel)**	Investigating chronic liver diseases and cancer by multimodal spectroscopy, **François Le Naour (Paris, France)**
coffee break		
Pharmacokinetics and biodistribution in FVB mice of radioactive and fluorescent triply-labeled lipid, **Juliette Merian (Grenoble, France)**	Hyperpolarized 13C MRS detects early changes in tumor metabolism following anti-angiogenic therapy, **Sarah Bohndiek (Cambridge, United Kingdom)**	Intraoperative immunophotodetection to improve peritoneal surface malignancy diagnosis and treatment, **Andre Pelegrin (Montpellier, France)**
Image guided surgery by means of near-infrared fluorescence, **Christiane Wenk (Grenoble, France)**	6 Years longitudinal MRI and 1H single voxel MRS follow-up in 26 Patients with oligodendroglial tumor, **Jean-Marc Constans (Caen, France)**	New multispectral imaging method for fluorescence molecular diagnostics, **Athanasios Sarantopoulos (Munich, Germany)**
In vivo gene delivery and imaging of NF-kB regulation pathway in acute lung inflammation mouse model, **Fabio Stellari (Parma, Italy)**	Magnetic resonance imaging permanent magnets, **Dimitris Sakellariou (Gif sur Yvette, France)**	Near-Infrared fluorescence imaging of colorectal liver metastases using indocyanine green, **Joost van der Vorst (Leiden, The Netherlands)**
Bimodal ultrasound/optical instrumentation and fluorescent molecular probes to improve prostate cancer diagnosis, **Isabelle Texier (Grenoble, France)**	Multifunctional Nanoparticles for MR imaging, **Fabian Kiessling (Aachen, Germany)**	Application of fluorescence in surgery; expectations and realistic approaches, **Philippe Rizo (Grenoble, France)** High resolution real-time imaging of small animals by Multispectral Optoacoustic Tomography (MSOT), **Adrian Taruttis (Munich, Germany)**
Targeted Nanoparticles, optical imaging and cancer, **Jean-Luc Coll (Grenoble, France)**	New perspectives for imaging molecules and metabolism in vivo. **Rolf Gruetter (Lausanne, Switzerland)**	Listening to light and seeing better, **Daniel Razansky (Munich, Germany)**
LUNCH		**CONCLUSION** **LUNCH / Departure**
FREE TIME SLOT		
Ultrasound induced mechanical release from paramagnetic liposome for MRI new drug delivery protocols, **Pierangela Giustetto (Torino, Italy)**	Hyperpolarized acetate as a metabolic tracer for skeletal and cardiac muscle energetics, **Jessica Bastiaansen (Lausanne, Switzerland)**	
MRI-guided HIFU-mediated drug delivery to tumors with temperature-sensitive liposomes, **Mariska De Smet (Eindhoven, The Netherlands)**	Shining a new light on brain glucose metabolism using synchrotron x-ray fluorescence imaging, **Carole Poitry-Yamate (Lausanne, Switzerland)**	
Focused ultrasound induced extravasation: in vivo SPECT/CT imaging and quantification, **Pedro Sanches (Eindhoven, The Netherlands)**	Optical imaging of oral squamous cell carcinoma and cervical lymph node metastasis, **Pieter van Driel (Leiden, The Netherlands)**	
Nanomedicine, theranostics and image-guided combination therapies, **Twan Lammers (Aachen, Germany)**	Translational optical imaging in oncology: fact or fiction, **Gooitzen van Dam (Groningen, The Netherlands)**	
Nanoprobes for imaging drug release, **Enzo Terreno (Torino, Italy)**	Image guided surgery of sentinel lymph nodes and solid tumors, **Alexander Vahrmeijer (Leiden, The Netherlands)**	
ENCITE - open discussion on Molecular Imaging	Surgical fluorescence imaging with targeted fluorochromes: advancing towards clinical use, **George Themelis (Munich, Germany)**	
DINNER	**"Repas savoyard"**	

SCIENTIFIC PROGRAMME COMMITTEE – TOPIM'11

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THANKS FOR YOUR SUPPORT

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the European Network for Cell Imaging and Tracking Expertise

ENCITE

SEVENTH FRAMEWORK PROGRAMME

day one: monday january 17, 2011

18F-FLUOROMISONIDAZOLE PET TO ASSESS TUMOR HYPOXIA DURING RADIOCHEMOTHERAPY FOR HEAD AND NECK CANCER

Wiedenmann, N. [1] ; Hentschel, M. [1] ; Bucher, S. [1] ; Mix, M. [2] ; Adebahr, S [1] ; Offermann, C. [1] ; Nestle, U. [1] ; Weber, W. [2] ; Grosu, A.L. [1]
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Introduction: Tumor hypoxia is a common feature in locally advanced tumors of the head and neck that is correlated with a more malignant tumor phenotype as well as increased resistance to radiation therapy. Invasive oxygenation measurements by Eppendorf probes have found tumor hypoxia to be associated with inferior survival after radiation treatment [1]. Radiobiological research has shown fractionated radiation treatment to have the potential to alleviate tumor hypoxia over the treatment course (tumor reoxygenation) which has also been identified as an important factor for treatment success.However, serial assessment of tumor oxygenation during fractionated radiation treatment is scarce. The hypoxia marker [18]F-fluoromisonidazole (F-MISO) allows non-invasive PET imaging of tumor hypoxia and is currently used in clinical trials. The purpose of the present study was to assess the ability of F-MISO PET to noninvasively visualize the time course of tumor hypoxia during radiation therapy and to correlate the PET findings with treatment outcome.

Methods: A prospective serial imaging study was conducted in 16 patients undergoing definitive radiochemotherapy (RCTx) for locally advanced head and neck cancer. Radiation treatment was delivered to a total dose 70 Gy. Cisplatin was administered in week 1, 4, and 7. CT, MRI and 18F-FDG-PET were acquired prior to treatment and MRI was repeated in week 5. Tumor hypoxia was visualized by F-MISO-PET at baseline and in treatment week 2 (22 +/-7.5 Gy) and 5 (56 +/-8.6 Gy). Static F-MISO scans were acquired 2.5h p.i. Study endpoints were the visual and quantitative assessment of F-MISO tumor uptake and local tumor control. F-MISO uptake was visually scored from 0 (no hypoxia) to 3 (significant hypoxia). The index of SUVmax (tumor) / SUVmean (muscle) was calculated for each patient. Mean and median indices for all patients were quantified for the first (pre-treatment), second (week 2) and third (week 5) F-MISO PET investigation. Tumor volumes were determined for FDG PET scans and the coregistered F-MISO scans. For follow-up patients were evaluated clinically and by MRI every 3 months.

Results: Mean summed tumor and lymphnode volumes per patient were determined by FDG PET as 26.5 +/- 22 ml. The mean SUVmax indices decreased from 1.9 (pre-treatment) to 1.6 (wk 2) and 1.3 (wk 5). Mean hypoxic tumor volumes declined from 15.8 ml (baseline) to 6.3 ml (wk 2) and 0.4 ml (wk 5). At a mean follow up time of 10 months local failure or absence of tumor response was observed in 4 pts., distant failure was seen in 1 pt. No local recurrence was seen when the last F-MISO scan indicated complete tumor reoxygenation at the end of treatment.

Conclusions: Significant reduction of tumor hypoxia was found in the majority of patients. The current follow-up data indicate a correlation between persistent tumor hypoxia visualized by F-MISO PET and inferior outcome.

References: Nordsmark et al. (2005). Prognostic value of tumor oxygenation in 397 head and neck tumors after primary radiation therapy. An international multi-center study. Radiother Oncol 77(1)

[18F]FALLYPRIDE BINDING IN THE MOUSE BRAIN: TEST-RETEST AND EFFECTS OF REGISTRATION

Bahri, M.-A.; Geuzaine, A.; Warnock, G.; Goblet, D.; Tirelli, E.; Lemaire, C.; Seret, A.; Luxen, A.; Salmon, E. ; Plenevaux, A.
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Introduction: The quantification of in vivo receptor kinetics with PET tracer experiments is an intricate and challenging problem especially for small animals such as rats and mice. A test-retest scan is usually set up in order to confirm an observed experimental effect or to examine the reliability of the experiment design. Inadequate processing of the image data may also mask small effects. The purpose of this study was to investigate the effect of image registration on [18F]fallypride binding potentials calculated from PET mouse test-retest data.

Methods: Seven C57BL/6J male mice (9-14 weeks) were scanned under isoflurane anaesthesia for 60 min (list mode) with the Siemens Focus 120 microPET scanner after [18F]fallypride injection (17-25 MBq iv). Subsequent PET recording sessions, identical to the first, were obtained in individual mice at an interval of 7-10 days. Dynamic data were reconstructed using filtered backprojection with all corrections except for scatter. Since there is little anatomical information in the [18F]fallypride images, the average of the first five minutes of the scan was registered to a high-resolution MRI mouse template [1].

Registration was performed in three different ways: Reg1: all images were registered manually to the MRI template; Reg2: test images were registered manually to the MRI template and then an automated rigid matching was applied to register retest images to test images; Reg3: an [18F]fallypride template was created in the same space as the MRI template, and all test retest images were automatically registered to this [18F]fallypride template. Time activity curves (TACs) were extracted for striatum and cerebellum using predefined volume of interest (VOI) templates. Spherical VOIs (2-mm diameter) positioned manually in the striatum and cerebellum were also used to generate individual TACs. Binding potentials (BPND) were calculated with simplified reference tissue model (SRTM) [2] using the cerebellum as reference tissue. Image processing and kinetic analyses were performed using PMOD 3.1 software (PMOD Technologies Ltd, Zurich, Switzerland). Binding potentials obtained for test-retest with the different registration methods were compared in terms of variability.

Results: Reg1, Reg2 and Reg3 produced comparable results with BPND values of approx. 2.5 with a test-retest mean percent variability of 14.4±8.5, 13.2±8.5, 16.0±7.3, respectively. However, the sphere method produced a higher BPND of approx. 4 with a mean variability of 12.2±8.3.

Conclusions: Sub-optimal registration affected the quantification of in vivo receptor kinetics with [18F]fallypride. The absence of anatomical information in the [18F]fallypride image and the lack of a homogeneous tracer distribution, even during the earlier minutes of the scan, lead to erroneous automatic registration. A FDG scan after each [18F]fallypride test could improve registration as FDG provides a more homogeneous brain image. Variability in the data could also result from stress induced by anaesthesia or the experimental environment.

References:[1] Ma et al. (2005) Neuroscience 135:1203-1215; [2] Lammertsma et al. (1996) Neuroimage 4:153-158.

MOLECULAR IMAGING OF MR-HIFU THERMALLY ABLATED TISSUE

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Introduction: Magnetic Resonance guided High Intensity Focused Ultrasound (MR-HIFU) is one of the promising techniques for controlled non-invasive tumor treatment by selective tissue heating deep inside the human body. Tumor thermal ablation with MR-HIFU is currently introduced to the clinic to treat both benign (e.g. uterine fibroids) and malignant tumors (e.g. breast). Treatment of malignant tumors is even more critical as it requires complete coverage including tumor border zone treatment with high spatial and thermal precision to prevent local reoccurrence. The clinical translation of this technique requires sensitive, non-invasive methods for tumor margin assessment post treatment [1]. Our work aims to develop thermal ablation protocols for malignant tumors in rats and to subsequently evaluate treatment outcome using nuclear imaging techniques. We adapted a clinical MR-HIFU system for treatment of small animals to allow a rapid translation of the developed protocols to the clinic. Here, we present controlled thermal ablation of malignant tumors in rats using a clinical MR-HIFU system and a first treatment evaluation using MRI, PET and histology.

Methods: Tumors were inoculated subcutaneously on the hind limb of rats (9L rat glioma). All animals were positioned in a dedicated small animal 4-channel MR-HIFU volume coil that was used as add-on to a clinical 3T MR-HIFU system (Sonalleve, Philips Healthcare, Vantaa). The target area was volumetrically heated with continuous wave ultrasound (1.44 MHz) to T > 328 K for 30 seconds (n=5, Pac=30 W). Temperature changes were monitored near-realtime using proton resonance frequency shift thermometry. The direct treatment effect was monitored with dynamic contrast enhanced (DCE-) MRI using Gd-DTPA. [F-18]FDG PET imaging was preformed for sensitive, quantitative therapy monitoring at different time intervals post treatment. Excised tumors were further evaluated in histology.

Results: The target temperatures were readily achieved while changes in body temperature remained less than 1 K. From the change in uptake kinetics [2] of the Gd-DTPA pre and post ablation the volume of viable tumor was calculated, indicating an affected area of 371±123 mm3 (Δtissue volume with ktrans ≥0.04 min-1). PET [F-18]FDG imaging showed a strong decrease in metabolic activity of the treated tumor directly after treatment and FDG uptake in the remaining viable tumor tissue at the border zone. Furthermore, both T2-weigthed MR and [F-18]FDG PET showed inflammation of the muscle tissue surrounding the tumor. The treatment outcome was further assessment with histology using NADH-diaphorase staining, which showed a sharp demarcation between viable and non-viable cells.

Conclusions:We demonstrate that ablation treatment in small animals can be performed on a clinical MR-HIFU system. Residual tumor tissue can readily be detected directly post HIFU treatment with PET 18F-FDG imaging. Future work will be devoted to establish a set of nuclear imaging biomarkers to monitor thermal therapies, with an initial focus on differentiating residual tumor mass from inflammation.

Acknowledgement: This research was supported by the Center for Translational Molecular Medicine (VOLTA).

References:1) Schmitz et al. J. Am. Col. Radiol. 2009; 2) Tofts et al. MRM 17, 1991

IN VIVO STUDY OF CEREBRAL ISCHEMIA USING MULTIMODAL ULTRAFAST ULTRASOUND IMAGING

Mace, E [1]; Martin, A [2]; Montaldo, G [1]; Tavitian, B [3]; Tanter, M [1]

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Introduction: Pathologies such as perinatal anoxia can induce severe cerebral ischemic lesions on newborns. Available modalities such as MRI or scanner can detect these lesions but sometimes only one week after birth, so there is the need for an earlier diagnosis. In this study, we investigated in vivo on a rat model of focal cerebral ischemia the potential of new imaging modes developed on ultrafast ultrasound scanners to detect ischemic lesions at an early stage by studying two parameters: the elasticity and the perfusion of brain tissue.

Methods Focal cerebral ischemia was obtained by the transient 2 hour occlusion of the middle cerebral artery in the right hemisphere of the rat brain [1]. For different times after the occlusion (control, 1, 2, 4 and 7 days, 4 animals per time), the rat was trepanned and both a Shear Wave Imaging and an Ultrafast Doppler scan were performed using an ultrafast ultrasound scanner. Shear Wave Imaging consists of generating a shear wave and tracking its propagation to map local brain stiffness [2]. Ultrafast Doppler is a completely new ultrasound mode providing maps of cerebral blood flows at very good spatial resolution (100 µm x 100 µm x 200 µm).

Results High resolution maps of brain elasticity were obtained in 3D for each animal using Shear Wave Imaging. The ischemic lesion was found to be softer compared to healthy brain tissues. Moreover, a constant decrease of elasticity was observed over time. This difference was significant 2 days ($p < 0.05$), 4 days ($p < 0.01$) and 7 days ($p < 0.001$) after occlusion compared to the control group. Ultrafast Doppler revealed an important increase in cerebral blood flow in the ischemic lesion compared to the control hemisphere. This increase in blood supply was observed only minutes after reperfusion. It peaks 48h after the occlusion (+46%, $p < 0.001$) and is still present at day 7 (+24%, $p < 0.05$). Here again, high resolution maps of brain vascularization were obtained in 3D. Ultrafast Doppler was also proved useful for monitoring the occlusion.

Conclusions Multimodal ultrafast ultrasound imaging is proved relevant for imaging ischemic lesions. New quantitative information on ischemic tissues was found: these damaged tissues are softening over time and are hyperperfused, particularly in the first 48h after the stroke. These results open wide prospects both in neuroimaging for making new findings in the physiopathology of cerebral ischemia and in clinics for improving the diagnosis of perinatal anoxia. Indeed, as planned in future work, these new imaging modes can be easily applied to brain imaging on newborns through the fontanel using portable ultrasound scanners.

References[1] C. Justicia et al, "Anti-VCAM-1 antibodies did not protect against ischemic damage either in rats or in mice." J Cereb Blood Flow Metab, vol 26, pp 421-32, no. 3, Apr 2006[2] J. Bercoff et al, "Supersonic shear imaging: a new technique for soft tissue elasticity mapping." IEEE Trans Ultrason Ferroelectr Freq Control, vol. 51, no. 4, pp. 396–409, Apr 2004

FOCUSED ULTRASOUND MEDIATED TARGETED DRUG DELIVERY

Moonen, C.

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Objectives: The primary goal is to increase the therapeutic index of potent, often toxic treatments through personalized image-guided treatment, ultimately decreasing adverse effects of drugs by better controlling the pharmacokinetics (PK) and pharmacodynamics (PD) of therapy. This is achieved by locally triggering the deposition or activation of drugs via image guided ultrasound triggers.

Introduction: Ultrasound can be focused within a region with a diameter of about 1 mm. The bio-effects of ultrasound can lead to local tissue heating, cavitation, and radiation force, which can be used for 1) local drug release from nanocarriers circulating in the blood, 2) increased extravasation of drugs and/or carriers, and 3) enhanced diffusivity of drugs. When using nanocarriers sensitive to mechanical forces and/or sensitive to temperature, the content of the nanocarriers can be released locally. Thermo-sensitive liposomes have been suggested for local drug release in combination with local hyperthermia more than 25 years ago. Microbubbles may be designed specifically to enhance cavitation effects. Real-time imaging methods, such as magnetic resonance, optical and ultrasound imaging have led to novel insights and methods for ultrasound triggered drug delivery. Image guidance of ultrasound can be used for: 1) target identification and characterization; 2) spatio-temporal guidance of actions to release or activate the drugs and/or permeabilize membranes; 3) evaluation of biodistribution, pharmacokinetics and pharmacodynamics; 4) Physiological read-outs to evaluate the therapeutic efficacy.

Methods: Thermosensitive liposomes have been suggested for local drug release in combination with local hyperthermia more than 25 years ago. Liposomes may carry both hydrophilic and hydrophobic drugs in their aqueous interior and lipid bilayer membrane, respectively. The circulation half-life may be increased by incorporating polyethylene glycol (PEG)-lipids in the bilayer. Nanoparticles may be designed specifically to enhance cavitation effects. Most microbubbles consist of air- or perfluorocarbon-filled microsphere stabilized by an albumin or lipid shell with a size in the range of 1-10 µm. Drugs can be attached to the membrane surrounding the microbubble, they can be imbedded within the membrane itself, they can be bound non-covalently to the surface of the microbubble and can be loaded to the interior of the microbubble, either in an oil or aqueous phase.

Results: Several recent publications have shown that ultrasound triggered delivery is feasible (reviewed by 1,2). Real-time imaging methods, such as Magnetic Resonance, optical and ultrasound imaging may lead to novel insights and methods for ultrasound triggered drug delivery. Image guidance of ultrasound (see e.g. ref 3) can be used for: 1) target identification and characterization; 2) temporo-spatial guidance of actions to release or activate the drugs and/or permeabilize membranes; 3) Evaluation of biodistribution, PK/PD; 4) Physiological read-outs to evaluate the therapeutic efficacy.

Conclusion: The bio-effects of (Focused) Ultrasound can be used for various aspects of local drug delivery and cellular uptake from circulating nanocarriers. MRI guided FUS is particularly useful in case of thermo-sensitive drug nanocarriers. Real-time ultrasound and optical imaging are leading to new insights to increase the therapeutic window with ultrasound.

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NEW PERSPECTIVES IN BIOMEDICAL ULTRASOUND

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The advent of ultrafast ultrasonic scanners paves the way to new imaging modalities for the screening and diagnosis of many human pathologies. By reaching very high frame rates of several thousands frames per second, Ultrasound enables to track in real time transient phenomena such as the propagation of mechanical vibrations in our organs. In particular, imaging shear waves propagation enables to map quantitatively the local stiffness of organs. Such shear waves are generated deep in our body using the concept of remote palpation induced by the radiation force of focused ultrasonic beams. Clinical applications of this new modality are growing extremely fast, from breast cancer diagnosis to liver fibrosis staging or tissue characterization in cardiovascular applications. Beyond quantitative elasticity mapping, ultrafast Ultrasound imaging permits to achieve very high sensitivity maps of Blood flows leading to the concept of ultrasonic angiography.

Finally, Ultrasound can also be used to destroy tissue and its potential interest for the non invasive treatment of brain disorders, Blood brain Barrier Opening, or improved drug delivery will also be highlighted.

ULTRASOUND-INDUCIBLE FLUORESCENT PARTICLES FOR INTERNAL TATTOOING

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Introduction: The spatial concordance between a lesion depicted in a radiological image and its localization in the operating room is still a major issue in therapy. As the sensitivity of medical imaging modalities is constantly improving, clinicians are now increasingly confronted with tumors that are detected by the imaging device but which remain invisible and unpalpable during surgery. Despite registration methods such as guiding wires, resecting those diseased tissues remains challenging. The objective of this study is to noninvasively deposit large quantities of optical markers at a precise location which could then be identified during surgery. We propose to use novel injectable droplets carrying important payloads of contrast agent and that are inducible by ultrasound. As opposed to conventional microbubbles or liposomal agents, the proposed carriers are formed as a double-emulsion. They are created by enclosing an aqueous nanoemulsion (2/3 of the volume) within a matrix of perfluorocarbon oil. Any hydrophilic marker or chemotherapeutic agent can then be carried to the site where the droplets are converted by ultrasound pulses. Moreover, the perfluorocarbon layer act as a barrier between the content of the droplets and the external medium, protecting it against undesired release, but also from chemical or enzymatic reactions.

Methods: In this study, we describe the fabrication and the characterization of composite droplets that are inducible by a clinical imaging scanner at 5 MHz. The droplets were monodisperse double emulsions produced in a microfluidic system, consisting of aqueous fluorescein in perfluorocarbon in water. Their conversion by ultrasound was both induced and measured using a clinical elastography scanner (Supersonic Imagine). The diffusion of the fluorescein after release was observed under blue illumination with a macrocamera. Ultrasound-triggered release was also observed in-vivo after the injection of the droplets in the chicken embryo.

Results: The composite particles are monodisperse with a diameter of 5 microns (±5%). The fluorescein-containing water represents about 70% of the particles' content and they are stable for more than 2 weeks. When submitted to 5 MHz imaging focused pulses, the droplets vaporize in-vitro at 1.4 MPa peak-negative pressure and eject their content. After several seconds, a brightly fluorescent dot (0.5 mm diameter) is observed at the focus of the transducer. Ultrafast ultrasound imaging (6000 images/seconds) showed an hyperechoic region after the conversion of the droplets. When the focus is moved electronically, the release of fluorescein allows painting of patterns within the opticell plate. Experiments in chicken embryo demonstrate that sufficient fluorescein can be released and accumulated to be observed under the naked eye.

Conclusion: Such internal tattooing under ultrasound guidance could become a precious tool for surgical resection of tumor. These ultrasound-inducible double emulsions could also be used to deliver large amounts of contrast agents, chemotherapy and genetic materials in-vivo using a simple ultrasound scanner. Adding targeting moieties, such as antibodies or toxin subunit, on the membrane of these carriers could improve additionally the specificity of the delivery.

CAROTID ARTERY PLAQUE CHARACTERIZATION BY CONTRAST-ENHANCED ULTRASOUND

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Introduction: MRI and CT are current standard techniques to in-vivo evaluate plaque morphology, but they are expensive and unsuitable for long-term surveillance and monitoring. Also, such techniques cannot provide insights relative to the plaque build-up process and associated inflammation state. To overcome the limited tissue discrimination of the conventional ultrasound imaging, we adopted a scan protocol based on contrast-enhanced (CEUS) images. Validation of the methodology was made against histological reporting of the plaque specimens.

Methods: Twenty carotid plaques relative to subjects elected for surgical endarterectomy were scanned. A small volume (about 1.5 ml) of contrast agent (SonoVue, Bracco, Italy) was injected intravenously and B-Mode images were acquired before and after injection. The portion of the wall corresponding to the plaque was automatically analyzed and color-coded intensity was assigned to a specific tissue. The rationale of this technique is that poorly perfused tissues (like lipids) show a lower contrast enhancement with respect to highly perfused tissues (such as fibrous and muscular tissue and inflammation regions). Color-code was assigned comparing of the echogenicity change from before to after contrast injection. Plaque specimens were cut into slices of 0.3 mm of thickness and H&E stained. An experienced pathologist reported each slice by a color code. Correlation of the histology color-coded report and of the CEUS analysis was performed.

Results: All 20 CEUS images were successfully processed by our automated technique. Plaque components that can be effectively identified are: thrombi, lipids, fibrous/muscular tissue, and calcium. Overall the errors on 20 plaques between automated classification and histology were: 3.1% ± 1.1% for thrombus, 4.2% ± 1.5% for lipids, 5% ± 3.4% for fibrous/muscular tissue and 3.2% ± 1.0% for calcium. This automated methodology showed maximal accuracy in the characterization of soft and unstable plaques, but limited performance on highly calcified plaques. Results also showed a high correlation between thrombus and inflammation state. In CEUS imaging, thrombus is characterized by a signal decrease from pre to post-contrast images.

Conclusions: Despite the need for further investigation and a quantitative 3-D evaluation of the results, this methodology showed encouraging results. Better discrimination of plaque composition was achieved by CEUS imaging, compared to conventional US imaging alone. Better discrimination of inflammation could be acquired by using specific molecular contrasts. This analysis architecture is undergoing validation in a Neurology Division and is aimed at being used for the follow-up of patients and quantification of drug therapy effects.

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SIMULTANEOUS ESTIMATION OF INPUT FUNCTIONS: THE B-SIME METHOD

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Introduction: Using Positon Emission Tomography for molecular imaging processes in vivo requires the knowledge of the arterial plasmatic activity concentration (PTAC). The reference method to obtain it is arterial sampling, not applicable in clinical studies because invasive and dangerous. Feng et al. [1] introduced the Simultaneous Estimation (SIME) method which deconvoluates the 18-FDG-three-compartments model. The present work proposes an improvement of the SIME through a bootstrap approach taking as input up to 23 structures, instead of 3 or 4.

Methods and material: Feng's SIME is the minimization of a cost function phi representing the differences between the estimated input function and the real one, plus the distance of the estimated input function to input venous blood samples (BS), if any. The Bootstrap-SIME method (B-SIME) consists in five steps: (A) From one set of n>3 kinetics, all possible sets of three different TTACs are built. (B) The 400 best-ranked sets according to the three following criteria are chosen: noise and Partial Volume Effect (PVE) affection, TTACs differences. (C) For each of the selected sets, a SIME is performed. (D) The final values of the analytical input function parameters are obtained by taking the weighted median of the values of the corresponding parameters in the results of C, the weights being equal to the inverse of phi at found minimum. The method was validated on two datasets: simulated and real datasets of the human brain. The simulated dataset was composed of 100 sets of 16 TTACs each generated using the three-compartment model and affected with realistic noise (1 to 20%). The real dataset included four healthy subjects with one T1-MRI image segmented into 16 structures and a PET image acquired on an Ecat HR+ PET system. Mean TTACs were extracted and corrected for PVE [2]. The figure of merit was the relative difference in area under curves (dAUC) between the estimated and reference curves.

Results: For simulated data, B-SIME (dAUC(1BS)=0,31xN+0,015, dAUC(noBS=0,58xN+0,077, where N is the noise percentage) achieved better performances than SIME (dAUC(1BS=0,32xN+0,045, while for dAUC(noBS) varied randomly between 1% and 53%). For real data, dAUC(1BS) was lower than 8% for three subjects over four for B-SIME, while for SIME dAUC(1BS) was greater than 70% . The mean gain using the bootstrap was 7.8.

Conclusions: The B-SIME method, improvement of the SIME, obtained good results for the estimation of the PTAC, especially when using one late venous blood sample, for simulated and real data. Better results are expected using more blood samples.

Acknowledgment Thanks to the French National Research Agency for funding.

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CIRCADIAN TIMING OF CANCER TREATMENTS

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Chronotherapeutics involve the administration of treatments according to circadian rhythms. Circadian timing of anticancer medications has been shown to improve treatment tolerability up to 5-fold and to double efficacy in experimental and clinical studies. However the physiological and the molecular components of the circadian timing system (CTS), as well as gender, critically affect the success of a standardized chronotherapeutic schedule. In addition, a wrongly timed therapy or an excessive drug dose disrupts the CTS. Therefore, a non-invasive approach is needed to accurately detect, to monitor and to image circadian rhythms in cells, mice and patients. The ultimate aim is to provide a dynamic assessment of the CTS and help personalize chronomodulated drug delivery schedule in cancer patients. We show the relevance of rest-activity and body temperature as robust circadian biomarkers and/or critical synchronizers of molecular circadian clocks and clock-controlled pathways in experimental models and patients.

In cancer patients, infrared imaging guides the location of temperature patches first, which later on record local skin temperature every 15 sec for several days before, during and after cancer therapy in patients. Subsequent wavelet-based analysis of circadian and ultradian dynamics reveals early warning signals of alterations, which are potential pharmacodynamic indicators for the subsequent personalization of cancer chronotherapeutics.

MOLECULAR IMAGING OF IN VITRO INTERACTIONS BETWEEN CIRCADIAN CLOCKS, METABOLISM AND CELL CYCLE

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Introduction: Circadian clocks rhythmically control both cell cycle and anticancer drug metabolism in healthy tissues over the 24 hours. As a result circadian rhythms characterize gene and/or protein expressions of cell cycle regulators such as wee1 and p21, as well as enzymes involved in anticancer drug metabolism such as UGTA1A and Top1. The circadian synchronization of cell cultures with serum shock or dexamethasone enable the identification of the molecular interactions between circadian clocks, cell cycle and drug metabolism, and their possible disruption in cancer cells. Dynamic imaging tools further allow the visualization of these interactions, and lend themselves to further complex time series analyses and modelling.

Methods : Here we focus on studies involving synchronized cancer cells, following modification with circadian clock and/or cell cycle reporter. This system allowed to measure the dose-dependent effect of anticancer treatments on the circadian clock of cells modified with luciferase reporter controlled by the promoter of core clock gene Period (Per2), using the Lumicycle (which measure in real time bioluminescence of luciferin enhanced by luciferase activity). Interactions between circadian clock and cell cycle were further investigated after cell transformation with combination of fluorescent clock reporter and Fluorescent Ubiquitin-based Cell Cycle Indicator reporters (FUCCI, two fluorescent reporters one for G1 phase and one for S/G2/M phases) within the ERASYSBIO+ project C5Sys.

Results : In a first step, we characterize circadian rhythms in synchronized cultures of colon Caco-2 cancer cells, and develop a mathematical model to define period and acrophase of the oscillation. A prominent rhythmic mRNA expression was demonstrated for clock genes Rev-erb□,Bmal1 and Per2 ($p<0.001$), with a mean period length of 26.6 □ 0.3h. The acrophase φ of Rev-erb□ occurred at 10.4 h and that of Bmal1 was located at 17.0 h, a finding which supports their known reciprocal regulation. Rhythmic mRNA expressions were significantly validated for drug transporter and enzymes involved in drug metabolism such as Abcb1 (φ=16.1 h), Ugt1a1 (φ=14.7 h), and Top1 (φ=15.6 h). In a second step, the modification of these cells with a clock reporter aims at the elucidation of treatment effects on clocks at a population level.Van der Horst et al., our partner in C5Sys (EMC, Rotterdam) has jointly modified both circadian clock (Rev-erbα reporter) and cell cycle (FUCCI reporter), in order to simultaneously visualize the temporal dynamics of cell cycle and circadian clock in an individual cell.

Conclusion : In vitro cell synchronization coupled with molecular imaging allows the study of molecular interaction between cell cycle, anticancer drug metabolism and circadian clock at cell population level or individual cell level, so as to provide new dynamic targets for chronotherapeutic interventions.

Acknoledgement : We thank Bert Van der Horst's team for providing modified cells. This work was supported by European program ERAsysBio+ (C5sys program) and ARTBC international.

IMAGE QUALITY EVALUATION FOR 124I IN THE MICROPET FOCUS 120 SCANNER USING THE NEMA NU4-2008 PHANTOM

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Introduction: Physical properties of 124I such as its high positron energy, corresponding large positron range in tissue and the fraction of the single γ-photons emitted may have detrimental effects on the PET image quality. The purpose of this work was to evaluate the image quality for 124I in the small animal microPET Focus 120 scanner using the NEMA NU4-2008 image quality phantom [1].

Methods: The methodology and phantom used are described in section 6 of the NEMA NU4-2008 document [1]. All microPET Focus 120 default settings were used. Moreover, the 250-590 and 350-590 keV energy windows, with 2 and 6 ns timing windows were also studied. Scan duration was adapted to obtain a number of emitted positrons that met the NEMA NU4 specifications. The uniformity, the coefficient of variation (COV) expressing the ratio between standard deviation and the mean reconstructed value on the uniform region, the recovery coefficients (RCs) for the hot rods, and the spillover ratio (SOR) for the non-emitting water and air compartments were measured. Filtered backprojection (FBP), 2D ordered subset expectation maximization (OSEM2D), maximum a posteriori (MAP) and 3-dimensional filtered backprojection (3DRP) were compared.

Results: The COV decreased from about 12 to 8 percent when increasing the time window from 2 to 6 ns and appeared to be fairly dependent on the energy window. Regarding the SOR, 3DRP and OSEM2D seemed to be insensitive to changes in both the time and energy windows, while FBP, MAP and 3DRP showed some important SOR decrease when the time window was reduced or the energy window was narrowed. The SOR were always larger for the water compartment than for the air compartment and the closest to zero for the 2ns time window scatter corrected images. RCs were larger for the 2 ns window, with the 350-650 keV window and when the images were not scatter corrected. Scatter correction decreased dramatically RC especially for the smallest objects. FBP with scatter correction seems to be the best reasonable choice for 124I image reconstruction.

Conclusion: Although the 2 ns timing window gives higher RC and slightly lower SOR and combined with the 350-590 keV energy window gives the lowest SOR, the combination of the 350-650 keV and 6 ns windows seems to be the best compromise to obtain images with high contrast and low noise content. MAP appeared to be the best reconstruction method. If its long reconstruction time or non-linear behavior is of concern, FBP is a good alternative.

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RADIOPEPTIDES IN ONCOLOGY

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Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT) are nuclear imaging techniques used to image physiological and biological processes in humans and animals following the administration of radiolabeled compounds. Applications are e.g. found in oncology, neurology and cardiology. In oncology, molecular imaging using PET and SPECT plays an essential role in diagnosis and in balancing the clinical benefits and risks of radionuclide-based cancer therapy. Selective receptor-targeting radiopeptides have emerged as an important class of radiopharmaceuticals for molecular imaging and therapy of tumors that overexpress peptide receptors on the cell membrane. After such peptides labeled with diagnostic radionuclides bind to their receptors, they allow clinicians to visualize receptor-expressing tumors non-invasively. Peptides labeled with therapeutic radionuclides could also eradicate receptor-expressing tumors.

The somatostatin receptors, which are overexpressed in a majority of neuroendocrine tumors, represent the first and best example of targets for radiopeptide-based imaging and radionuclide therapy. The somatostatin analog 111In-octreotide permits the localization and staging of neuroendocrine tumors that express the appropriate somatostatin receptors. Newer modified somatostatin analogs are successfully being used for tumor imaging and for radionuclide therapy. For further evaluation of tumor receptor expression and to increase the value of cancer targeting using radiopeptides, researchers have introduced and evaluated different radiolabeled analogs of other peptide families such as cholecystokinin (CCK), gastrin, bombesin, substance P, vasoactive intestinal peptide (VIP), and neuropeptide (NP)-Y analogs.

For these peptide analogues, receptor imaging and radionuclide therapy can be combined in a single probe, called a “theranostic”. The further development of such theranostics could greatly advance the development of personalized treatments. Improvements in the field can be further expected from new compounds with higher or broader receptor affinity, induction of increased receptor expresion on tumor cells, and the use of combination therapy, especially combinations of different radiolabeled peptides and combinations of radiolabeled peptides plus chemotherapy or radiosensitizer pretreatment.

NEW IMAGING PROBES FOR PET AND SPECT BASED ON RADIOMETALS

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Introduction: In the development of nuclear probes for imaging and targeted radionuclide therapy radiometals play an important role. One of the reasons is the possibility to provide probe precursors as lyophilised kits allowing radiolabeled probes to be produced upon addition of a solution of the radiometal.

Nuclear imaging benefits from the development of hybrid instruments such as SPECT/CT and PET/CT obtaining both morphologic and functional information. Among γ-emitters for SPECT ^{99m}Tc is the radionuclide of choice, while ^{111}In is often used as a surrogate of some major therapeutic radionuclides whereas.

Positron emission tomography (PET) is the most sensitive imaging modality in diagnostic radiology and provides quantitative information. It relies on the molecular targeting of disease using a specific probe labelled with a positron emitter. The most widely used positron emitters are ^{18}F ($t_{1/2}$ = 108 min) and ^{11}C ($t_{1/2}$ = 20 min) which are produced by cyclotrons. Their drawback often is the short half-life which may not match with the pharmacokinetics of slowly localizing molecules. In addition ^{18}F-and ^{11}C-chemistry is often time-consuming.

Three metallic positron emitters have recently received particular attention: ^{68}Ga ($t_{1/2}$ = 68 min), ^{64}Cu ($t_{1/2}$ = 12.7 h) and ^{89}Zr ($t_{1/2}$ = 78.4 h), covering a relatively broad spectrum of half-lives and are therefore useful for the study of a range of molecules with different pharmacokinetics. ^{68}Ga is of particular interest as it can be produced from a commercial generator providing a cyclotron independent source. ^{64}Cu can be produced in high quantity by small medical cyclotrons; it has an intermediate half-life. ^{89}Zr is particularly well suited for long circulating proteins such as monoclonal antibodies.

Methods: Target molecules were assessed using autoradiography; they are mainly from the G-protein coupled receptor family. Targeting ligands discussed are peptidic agonists and antagonists targeting the bombesin (prostate, breast cancer) and somatostatin (neuroendocrine tumors) receptor families as well as integrins (several tumors). Agonists/Antagonist potency was determined by functional assays such as Calcium flux measurements and immunofluorescence. Pharmacokinetics was determined in animal models bearing subcutaneous tumor xenografts. Clinical translation was possible in a few cases.

Results: Peptide-chelator conjugates with high affinity to the targeted receptors were synthesised and labeled with 64Cu, 68Ga, 99mTc and 111In. Surprisingly antagonists target more binding sites than agonists in vitro which consequently results in higher tumor uptake in the animal models but also in a long tumor retention time. When comparing 68Ga labeled PET tracers with the corresponding 64Cu labeled molecules the latter seem preferable as they allow PET imaging at later time points with improvement of contrast. For SPECT 99mTc was successfully used when coupled with a tetraamine chelator exhibiting very convincing pharmacokinetics. 111In is best suited when used along with the “universal” chelator DOTA.

Conlusion: A series of new peptide based radiotracer for SPECT and PET were developed for less frequent but also for major human tumors. A few of them were selected for clinical translation. Early human use indicates that they may have a future impact in patient care.

RADIOLABELLED CHOLECYSTOKININ-2 RECEPTOR (CCK2-R) BINDING ANALOGUES; PRECLINICAL AND CLINICAL EVALUATION OF TUMOUR AND RENAL UPTAKE

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Introduction: Overexpression of CCK2-R on tumours such as medullary thyroid carcinoma (MTC) can be used for diagnostic purposes using peptide receptor scintigraphy and for radionuclide therapy (PRS and PRRT). Scintigraphy using several radiolabelled CCK2-R-targeting analogues has been reported [1,2]. However, during the first clinical PRRT studies using ^{90}Y-minigastrin-0 nephrotoxicity was a major concern [3]. Therefore, suitable radiopeptides should exhibit high tumour and low renal uptake. In the current study four peptide analogues were investigated in rats. The effect of Gelofusine on renal uptake was tested as well. Three of these radiopeptides were applied in clinical PRS.

Methods: In CCK2R-expressing CA20948 tumour-bearing Lewis rats (n=3/group) four gamma-ray emitting radiolabelled CCK2-R targeting analogues were investigated in biodistribution (3-10 MBq/0.1 µg per rat) and imaging studies (20-40 MBq/0.3 µg per rat), with or without co-injection of Gelofusine. Three compounds ^{111}In-DOTA-(D)Asp-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (^{111}In-CCK), ^{111}In-DOTA-(D)Glu-$(Glu)_5$-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2 (^{111}In-MG-0) and ^{111}In-DOTA-(D)Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2 (^{111}In-MG-11) were used as model for ^{177}Lu-labelled analogues and one compound ^{99m}Tc-N_4-Gly-(D)Glu-$(Glu)_5$-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2 (^{99m}Tc-Demogastrin-2) as model for the ^{188}Re-labelled therapeutic beta-particle emitting counterpart. Specificity control was performed using excess unlabelled gastrin.

Six MTC patients were consecutively scanned using 200 MBq ^{111}In-CCK and ^{111}In-MG-11 and 800 MBq ^{99m}Tc-Demogastrin-2 to visualize tumour lesions and quantify renal uptake as mean of posterior and anterior views, corrected for attenuation based on CT.

Results: In rats at 4 h p.i. the tumour uptake varied from 0.1-0.3 %ID/g (^{111}In-MG-0>^{111}In-MG-11=^{99m}Tc-Demogastrin-2>^{111}In-CCK). Renal uptake was ~0.5% ID/g for ^{111}In-CCK and ^{111}In-MG-11 and ranged from 15-20% ID/g for ^{111}In-MG-0 and ^{99m}Tc-Demogastrin-2. Excess of gastrin blocked specific tumour uptake but not the renal retention. Gelofusine co-injection drastically reduced renal retention of ^{111}In-MG-and ^{99m}Tc-Demogastrin-2 but no effect was found for ^{111}In-CCK and ^{111}In-MG-11. The washout from the kidneys of ^{111}In-CCK, ^{111}In-MG-11 and ^{99m}Tc-Demogastrin-2 was fast, whereas renal retention of ^{111}In-MG-0 remained high until 24 h p.i. ^{111}In-CCK and ^{111}In-MG-11 did not show all tumour lesions in patients. ^{99m}Tc-Demogastrin-2 visualized not only all known but also unknown tumour lesions. Scintigrams of three out of the six patients could be used to quantify renal retention, resulting in mean values of 0.6 (^{111}In-CCK), 0.4 (^{111}In-MG-11) and 0.5 (^{99m}Tc-Demogastrin-2) %ID per kidney at 4 h p.i.

Conclusions: CK2R-scintigraphy with radiolabelled analogues such as ^{99m}Tc-Demogastrin-2 is very promising in MTC patients and is still being used in patients. Renal uptake of this analogue was unexpectedly low. Further research into therapeutical application of CCK2R-binding analogues in patients with inoperable MTC lesions is warranted. Renal uptake of these analogues has to be tested in patients. In case of unfavourable renal uptake of potential therapeutic analoques this might be reduced by co-injection of Gelofusine as was demonstrated in rats for the $(Glu)_5$ containing analogues.

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OLIGOPROLINES AS MULTIVALENT SCAFFOLDS FOR TUMOR TARGETING VECTORS

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Herein we present a novel approach towards multi- and heterovalent radiotracers for tumor targeting. Multivalent tumor tracers are envisioned to provide higher affinity and longer residence times on the tumor cell compared to established monovalent agents [1]. Heterovalent ligands may offer tailor-made targeting to meet the molecular phenotype of the tumor [2]. In our approach azido-functionalized oligoprolines are used as helical, conformationally well-defined scaffolds to display peptidic targeting vectors [3]. As targeting vectors, bombesin agonists and antagonists with a high affinity towards gastrin releasing peptide receptors (GRP-R), which are abundant on most breast and prostate cancer cells, are employed [4]. Conjugation to the scaffold has been achieved via copper catalyzed "click chemistry". The modular synthesis of the molecules is performed on solid support. Additionally, a DOTA-chelator was introduced for labelling with different radiometals, which allows for molecular imaging via PET and SPECT. A series of molecules, differing in type and composition of the targeting vectors (for examples see figure 1) has been synthesized. In vitro studies with 177Lu labelled ligands show excellent binding and internalization. PET images of mice with 68Ga labelled ligands demonstrate the specific uptake in the tumor, underlining the ability of oligoprolines to serve as scaffolds with beneficial influence on the biological profile of the tracer.

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LIGHT-ACTIVATED FOCAL VASCULAR OCCLUSION OF TUMOR BLOOD SUPPLY: A NOVEL CANCER THERAPY APPROACH

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Obstruction of Blood Vessels (BV), the lifeline of solid tumors is long been thought to be an effective strategy for cancer therapy. The two major approaches to achieve this objective include anti-angiogenic and anti-vascular therapies. While the first relies on interference with the development of tumor neo-vessels by chronically administered angiogenesis inhibitors, the second attempts to functionally inactivate existing BV that nourish the tumor, by cytotoxics. However, no real curative modality has thus far been successfully adopted as a monotherapy.

During the last decade our labs developed an anti-vascular treatment modality for focal treatment of prostate cancer that occludes tumor BV in a matter of minutes, leading to rapid tumor ablation (days), termed focal vascular occlusion (FVO). This therapy is currently entering phase III clinical trials in France and other European countries. In this treatment protocol, the Pd-bacteriochlorophyll derived photosensitizer (Tookad or lately Tookad-soluble (TS)) is i.v infused (~10 min) to the patient, followed by interstitial focal laser guided illumination of the tumor (763/755nm, respectively) for 10-20-min. Upon perfusion of the locally illuminated tumor, the circulating photosensitizer (that does not extravagate) generates reactive oxygen species (ROS). These include mainly superoxide and hydroxyl radicals (1) that are confined to the lumen of the tumor feeding arteries (FA), with consequent consumption of circulating oxygen (2). These primary events are in sharp contrast to other anti-vascular therapies including common PDT in two aspects: (i) No singlet oxygen is involved and (ii) The primary ROS generation is extra cellular, not within the target tumor cells. Some other differences between FVO and photodynamic therapy (PDT) will be discussed. To study the mechanistic aspects of the photoactivted FVO, we chose to use complementary imaging approaches to resolve the instantaneous responses to the primary photochemical events in-situ, concentrating on the hemodynamic response, the pathophysiology and chemistry of the concerted progress of therapeutic damage induced. The chosen imaging techniques were restricted to record “online” and “non invasive” so as to permit data acquisition in the second-to minute time resolution using the intact non-injured mouse ear lobe tumor model. The methods used included Fluorescent Intra-vital Microscopy (FIVM) Dynamic Light Scattering Imaging (DLSI), MRI and IVIS. We found that the interactions of these radicals, in addition to the resulting hypoxia, boost local nitric oxide (NO•) release from circulating S-nitrosothiols (mainly de-oxyhemoglobin) with consequent vasodilatation. The diffusion-controlled chemical reactions between the ROS and NO• result in the formation of reactive nitrogen species (RNS), notably the physiologically cytotoxic peroxynitrite. The decline in NO• levels promotes endothelin-1 assisted vasoconstriction, while ROS/RNS interaction with erythrocytes leads to blood clotting.

Jointly these ultra-fast processes culminate with arterial stasis within 60s to a few min respectively in mice and humans. Thus, a single treatment session suffices to accomplish ablation and cancer cure, in the range of 80-100% success in mice (using various tumor models) and >80% in man treated for focal prostate cancer.

REAL-TIME MONITORING OF TUMOR VASCULAR OCCLUSION BY VASCULAR TARGETED PHOTODYNAMIC THERAPY

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The distinctive morphological and functional characteristics of tumor versus normal vasculature render the tumor vasculature an attractive target for tumor therapy. Nevertheless, current antiangiogenic and antivascular therapies are facing difficulties that withhold them from being curative. These treatment approaches, commonly based on VEGF/VEGFR activities, are likely to target only certain subsets of tumor blood vessels, sparing the relatively large feeding arteries (FA) and draining veins (DV), that transverse the tumor rim and are not VEGF-dependent. Consequently, these particular vessels provide a formidable target for novel anti-vascular therapies such as Vascular-Targeted Photodynamic Therapy (VTP) with bacteriochlorophyll based photosensitizers, Tookad and Tookad-soluble® (TS). Following venal infusion the circulating photosensitizer is locally illuminated, leading to prompt vascular occlusion, tumor eradication followed by healing and cure in a matter of weeks. This therapy shows significant clinical efficacy in treatment of localized prostate cancer and is presently entering phase III clinical trials. In this study, the vascular response to VTP in the mouse ear tumor model was studied under controlled conditions by two complimentary imaging methodologies, Fluorescent Intravital Microscopy (FIVM) and Dynamic Light Scattering Imaging (DLSI). The VTP process started with prompt vasodilatation and a transient blood-rate upflow in the FA followed by simultaneous vasoconstriction and blood clotting on the inner vessel wall. Vessel dysfunction and the sharp decline in the flow rates, culminated with FA occlusion by ~60 seconds of VTP onset. Following a single treatment session the success rates and tumor cure are 80-100% in mice (for various tumor models) and >80% in man treated for focal prostate cancer. The results of this study provide new insight into the hemodynamic response to VTP and substantiate our understanding towards the development of online, intra-operative guiding tools that will allow online monitoring VTP in action.

This study was supported in part by Steba Biotech, France.

ADVANCED IMAGING TECHNIQUES FOR GUIDING RADIOTHERAPY AND FOLLOW-UP IN GLIOMAS

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Background: glioma patients are referred from the neurosurgeon to the radiation oncologist (RO) for adjuvant treatment in different situations: after biopsy, after a partial surgery or after a macroscopic "radical" resection. Consequently, in terms of ballistic, in the last case, there is no "gross tumour volume" (GTV) to be irradiated as a visible "target volume" and the radiation planning is only based on a "clinical tumour volume" (CTV) which is supposed to include all the microscopic areas potentially infiltrated. A planning target volume (PTV) is added by the physicist to the CTV, considering the daily random error when setting the patient under the machine and the penumbra produced by the radiation particles. First goal of RO is to deliver the "good dose" in the "good place" (the PTV), sparing a maximum of normal tissue, especially the "Organs at risk" (OaR), as brainstem, optic chiasm, cochlea and spinal cord. For the RO, gliomas have three specific characteristics: they are mainly *infiltrative* (CTV is much larger than GTV), highly *heterogeneous* and particularly *radio resistant*, recurring mostly locally.

Ongoing EORTC guidelines: modern radiation therapy planning (RTP) basically needs contrast-enhanced computed tomography (CT) scan and *anatomical* Metabolic Resonance Imaging (*a*MRI) to define GTV / CTV and OaR. Even if sophisticated image co-registration permits accurate mapping of new modalities to a single coordinate system (CT is still the "reference"), target volume definitions have not incorporated new advances in Functional and Metabolic Imaging (FMI). However, it is clear now that "abnormal areas" enhanced in T1-gadolinium (for grade 4 gliomas) and / or T2 hyper-intensity in aMR (for grade 2 and 3) are only "anatomical views" of brain tissue. For example, a hot debate is still ongoing for differentiating "peritumoral edema" from "specific infiltration" or "radiation necrosis" from true recurrence.

Combining CT / ***a***MRI with FMI could help for a better RTP and follow-up at less in three ways: (1) improving target volume delineation by *providing additional information* regarding abnormal biological and metabolic activities corresponding to new "areas at highest risk of recurrence" (2) assessing *functional / metabolic responses* of the tumour *during the course* of chemo-RT and/ or *very early in the follow-up*, identifying "treatment responders" from "resistant", thereby allowing fast "adaptive chemo-RT" with intensification or re-optimization of RTP (3) *in recurrent gliomas* retreated with RT, decreasing the risk of geographic miss and the volume of normal brain irradiated.

FMI: metabolic Positron Emission Tomography (PET), MR spectroscopy (MRS) and MR Perfusion / Permeability imaging are basically *different and complementary* advanced imaging tools.18-fluoro-deoxy-D-glucose (FDG) PET is routinely available and able to detect the increased *cellular* glucose metabolism in brain tissue, but its use in RTP is complicated by the high level of intrinsic glucose uptake specially in the cortical-subcortical areas, where grade 2 and 3 gliomas are mainly located. However, newer tracers provide better differentiation of the tumour from brain background signals than FDG, as carbon 11 (^{11}-C) methionine (MET) PET, 18F-fluorothymidine (FLT) and 18F-fluoroethyl-L-tyrosine (FET) PET. They provide *a direct reflect of the cellular proliferation rate*, thereby potentially defining tumour extension better than *a*MRI. It was demonstrated in glioblastoma patients (GBM) that abnormal high MET activity extends largely beyond the abnormal lesion identified from *a*MRI; recently, a significant correlation was showed between initial areas of increased MET uptake and the subsequent sites of failure. At less two prospective studies (one for primary, one for recurrent GBM) will incorporate PET into "clever" escalating-dose trials. Moreover, FLT PET could provide an early imaging "biomarker" of response to bevacizumab treatment in patients with recurrent GBL and predict overall survival better than conventional factors.

MRS detects proton metabolites in tissue *in vivo* and provides information on *cell proliferation*, cell membrane breakdown, *neuronal activity* and *necrosis*. It could be a useful adjunct to *a*MRI in delineating the extent of disease, especially in GBL. As a recent study showed that initial areas of "high activity" (with high peak in choline-containing compounds and reductions in N-acetylaspartate) were correlated with sites of relapse, a French multicentric Phase III study is planned, testing the hypothesis of a better local control through a "boost" dose (beyond 60 Gy) delivered only in MRS defined "highest-risk" areas.

MR Perfusion and Vascular Permeability. Perfusion estimate using relative Cerebral Blood Volume (rCBV) item and microvascular leakage (MVL) as reflecting permeability *in tissue* were correlated with *angiogenesis* and aggressiveness. They are proposed as "easy-to-use" tools *for grading*, as biomarkers *for prognostic* evaluation and *to differentiate post-treatment modification* from true recurrence. It was showed that both high rCBV values and MVL could be independent prognostic factors; we have planned an EORTC side-study for a *prospective* confirmation in unfavourable anaplastic glioma pts. In a large series of GBL, we have identified different sub-groups in terms of Perfusion / Permeability maps and will present preliminary results. A clinical implementation of Perfusion cartography into a real "multi-modal" RTP will also be presented.

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IN VIVO IMAGING OF AKT1 SIGNALING IN ANGIOGENESIS

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Akt1, also known as PKBalpha, is an intracellular kinase that phosphorylates target substrates to regulate crucial aspects of growth and survival. Akt1, a protein kinase, is a major mediator of angiogenic signaling, acting downstream of vascular endothelial growth factor (VEGF). Akt1 deficient mice are smaller, have increased neonatal mortality (Cho et al., 2001; Chen et al., 2001) and exhibited long bone mineralization defects (Kawamura et al. 2007; Ulici et al., 2009). Moreover, Akt1 controls heart size and function, and Akt1 null mice exhibit heart defects and abnormal cardiomyocyte proliferation (Cho et al. 2001, Chang et al., 2010). Noninvasive imaging is an important tool to answer many questions on deficient Akt1 protein function in Akt1 deficient mice.

The purpose of this study was to use on the one hand macromolecular DCE-MRI to examine in vivo vascular changes at the site of long bones in growing Akt1 deficient mice, and on the other hand asses cardiac function in embryonic mice using ultrasound and in adults using cardiac MRI, in an attempt to find a reason for the small size and in utero and neonatal mortality of Akt1 deficient mice.

Reduction of metaphyseal blood vessel invasion was shown using macromolecular DCE-MRI validated by histology, concomitant with aberrant trabeculae and shorter long bones demonstrated by ex vivo µCT and µMRI. Surprisingly, also heterozygous mice, containing only one Akt1 allele also exhibited a reduction of blood vessel invasion. At embryonic day E16.5 and 18.5, in utero echocardiography revealed heart defects in Akt1 deficient and heterozygous mice. Cardiac function of adult Akt1 null mice assessed by cardiac MRI, appeared normal, whereas adult heterozygous mice exhibited a modest myocardial hypertrophy, validated by histology.

Here, we showed in vivo, in growing Akt1 deficient and heterozygous mice deficient blood vessel invasion resulting in aberrant long bone formation, explaining their smaller overall size. We also demonstrated during development and in adulthood, slight alterations of Akt1 deficient and heterozygous hearts, both structurally and functionally. Cardiac defects during embryonic development could be a reason for increased neonatal mortality.

ANNEXIN-BASED APOPTOSIS IMAGING: PITFALL IN THE ASSESSMENT OF ANTI-ANGIOGENIC THERAPY?

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Introduction: Molecular apoptosis imaging is frequently discussed to be useful for monitoring cancer therapy in vivo. Thus, the aim of this study was to analyze the accuracy of apoptosis imaging for the assessment of anti-angiogenic therapy response. In order to evaluate the therapy effects on the vasculature, the analysis of the tumor vascularization was included.

Methods: AnnexinVivo 750 (apoptosis) and AngioSense 680 (vascularization) concentrations were determined by Fluorescence Molecular Tomography (FMT2500) in nude mice with subcutaneous epidermoid carcinoma xenografts (A431) after 4 days of anti-angiogenic treatment with the multispecific tyrosine-kinase inhibitor SU11248. Tumor localization was facilitated by parallel morphological µCT-scans. 3D FMT data were compared with corresponding 2D fluorescence intensities. In addition, tumor vascularization was analyzed by contrast-enhanced ultrasound (25 MHz) using the 2D MIOT technique. In vivo-data were validated on corresponding histological sections (CD31-staining: vascularization; TUNEL-staining: apoptosis). A Student´s t test was applied for statistical analysis.

Results: After 4 days of therapy, strikingly lower concentrations of AnnexinVivo were measured in treated tumors compared with controls (Annexin/volume 3D: control: 81.3 ± 73.7 pmol/cm3, therapy: 27.5 ± 34.7 pmol/cm3; 2D: control: 13 ± 15 FI/cm2; therapy: 11 ± 7 FI/cm2). The in vivo-data clearly contradicted histological analyses, demonstrating a significantly increased apoptosis in the treated group (TUNEL+ area: control: 0.011 ± 0.014%, therapy: 0.461 ± 0.194%; $p<0.001$) as expected. Lower concentrations of the blood pool marker AngioSense were detected in treated tumors compared with untreated controls (AngioSense/volume 3D: control: 13.8 ± 26.2 pmol/cm3, therapy: 6.3 ± 7.3 pmol/cm3; 2D: AngioSense/area: control: 38 ± 37 FI/cm2, therapy: 15 ± 17 FI/cm2). These data were confirmed by ultrasound, demonstrating a significantly reduced vascularization in treated tumors (control: 285.3 ± 107.5 a.u./mm2 therapy: 87.3 ± 23.9 a.u./mm2; $p<0.01$). Accordingly, histological analyses revealed a significantly reduced vessel density in treated tumors (area CD31+: control: 4.8 ± 0.50%, therapy: 1.7 ± 0.37%; $p<0.001$).

Conclusions: In vivo apoptosis imaging fails in assessing increased apoptosis in response to an anti-angiogenic treatment. This can be explained by the significant breakdown of the vasculature in response to treatment, thereby impairing the delivery of the annexin probe to the tumor tissue and resulting in a low probe accumulation. These data favor the application of apoptosis imaging for therapy control only in therapies that do not severely interfere with the vasculature.

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MOLECULAR CT IMAGING OF CANCER USING TARGETED GOLD NANOPARTICLES

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Introduction: traditional medical imaging employs physical methods, such as X-rays and MRI. A quantum leap in sensitivity, selectivity and effectiveness is being achieved by mimicking Nature: Molecular recognition and molecularly targeted nanoparticles are revolutionizing cancer detection, diagnosis and therapy. Here we describe a new class of Computed Tomography (CT) contrast agents, which will enable cancer diagnostics that are based on molecular markers rather than anatomical structures, while using an ordinary clinical CT machine.

The method is based on the development of immuno-targeted gold nano-probes that selectively and sensitively target tumor specific antigens, while inducing distinct contrast in CT imaging (increased x-ray attenuation). In this work we use a head and neck cancer model that overexpress EGFR.

Results: an in vivo molecular CT of tumor bearing mice is presented. We demonstrate that the attenuation coefficient for the molecularly targeted tumor is over 3 times higher than for identical but untargeted tumor.

Conclusions: We show that we are capable to detect micro-tumors, based on their molecular markers. In addition, molecular CT imaging and structural CT imaging can be simultaneously performed. This novel technique may facilitate early cancer detection based on molecular markers and will provide researchers a new technique to investigate in vivo the expression and activity of cancer related biomarkers and molecular processes.

MULTI-MODAL (MRI/PET/ALA-OI) IMAGING-BASED NEUROSURGERY OF GLIOMAS

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- New aspects in PET diagnostics for gliomas and experimental treatment options by using 5-ALA in glioma stem cells -

Cerebral gliomas are locally invasive tumors that have poor prognosis despite treatment with a combination of surgery, radiotherapy, and chemotherapy. Cytoreductive surgery is generally thought to prolong survival and appears to be essential for the efficacy of modern adjuvant treatment. Today, MRI is the most important diagnostic tool for assessing cerebral gliomas but the differentiation of glioma tissue from surrounding edema is unreliable particularly when the tumor is not sharply demarcated from normal brain tissue, and when the blood-brain barrier remains intact. The contrast-enhanced area provided by T1-weighted images after injection of Gd-DTPA usually represents the volume that neurosurgeons plan to remove. This area, however, may not accurately reflect malignant tumor tissue and serial biopsies have revealed tumor cells several centimeters distant from the contrast enhancing margin.

PET using radiolabeled amino acids shows improved imaging of tumor extent of cerebral gliomas for treatment planning and for biopsy guidance compared with MRI. A number of studies have proven the clinical value of ^{18}F-FET PET to determine the extent of cerebral gliomas for treatment planning and biopsy guidance and to detect tumor recurrences. Furthermore, there are significant differences in the dynamics of FET uptake between low and high grade gliomas. Parameters addressing the different kinetic pattern allow discrimination with high diagnostic power between these prognostically different groups.

The complete resection of cerebral gliomas is hampered by the fact that viable tumor tissue is often difficult to distinguish from brain tissue during surgery. In recent years, 5-aminolevulinic acid (5-ALA) has emerged as a metabolic marker of malignant glioma cells that can be used intra-operatively for identifying tumor tissue. 5-ALA is a natural biochemical precursor of hemoglobin that elicits synthesis and accumulation of fluorescent porphyrins within malignant glioma tissue. By using a modified neurosurgical microscope to visualize porphyrin fluorescence, residual malignant glioma can be identified intra-operatively. A multicenter study has shown that a significantly larger number of “complete” resections defined as absence of contrast enhancing tumor on early postoperative MRI can be achieved using 5-ALA guided resection, compared to conventional microsurgery. Consecutively, immense attention is focused on basic research as well as translation into clinical use for neural stem cells and their therapeutic potential when appropriately manipulated. 5-ALA based photodynamic therapy (PDT) indicates cell death in C6 glioma spheroid model and can improve clinical outcome in patients suffering from recurrent glioblastoma and causes long-sustaining responses.

IMAGING GUIDED TRANSCRANIAL MAGNETIC STIMULATION

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Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive method for modulation of cortical activity. Single pulses applied over specific parts of the motor cortex, induce muscle contractions in the respective body parts. Rhythmic repetition of stimuli is called repetitive TMS (rTMS) and can induce changes of cortical excitability that outlast the stimulation period. Since the magnetic field decreases rapidly with increasing distance from the coil only superficial cortical brain areas can be directly stimulated. However stimulation effects can propagate to remote functionally connected brain areas.

Single pulse TMS is used for diagnostic purpose in neurology. Repetitive TMS has been investigated as an innovative non-invasive treatment method in neurologic and psychiatric diseases. rTMS has also been used in cognitive neuroscience, mainly in connection with functional imaging. Functional imaging studies are methodologically restricted to a correlational approach. rTMS enables to actively interfere with specific brain areas, which have been identified by functional imaging. By using this method the relevance of these brain regions for the task under study can be identified.

Also the use of rTMS for the treatment of neuropsychiatric disorders requires exact knowledge of disease-related alterations of brain function. Based on this knowledge rTMS can be used either for normalizing pathological alterations or for enhancing compensatory mechanisms. Finally effects of rTMS can be assessed by neuroimaging before and after the intervention. The best method for exactly positioning the rTMS coil over the target area is the use of a neuronavigation system. By using functional imaging data of an individual person, the target area can be exactly identified; the fusion with structural imaging data enables the exact coil positioning

ULTRASOUND INDUCED DRUG DELIVERY

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Medical imaging technologies are moving forward into therapeutic applications and start to play a crucial role in image-guided interventions. MRI plays here a pivotal role thanks to its superb resolution and a variety of techniques that allow imaging of very different contrast mechanisms as well as functional or temperature information. Consequently, MRI has been used in a variety of interventional procedures and drug delivery applications. From interventional perspective, ultrasound provides a non-invasive therapeutic option where either pressure pulses or local heating with ultrasound can be exploited to induce local delivery of drugs. The choice for pressure or temperature sensitive drug delivery is dictated by the medical application and the nature of the drug, it furthermore asks for different imaging modalities to control the therapeutic intervention. Temperature-sensitive systems like liposomes release a drug upon temperature rise induced by ultrasound. The local heating as well as drug delivery can be quantified by MRI using either T1, CEST, or Fluorine imaging, making this the preferred image guidance technology. Pressure-induced drug delivery can be mediated by microbubbles that are co-administered with drugs. Here, ultrasound imaging is here the preferred modality for image guidance. Ultrasound-induced drug delivery has the potential to increase the therapeutic window in chemotherapy, and may be an enabling technology to solve the outstanding challenge of pharma industry to deliver oligonucleotide-based drugs. This talk will present an overview of image guided drug delivery with focus on MRI-high focused ultrasound.

day three: wednesday january 19, 2011

FUNCTIONALIZED NANOPARTICLES FOR BIOMOLECULAR IMAGING AND SENSING

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Gold nanoparticles have been used for several decades as labels in immuno-electron microscopy. In the last decades, progress in the synthesis, functionalization and optical detection of these particles have opened a range of new applications in imaging and sensing. For most of these applications, the capping layer is a critical feature as it provides colloidal stability and control specific and unspecific interactions.

The presentation will cover three related themes of research. First, I will present the synthesis and characterization of peptide-capped gold nanoparticles [1] and discuss our efforts to generate complex and controlled nanomaterials based on self-assembly of these small molecules at the surface of the particles. Then, I will report on the entry and fate of the particles in live cells, with a particular focus on the fate of the capping layer [2]. Finally; I will discuss our current attempts to break the barrier of endocytotic trapping of the nanoparticles using a variety of methods including toxins, signalling peptides and direct injection.

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MOLECULAR IMAGING OF ATHEROSCLEROTIC PLAQUES

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Acute coronary syndromes and ischemic strokes are most often caused by the fibrous cap disruption of atherosclerotic plaques. Sudden exposure of the underlying atheromatous content to blood triggers the formation of a thrombus that can occlude the artery or embolize distally leading to myocardial or cerebral necrosis. Typical features of vulnerable plaques include a large lipid core, a thin fibrous cap, positive remodelling and presence of inflammatory cells and neo-angiogenesis. Identification of atherosclerotic plaques at risk of rupture could be useful for risk stratification and early implementation of therapies aimed at plaque stabilization.

One major hurdle in detecting high-risk atherosclerotic plaques in coronary and carotid arteries is the lack of an imaging modality that allows for the identification of biological activities in addition to morphological aspects of atherosclerotic plaques with high spatial resolution. Based on an approach similar to radiotracers synthesis for nuclear medicine, this limitation has stimulated the development of contrast agents for magnetic resonance imaging (MRI) and computed tomography (CT) that target biological activities present in vulnerable plaques. We will discuss the advantages and drawbacks of these new imaging approaches.

Despite its low spatial resolution, nuclear imaging techniques offer a high sensitivity for the detection of biological activities in atherosclerotic plaques. Accumulation of 18Fluoro-deoxyglucose has been detected in atherosclerotic plaques with positron emission tomography (PET) and is associated with the presence of inflammatory cells in plaques. We will give a comprehensive overview of the latest results of clinical studies based on FDG-PET for the evaluation of atherosclerotic plaques.

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

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

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

Results More than 98% of the radiotracers and 520 molecules of DiD are encapsulated in the lipidots. Particle loading does not modify its physicochemical properties: 55 ± 1 nm diameter with a polydispersity index of 0.19 ± 0.01, and long-term stability (> 5 months). Injections of free radioisotopes or free DiD in FVB mice leads to their accumulation mainly in lungs, and less extendingly in spleen and liver, and for free DiD in spleen and liver. Injection at the same dose of the triply-labeled lipidots leads to a different biodistribution, evidencing a nanovectorization effect. The particle integrity seems to be preserved in blood and the nanoparticles are rapidly captured by the liver (t1/2 around 30 minutes). All the 3 tracers are uptaken in liver, adrenals and ovaries, and for longer times (> 48h), fluorescence is observed in uterus. In the PyMT model, lipidot accumulation is rapidly observed in the tumour site (around 5 h), certainly due to the Enhanced Permeability and Retention (EPR) effect of the nanoparticles.

Conclusion Lipidots modify the biodistribution of the tracers they encapsulate. Both radioactivity and fluorescence tracers have the same biodistribution up to 3 hours post-injection, indicating the nanoparticle integrity for this time lapse. Lipidots, thanks to their nanometric size, take advantage of the EPR effect and passively accumulate at tumour sites. The next development step lies in the design of ICG and 18F labelled particles to take benefit of their biodistribution for multimodality imaging, combining PET tumour localization firstly, followed after radioactivity decrease, by fluorescence guided surgery.

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IMAGE GUIDED SURGERY BY MEANS OF NEAR-INFRARED FLUORESCENCE

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Introduction: So far, surgical intervention is still the method of choice in cancer treatment. However, recurrence of cancer after incomplete tumor surgery remains a critical point. We developed a per operative detection system made of a near-infrared fluorescent tracer, called RAFT-c(RGD)4, that light-up infra millimeter tumor nodules but also helps the surgeon to delineate the tumor margins. The aim of the present study is to demonstrate the very significant improvement in primary tumors and metastasis resection in preclinical animal models, from mice to rats and finally in a veterinarian "clinical phase I/II" in cats. In addition, there is also a way to improve the quality of the fluorescent probe and method.

Methods: Luciferase positive cancer cells were injected in the peritoneal cavity of nude mice resulting in the formation of small peritoneal nodules. 24 hours after the intravenous application of a fluorescent tumor-targeting probe, the RAFT-c(RGDfK)4-Alexa Fluor 700, image guided surgery was done using the Fluobeam. This is a portable device for near-infrared (NIR) illumination and vision. The quality of the surgery was evaluated using bioluminescence for the detection of remaining tumor cells. At the same time the time of operation has been measured. This molecule and instrumentation are now being transferred to surgery of cats bearing natural fibrosarcoma.In parallel, additional peptidic sequences are currently being added to the RAFT-RGD molecule in order to augment its specificity.

Results: Under normal light, the surgeon detected and removed a mean of only 50.6 per cent of the nodules, which were visible under NIR light. The duration of the surgery was reduced from 19.3 minutes to 14 min when NIR light was used, whereas the sensitivity of the NIR system allowed a detection of nodules of 227 tumor cells. An additional tumor model of peritoneal carcinoma in mice has also been generated as well as new evolutions of the molecules that are currently being evaluated.

Conclusions: NIR image-guided surgery improved the quality of peritoneal carcinomatosis surgery by doubling the number of nodules detected and significantly reducing the duration of surgery.This is the first step towards real case surgery in cats and a very strong rational for translating this system in clinical phase I.

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IN VIVO GENE DELIVERY AND IMAGING OF NF-KB REGULATION PATHWAY IN ACUTE LUNG INFLAMMATION MOUSE MODEL

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NF-kB plays a central role in immunity, inflammation, development, cell survival and has been indicated under a number of pathological conditions of lung disease, including asthma, chronic bronchitis, and chronic obstructive pulmonary disease (COPD). In this study, we assessed the in vivo activation of NF-kB signaling in lung tissue using a bioluminescence imaging system (IVIS) to monitor activation of an NF-kB promoter in response to lipopolysaccharide (LPS) stimulation. A plasmid contained responsive elements (RE) of NF-kB and luciferase as a reporter gene has been delivered intravenously in nude mice at the concentration of 40 ug per mouse using in vivo-jetPEI™ from Polyplus as a transfectant agent. One week after DNA delivery the transient transgenic mice had been imaged in order to check the baseline activation of the NF-kB pathway. The day after, the mice have been treated with LPS 15 ug per mouse intratracheally and the lungs imaged using bioluminescence (BLI) at 2, 4, 7 and 24 hs. The ability of the IKK2 inhibitor MLN120B orally administered at the dose of 300 mg/kg to counteract NF-kB activation has been evaluated. The maximum peak of NF-kB activation was reached at 4 hs with 7-10 folds of induction in comparison to the saline group and at 24 hs the signal dropped down at basal level. In the group treated with MLN120B was observed a 50% inhibition of LPS-induced NF-kB stimulation, an effect that was in good agreement with the inhibition of p65 nuclear translocation evaluated ex vivo in lung homogenates. In this experiment we showed that is feasible to monitor NF-kB activation in vivo in lung tissue in a non-invasive way by BLI and create a new in vivo tool for drug discovery process.

BIMODAL ULTRASOUND/OPTICAL INSTRUMENTATION AND FLUORESCENT MOLECULAR PROBES TO IMPROVE PROSTATE CANCER DIAGNOSIS

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The prostate cancer diagnosis is normally established further to the histological study of biopsies. These biopsies are prescribed according to the patient's clinical signs and high dosages of PSA. Biopsy sample-taking is, in most cases, guided by the ultrasonic imaging which is used to ensure to sample the whole prostate volume. Despite this, however, in some 30% of cases, the biopsies conducted do not reveal any traces of the tumor, thus resulting in a second or even a third series of sample removals, at several months' intervals. This delay may significantly complicate the following therapy. Development of a method designed to localize the tumor, or a suspect zone, within the prostatic tissue during the biopsy, would considerably improve its detection sensitivity, reduce the number of biopsies required, and avoid the second or third series of biopsies and months of delay.

To enable an early detection of prostate cancer, we have developed a bi-modal transrectal probe allowing fluorescence molecular imaging measurement to be added to the traditional ultrasound diagnosis. This instrument, combining two imaging methods, is to be associated with prostate cancer near-infrared fluorescent marker to enable the medical practitioner to localize precisely potentially cancerous zones and to produce far more accurately targeted and clearer biopsies. Among many commercially available near infrared optical contrast agents, only indocyanine green (ICG), has been approved since now by the United State Food and Drug Administration (FDA) for various medical applications. However, ICG instability (photo-degradation, thermal-degradation and low aqueous solubility) limits its application as a fluorescent marker for imaging purposes. New dye nanoformulations can bring several benefits: i) improve ICG optical and chemical stabilities, therefore resulting in better signal over noise, ii) modify the dye pharmacokinetics and prolong its retention in tumor tissues, thanks to the nanometric size of the cargo, iii) open possibilities to chemically modify the surface of the nanovectors, in order to improve dye tumor targeting and retention.

In this talk we will describe the bimodal imaging instrument and its associated localization algorithm that have been developed for prostate tumor detection in less that 1 minute and with an accuracy better than 1.5 mm and a precision of 0.5 mm, as well as ICG-loaded lipid nanoparticles (ICG lipidots), which present improved tumor over skin contrast in different mice models in comparison to free ICG.

Association of the bimodal ultrasound/fluorescence imaging instrument and the nanometric ICG lipidots should improve in the future prostate cancer diagnosis.

TARGETED NANOPARTICLES, OPTICAL IMAGING AND CANCER

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Early and accurate detection of tumors, like the development of targeted treatments, is a major field of research in oncology. The generation of specific vectors, capable of transporting a drug or a contrast agent to the primary tumor site as well as to the remote (micro-) metastasis would be an asset for early diagnosis and cancer therapy. Our goal is to develop new treatments based on the use of tumor-targeted delivery of large biomolecules (DNA, siRNA, peptides). We generated new targeting vectors (as the RAFT-RGD) and nanoparticles for drug and biomolecules delivery. To undertake this work, we also developed several optical imaging systems allowing the follow-up and evaluation of our vectorisation systems.

Near-infrared fluorescence (NIR ; 650-900 nm) can be imaged in 2D or 3D. The strong reflection of incident light and autofluorescence of the skin affect the sensitivity when working in reflectance. Switching to Fluorescence Molecular Tomography (FMT) mode greatly improves the quality of whole-body fluorescence imaging. It offers 3D volumetric imaging, true quantification very little affected by depth, optical tissue properties and heterogeneity, and autofluorescence. It is thus an emergent diagnostic tool for the localization and quantification of fluorescent probes, at a depth of a few cms, in some organs, like prostate. In such situations, this technique may be considered as an alternative to the classical ionizing radiation imaging techniques, or a complement to morphological imaging as ultrasounds. Very recently, clinical applications in surgery of cancer as well as for the diagnosis of human prostate cancer became realistic. All these recent issues will be presented.

ULTRASOUND INDUCED MECHANICAL RELEASE FROM PARAMAGNETIC LIPOSOME FOR MRI NEW DRUG DELIVERY PROTOCOLS

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The non invasive in vivo visualization of drug release triggered by externally applied ultrasounds (US) is an emerging topic in molecular imaging field. [1] Most of the reported studies were performed using nano- or micro-bubbles in which the presence of the gas-filled core makes cavitation (and US detection) possible. However, the use of imaging modalities with an improved spatial resolution and widerdiagnostic/therapeutic potential like MRI would be very useful. Liposomes, nanovesicles endowed with an aqueous core, are extensively used as drug delivery nanocarriers and are also under intense scrutiny in the field of MRI contrast agents where they represent one of the most promising nanoplatform for designing highly-sensitive probes.

The most straightforward approach to design liposomal MRI probes whose image contrast can report about the US-induced cavitation of the nanocarrier is the encapsulation of a large amount (300 mM) of a clinically approved Gd(III) agent in the vesicle. The ability of the encapsulated agent to generate a good T1 contrast is strongly limited by the water permeability of the vesicle membrane. Upon the probe release, the T1 “quenching” is removed and a contrast enhancement can be observed. In this work, we demonstrate that low frequency US (27.6 kHz) can induce a probe release much more efficiently than using high frequency waves (3 MHz). The evaluation of the probe release, monitored in vitro at 0.5 T on a fixed frequency relaxometer, was performed at different US frequencies, power supply of piezoelectrical transducer, acoustical intensity and insonation time. In addition to US insonation characteristics, the physicochemical properties of the liposomal membranes [2] were evaluated in terms of size, shape and internal concentration of Gd3 + complexes. Thus allowing the development of a model to predict the efficiency of the release mechanism.The in vitro work was followed by an ex vivo and in vivo [3] (on a xenografted B16 melanoma mouse model) MRI study that successfully demonstrated the potential of this approach to visualize the probe release following a local US application. The possibility to selectively trigger the release of the imaging reporter, as well as a drug, from a mixture of different nanocarriers could open new and intriguing therapeutic schemes able to improve the overall efficacy of the pharmacological treatment.

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MRI-GUIDED HIFU-MEDIATED DRUG DELIVERY TO TUMORS WITH TEMPERATURE-SENSITIVE LIPOSOMES

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Introduction. Temperature-triggered drug delivery is a promising treatment option in oncology, because it can improve the therapeutic efficacy and reduce 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]. 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. Temperature-sensitive liposomes (TSL) containing doxorubicin and 250 mM [Gd(HPDO3A)(H2O)] were prepared as described before [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 MR-HIFU experiments were performed on rats bearing a subcutaneous 9L tumor on the hind leg. TSLs were injected intravenously and local hyperthermia in the tumor was induced with focused ultrasound 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 using a Look-Locker EPI-sequence. At t=90 min after TSL injection the rats were sacrificed and blood and tumors were analyzed for doxorubicin and gadolinium concentrations by a doxorubicin extraction assay and ICP-MS, respectively.

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 ΔT1 and the concentrations of doxorubicin and [Gd(HPDO3A)(H2O)] in the dissected tumors [4].

Conclusions. The good correlation between ΔT1and 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. These results show that image guided therapy enables a way to quantify and monitor the drug delivery process and may serve as a tool in clinical decision making to personalize treatments.

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FOCUSED ULTRASOUND INDUCED EXTRAVASATION: IN VIVO SPECT/CT IMAGING AND QUANTIFICATION

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Introduction: Drug delivery of hydrophilic and charged macromolecular drugs, like pDNA or siRNA, presents one of the biggest challenges in pharma as these drugs need to overcome barriers like the endothelial layer of blood vessels and cellular membranes to reach their site of action. Focused ultrasound (FU) may present a solution for this delivery dilemma as it was recognized that the pressure waves of ultrasound can induce a transient permeability of the endothelial lining of blood vessels as well as in cell membranes. This effect is termed sonoporation and can be further enhanced through the cavitation of microbubbles (MBs) that are also used as ultrasound contrast agents. Though transient in nature, sonoporation facilitates local extravasation of drugs into the interstitial space that otherwise would stay confined to the vascular system [1]. However, little quantitative information is available to correlate kinetics of drug extravasation with the exact delivery protocol comprising ultrasound settings, MB characteristics and treatment schemes. In this work we have developed a method to image and quantify the ultrasound mediated extravasation of a radiolabeled model drug, 111In-DTPA-BSA, with dynamic SPECT/CT measurements in vivo. This approach allows to characterize the transient effects of sonoporation in real time.

Methods: Swiss mice were intravenously injected with polymer MBs followed by FU exposure (7-point hexagonal-shaped pattern, 2.5mm between points, 22 exposures per point - 1.2 MHz, 2MPa and 10000 cycles) in the left hind limb muscle. After a well defined waiting time dt ($0 < dt/min < 90$), the animals were injected with radiolabeled 111In-DTPA-BSA (ca, 30µg, 30-45MBq). The extravasation of 111In-DTPA-BSA was followed in vivo with SPECT scans over a time period of 1.5h followed by a CT scan. A post-mortem biodistribution study was performed by γ-counting to determine the %ID/g.

Results: Ultrasound mediated drug extravasation was followed with dynamic acquisitions up to 1.5h post-injection (p.i.). The drug showed an exponential accumulation in the treated tissue leveling off after ca. 40-60 minutes p.i. In contrast, the non-treated control tissue showed a decrease of activity due to blood clearance. The maximum amount of extravasated drug decreased exponentially as a function of waiting time dt with a characteristic time constant of t=18 min. For waiting times (dt) less than 10 min we find up to a 20 fold increase in accumulation of drug in treated tissue when compared to the control.

Conclusions: In this study, we characterized in vivo the ultrasound induced extravasation of a radiolabeled drug with SPECT. The enhanced extravasation effect is restricted to the FU-exposed tissue demonstrating controllable and localized delivery and furthermore transient in nature. Non-invasive imaging is well suited to correlate drug extravasation to FU parameters and to provide a feedback for protocol optimization. Ultrasound triggered extravasation is an enabling technology to induce local drug delivery across biological barriers.

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NANOMEDICINES, THERANOSTICS AND COMBINATION THERAPIES

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Nanomedicine systems are nanometer-sized carrier materials designed to improve the biodistribution of systemically applied (chemo-) therapeutics. By increasing drug localization at the pathological site, and by reducing its accumulation in healthy non-target tissues, the efficacy of the intervention can often be increased, while its toxicity can be attenuated. Various different nanomedicine formulations have been evaluated over the years, including e.g. liposomes, polymers, micelles and antibodies, and clear evidence is currently available for substantial improvement of the therapeutic index of anticancer agents. Using HPMA copolymers as a model nanomedicine system, and several selected examples of recently published proof-of-principle reports, we here show that besides for standard drug delivery purposes, nanomedicine formulations can also be used for image-guided drug delivery, as well as for tumor-targeted combination therapy.

NANOPROBES FOR IMAGING DRUG RELEASE

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The search for novel therapeutic technologies is essentially driven by the need to increase efficacy or improve safety and patient compliance. Drug delivery systems are primarily aimed at improving treatment of cancer, diseases of the respiratory and central nervous system and cardiovascular disorders, but many therapeutic agents have failed due to their limited ability to reach the target tissue and/or because they damage both diseased and healthy cells.

Therefore, there is a strong demand for developing in vivo, low invasiveness imaging protocols that can report about the fate of a given nanomedicine.

Besides the delivery of the therapeutic agent to the biological target, very important is also to visualize its release from the nanocarrier.

In this contribution, the most important strategies for designing nanoprobes able to generate an imaging response sensitive to the drug release will be described and discussed.

Emphasis will be mainly devoted on nanovesicular carriers (like liposomes) specifically developed to respond on endogenous or externally applied stimuli able to trigger the drug release.

Notes

day four: thursday january 20, 2011

IMAGING TUMOURS WITH HYPERPOLARIZED MRI

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Nuclear spin hyperpolarization techniques can increase sensitivity in the MR experiment by >10,000x. This allows us to image the location of labelled cell substrates in vivo, following their intravenous injection, and, more importantly, their metabolic conversion into other metabolites. The technique has the potential to offer new insights into tissue and tumour metabolism in vivo. We have been using this technique to detect the early responses of tumours to treatment.Patients with similar tumour types can have markedly different responses to the same therapy. The development of new treatments would benefit, therefore, from the introduction of imaging methods that allow an early assessment of treatment response in individual patients, allowing rapid selection of the most effective treatment [1].

We have been developing methods for detecting the early responses of tumours to therapy, which has included MR imaging of tumour cell metabolism using hyperpolarized 13C-labelled cellular metabolites. We showed that exchange of hyperpolarized 13C label between lactate and pyruvate, in the reaction catalyzed by lactate dehydrogenase, could be imaged in a murine model of lymphoma and that this flux was decreased in treated tumours undergoing drug-induced cell death [2]. We compared this method for detecting treatment response with measurements of fluorodeoxyglucose uptake [3]. We have also shown that hyperpolarized [1,4-13C]fumarate can be used to detect tumour cell necrosis post treatment in this tumour model [4].

More recently we have shown that the polarized pyruvate and fumarate experiments can detect early evidence of treatment response in a breast tumour model [5] and can detect very early responses to anti-vascular [6] and anti-angiogenic drugs. We have also shown that tissue pH can be imaged from the ratio of the signal intensities of hyperpolarized H13CO3- and 13CO2 following intravenous injection of hyperpolarized $H^{13}CO_3^-$. The technique was demonstrated with a study on the murine lymphoma model, which showed, as expected, that the average tumour pH was significantly lower than the surrounding tissue. Since pyruvate, fumarate and bicarbonate are endogenous molecules we expect this technique to translate to the clinic, where it could be used to image disease and response to treatment [7].

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IMAGING INTRA AND INTER-CELLULAR SIGNALING IN VASCULAR REMODELING

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Uni-cellular organisms rely on diffusion for transfer of molecules needed to maintain biological homeostasis. For larger, multicellular organisms, diffusion cannot cover the required distances needed for moving large amounts of material from organs specialized in food uptake and breathing, to the sites of their consumption. The primary role of the vasculature is to sustain tissue homeostasis by providing the routes for fast flow. However, needs are never static, and the vasculature must be able to maintain homeostasis in face of acute and chronic changes in tissue requirements. To accommodate these needs, the vasculature can adapt to acute changes by rapid functional responses, and can show high stability and capacity for significant plasticity as required for coping with chronic changes. Over the last years the mechanisms by which hypoxia triggers changes in blood flow and remodeling of the blood microvasculature by angiogenesis and vascular regression were elucidated. While blood vessels critically impact tissue (or tumor) survival and growth, it is increasingly recognized that proper function of the lymphatic vasculature is critical for prevention of edema and tissue damage. Similar to blood vessels, the lymphatic microvasculature must also be able to respond to microenvironmental cues.

However the mechanisms controlling homeostasis of the lymphatics are not well known. We apply molecular imaging tools for analysis of molecular pathways that control acute and chronic changes in remodeling and function of the lymphatic and blood vasculature.

HYPERPOLARIZED 13C MRS DETECTS EARLY CHANGES IN TUMOR METABOLISM FOLLOWING ANTI-ANGIOGENIC THERAPY

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Introduction: Targeting of tumor vasculature is an attractive option in cancer therapy, as the development of a blood supply is a rate limiting step in tumor progression [1]. Anti-angiogenic drugs block the growth of new tumor blood vessels; early response to these agents cannot be assessed by standard measures as they rarely evoke a change in tumor size [2]. Detection of response has therefore focused on metabolic and functional imaging, including Dynamic Contrast Enhanced MRI (DCE-MRI) measurements of tumor perfusion [3]. Previous work in our laboratory [4] has shown that a decrease in the flux of 13C label between hyperpolarized [1-13C]pyruvate and lactate, kP, is an early indicator of treatment response to the vascular disrupting agent Combretastatin-A4-Phosphate in murine lymphoma tumors, while hydration of hyperpolarized [1,4-13C]fumarate to malate correlates with tumor cell necrosis in the same model [4]. In the present study, we investigate whether these hyperpolarized substrates can detect response in two colon cancer models treated with the anti-angiogenic drug, Bevacizumab.

Methods: [1-13C]pyruvate and [1,4-13C]fumarate were hyperpolarized as described previously [4] and administered to mice bearing xenograft colon carcinoma tumors established by s.c. injection of 5x10^6 LoVo or HT29 cells. Animals were treated with 5mg/kg Bevacizumab at 0 and 48 h (to simulate twice weekly dosing); experiments were performed at 24 h after each dose (24, 72 h). All tumors were excised for histology; tumor enzyme activities and metabolite concentrations were measured in a separate cohort.

Results: Both hyperpolarized markers were sensitive to early metabolic changes following Bevacizumab treatment. In HT29 tumors, kP rose 1.7-fold after treatment, while in LoVo tumors, kP fell by nearly 40% (p=0.02). The differential tumor response stems from the greater vessel density and pericyte coverage of HT29s compared to LoVos (5). In support of this, biochemical assay of lactate concentration showed a 30% increase after treatment from 4.2±0.8µmol/g wet wt. to 5.5±0.8µmol /g wet wt. (p=0.07) in LoVo tumors indicating a hypoxic response but no change was observed in HT29s. Treatment did not induce tumor shrinkage in either model; kP was correlated with tumor size in the non-responding HT29 tumor (r^2=0.72). Furthermore, the ratio of malate to injected fumarate increased more than 3-fold after treatment in LoVo tumors (p=0.05). No background malate was seen in untreated HT29 tumors, but following Bevacizumab treatment, a low level of malate was observed (p<0.01). H&E stained tumor sections confirmed these findings, with both tumors exhibiting small abnormal or necrotic areas at 24 h, which were larger and had expanded by 72 h in the LoVo tumors.

Conclusion: These results suggest that hyperpolarized markers may inform on acute responses to anti-angiogenic therapy and more importantly, provide a means to differentiate responders and non-responders at an early stage in the treatment time course.

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6 YEARS LONGITUDINAL MRI AND 1H SINGLE VOXEL MRS FOLLOW-UP IN 26 PATIENTS WITH TUMOR

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Purpose: to better understand glial tumor metabolism and post chemotherapy, radiotherapy and antiangiogenic variation. To determine cerebral variation in MRS area, amplitude, and ratios of metabolites and spectral profiles during a 6 year longitudinal follow-up in 26 patients with oligodendroglial tumors (12) or gliomatosis (14) without hyperperfusion initially and treated with Temodal and to detect differences in infiltration, proliferation or lipids. Gliomatosis Cerebri (GC) is a challenging tumor, considered to have a poor prognosis and poor response to treatments.

Methods : MRI: Sagittal T1, axial proton density, T2, FLAIR, diffusion, 3D T1 3 planes after gadolinium. MRS : 1H, single voxel (6 to 12 cm3), PRESS with multiple TEs on a 1.5 T (GEMS) . Over 26 patients, 14 underwent radiotherapy and 5 antiangiogenic therapy.MRI. Data processing : SA/GE software and home-written automatic processing (SCI-MRS-LAB in Scilab cINRIA-ENPC open source code) yielding amplitudes, areas, ratios, and relative concentrations. Statistical analysis of longitudinal spectroscopic data (every 3 months over 78 months).

Results : quantitative studies in MRI with multi-spectral segmentation and tissular classification are ongoing. Without chemotherapy spectroscopic profiles worsen with increases in Choline/N-Acetyl-Aspartate (Cho/NAA), Cho/Cr and Myoinositol/Creatine (mI/Cr) ratios, decreases in NAA/Cr and sometimes with increases in lactate. After chemotherapy treated tumoral volumes, in MRI, change little between two exams while spectroscopic profiles and ratios do change. MRS could, in fact, be more sensitive than MRI and could, in some cases, be predictive of worsening. The water and creatine are quite stable, which could justify using them for some other ratios to quickly detect spectroscopic variations. Cho concentration increased in 6 patients with aggravation later in 4 gliomatosis and 2 oligodendrogliomas. There is also decrease Cho concentration in 4 patients before clinical improvement and MRI volumetric response. Spectroscopic and metabolic changes often come well before clinical deterioration and sometimes before improvement. Therefore, MRS could be more sensitive and could detect changes earlier than MRI and sometimes is predictive. Later in the evolution for 5 patients with hyperperfusion this one disappears but proliferation stayed very important.

Discussion and Conclusion :Temozolomide was well tolerated. MRI remained stable for all patients, except for two late partial responses. MRS showed variable ratio of mI/Cr, Cho/Cr and NAA/Cr at baseline. We observed a decrease in Cho/Cr ratio and an increase in NAA/Cr ratio for patients whose clinical condition improved and inverse results for those whose conditions deteriorated. These spectroscopic and metabolic changes occurred well before clinical deterioration and just before improvement. MRS allows non-invasive follow-up of treated cerebral tumors. There is a large variability, but repetition and modelisation of spectroscopic measurements during longitudinal follow-up could allow us to diminish it and to improve prognostic evaluation especially in some cases under antiangiogenic therapy. Studying the relationship between MRS measures, methionine PET, segmentation and perfusion parameters could lead to better understanding of therapeutic response, especially with regard to chemotherapy, radiotherapy and antiangiogenic molecules and in the future hypoxia modulators.

MAGNETIC RESONANCE IMAGING PERMANENT MAGNETS

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Introduction: There are many cases where the sample, object or subject under investigation, have spatial requirements that cannot be fulfilled with standard superconducting MRI magnets and the need for portable devices becomes apparent. Permanent magnets are well suited for portable, dedicated and low cost medical applications. However the theory of making homogeneous permanent magnets for MRI was not developed and only few prototypes were available.

Methods: We present an approach to portable magnet design based on an analytical theory that allows the design of arbitrary magnetic fields inside and outside of a permanent magnet [1]. The control of the field profiles can be mastered at any order, which leads to large regions of interest and deep object-penetration distances. This theory allows us to design, to measure and to correct permanent magnets for making MRI quality magnetic fields.

Results: We will present a low-cost cylindrical permanent magnet producing 0.1T and having high-uniformity (10ppm). This prototype serves as a demonstrator for the theory and for analysis of porous media [2]. We also present the first high-uniformity, linear-gradient (3.3 T/m), one-sided permanent magnet system, which was conceived using our theoretical framework. Measurements show that the magnitude of the field is 0.33 T and its profile is linear up to 5 ppm inside a 10 mm diameter spherical volume, positioned 20 mm from the surface of the magnet. The field can penetrate up to 5 cm inside an object with sufficient uniformity (3%) for diffusion measurements. The point spread function of the magnet is measured to be 15-20 microns [3]. Finally we present the first permanent magnet generating a homogeneous and strong magnetic field (0.22T) pointing along the magic angle [4].

Conclusions: Our novel theory is a powerful tool for designing MRI magnets, which can be easily optimized for organ-specific studies or for multi-modal imaging applications.

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MULTIFUNCTIONAL NANOPARTICLES FOR MR IMAGING

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Nanoparticles are frequently used as carriers for therapeutic drugs but can also be used as part of imaging compounds for various imaging modalities. In this talk, an overview will be given on nanoparticles that were successfully used for MR cell tracking and targeted imaging. 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 targeted imaging of transferring-receptors, αvβ3-integrins and apoptosis. However, there will also be a focus on recent developments of our group, where dual-functional nanoparticles are used for simultaneous fluorescence and MRI imaging. In this context, we will introduce fluorescent USPIO that target the riboflavin carrier protein (RCP), a target which is hardly used in context with imaging but which is highly expressed in activated endothelial cells and some prostate cancer cells. In comparison to USPIO that are targeted to receptors like αvβ3, a very fast and intense uptake of the RCP-targeted nanoparticles is observed, which can excellently be blocked in competition experiments. Cellular uptake of the particles can be confirmed by their fluorescence and the fluorescent label can also be used to detect the labelled cells histologically. The second example is about an MRI-ultrasound hybrid contrast agent. Here, USPIO are embedded into a poly(butyl-cyanoacrylate) microbubble. These microbubbles have excellent acoustic properties and exhibit a strong T2* contrast. By insonnation these microbubbles can be destroyed. This leads to proton access to the USPIO and as a consequence to a strong increase in T1-relaxivity. We consider using this bimodal probe to locally and temporarily increase the vessel permeability (mechanically due to microbubble destruction) and to visualize the amount of permeation by the accumulation of the USPIO using MRI.

NEW PERSPECTIVES FOR IMAGING MOLECULES AND METABOLISM IN VIVO

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Magnetic resonance imaging is subjected to continuous advances and discoveries, much of which are lead by either improved data acquisition techniques and/or higher magnetic fields or the detection of novel principles/contrast mechanisms altogether. This presentation will highlight on one hand the possibility to detect contrast agents at very low concentrations using a new approach to MR, based on hyperpolarized media using dissolution DNP. The potential will be demonstrated especially for long-lived nuclei with long T1, such as Lithium-6, Yttrium-89 and N-15, but also C-13 will be illustrated. The ability to detect low-concentration nuclei or rare spin nuclei opens new possibilities for molecular imaging. On the other side, for imaging molecules (metabolism) recent advances have allowed to attain in rodent brain a spatial resolution of below 1umol at 14.1 Tesla that matches or even exceeds that possible by nuclear imaging modalities, in particular PET. The quantitative nature of the MR spectroscopic imaging approach allows insights into cellular biochemistry and metabolism that complements the capabilities of PET and SPECT and opens new windows on characterizing disease evolution and treatment opportunities, as will be illustrated with the example of ischemia and cancer, among others.

HYPERPOLARIZED ACETATE AS A METABOLIC TRACER FOR SKELETAL AND CARDIAC MUSCLE ENERGETICS

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Introduction: Carnitine plays a fundamental role in regulating fatty-acid and carbohydrate metabolism and buffers the acetyl-CoA pool by rapid conversion into acetyl-carnitine via carnitine acetyl transferase (CAT) [1]. Directly monitoring acetyl-carnitine kinetics is thus instrumental towards unraveling its metabolic pathways and may be a valuable tool for differentiating between healthy and pathological metabolism. It has been demonstrated that the conversion of acetate into acetyl-carnitine can be probed in vivo following the infusion of hyperpolarized 1-13C acetate[2]. The conversion rates are directly correlated with the enzymatic activity of CAT. The aim was to characterize the dynamics of 1-13C acetate metabolism in skeletal muscle in vivo and to deduce the associated kinetics by applying a metabolic model.

Methods: A sample containing 4.5 M 1-13C acetate, 33 mM TEMPO, dissolved in d6-EtOD/D2O mixture was polarized in a DNP polarizer for 2.5 h[3,4]. The sample was dissolved, transferred to a separator-infusion pump and infused into the femoral vein of a rat in 9 seconds. Measurements were carried out on a 9.4T animal scanner. Single pulse acquisitions were recorded with a surface coil using 30° adiabatic RF pulses applied every 3 s with proton decoupling. Modified Bloch equations for one-site exchange were used to fit the hyperpolarized time courses to determine kinetic and decay rate constants.

Results: Time courses of acetate and its metabolic product, acetyl-carnitine, were detected in the skeletal muscle in vivo(n=3). The acetate signal decays 3 seconds after the end of infusion and the acetyl-carnitine signal increases to reach its maximum value at 12 seconds after infusion. Due to the high concentration of acetate following bolus injection, the conversion rate from labeled acetate to acetyl-carnitine was likely maximal. The model reflects the role of acetyl-carnitine as an energy buffer in resting skeletal muscle. The kinetic rate constant k reflects CAT activity, acetyl CoA synthetase activity and membrane transport of acetate, and is therefore an “apparent” rate constant. The decay rate constant of acetate R1,A does not correspond with its spin-lattice relaxation rate because it is a superposition of the inflow of hyperpolarized acetate and the spin-lattice relaxation rate. The peak intensities were fit to the solutions of the modified Bloch equations for one-site exchange. The kinetic rate constant was k = 0.09 ± 0.03 s-1, the characteristic decay rate R1,Ace = 0.012 ± 0.002 s-1 and R1,Acc = 0.093 ± 0.006 s-1.

Conclusion: We deduced the apparent relaxation rates and the kinetic rate constant for the conversion of acetate into acetyl-carnitine in healthy skeletal muscle in vivo. The conversion of acetate to acetyl-carnitine has been shown to reflect disturbed mitochondrial bioenergetics in several tissues [2]. Therefore, local instantaneous measurements of the conversion rate have the potential to distinguish healthy and pathological tissues and offer insight in the kinetics of altered metabolic states.

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SHINING A NEW LIGHT ON BRAIN GLUCOSE METABOLISM USING SYNCHROTRON X-RAY FLUORESCENCE IMAGING

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Regional increases in neuronal activity are correlated with regional increases in glucose metabolism (CMRglc) in mammalian brain, implying that a rapid and effective modulation of glucose metabolism occurs during and following functional responses to neural stimulation (1). Where this modulation occurs at the cellular level is yet unclear and is central to understanding the physiological mechanisms regulating brain glucose metabolism. To address this question, we exploited an emerging imaging method in medecine that operates at length scales reaching subcellular dimensions -- synchrotron based x-ray fluorescence spectromicroscopy -- to map and quantitate the stable fluorine isotope 19F in the phosphorylated deoxy-D-glucose (DG) molecule in glia and neurons in situ. Following exposure in vivo or ex vivo to 10mM 19F-DG under light and dark-adapted conditions (2), retinas and hypothalami from rat were chemically fixed, epon infiltrated, sectioned to 1-4µm thickness and then mounted onto EM grids surrounded by molybdenum. Low-energy x-ray fluorescence (LEXRF) measurements were performed at the Elettra Synchrotron Facility beamline Twinmic in Trieste, Italy with the objective to determine the intensity of secondary photon fluorescence emitted by 19F, carbon and oxygen; and their distribution through the laminar structured light-sensing retina and glucose-sensing hypothalamus. The x-ray microprobe exploited a Fresnel zone plate with 320µm diameter and an outermost zone width of 50nm. The sample was raster-scanned with respect to the microprobe, simultaneously collecting the emitted and transmitted photons using an annular array of 4 silicon drift detectors, while a neighboring CCD camera acquired phase contrast images of the scanned area (3). The fluorine signal was optimized by tailoring the photon beam (900eV) illuminating the zone plate. LEXRF maps ranged from 600 to 1100µm2 (600 to 1100 pixels2) with 45-60s dwell times/pixel. Deconvolution per pixel of the fluorescence energy emission spectrum, extending from 0.2 to 1.0 keV, was performed using the PyMCA Hypermet algorithm. Enzymatic spectrophotometric assays showed that DG in brain samples was predominantly phosphorylated, thereby confirming transport of 19F-DG into metabolically active cells and its subsequent transformation to 19F-DG6P by hexokinase. In LEXRF spatial maps of 19F, we detected 19FDG6P moreso in the radial glial cells than in neurons during glutamate neurotransmission in the retina, and neuroglycopenia in the hypothalamus. As expected, the intensity of secondary photon fluorescence emitted by carbon and oxygen remained relatively constant from both brain regions. However, that of fluorine was related to the glial cell volume. Given that almost all oxidative metabolism is fuelled by glucose combustion, it is a direct consequence of this study that fuel transport from the glia to the high oxidative capacity neurons must occur. We conclude that the approach used here will be able to address the controversy concerning the compartmentation of glucose metabolism in other brain regions.

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OPTICAL IMAGING OF ORAL SQUAMOUS CELL CARCINOMA AND CERVICAL LYMPH NODE METASTASIS

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Background: In head and neck cancer surgery, intraoperative optical imaging using near-infrared fluorescence imaging could help the surgeon to determine adequate tumor-free margins.[1]

Methods Tumor-specific near-infrared fluorescence agents targeting epidermal growth factor receptor (CW800 EGF)[2] or glucose transporter system (CW800 2-DG)[3] were administered to mice with tongue carcinoma and cervical lymph node metastases. Tumor growth was followed noninvasively by bioluminescence imaging. Fluorescence signals were compared to a control group of mice without tumor and to surrounding healthy tissue within histological sections.

Results Significantly higher fluorescence signals were found in tongue tumor and cervical lymph node metastases compared to control animals. Fluorescence correlated with histopathology. Tumor-to-background ratios ranged between 13.8 - 15.7 (CW800 EGF) and 4.6 - 33.9 (CW800 2-DG).

Conclusions Optical imaging using near-infrared fluorescence agents can be used to detect oral cancer and cervical lymph node metastases. This technique has potential to improve complete surgical resection of oral cancer by real-time image-guided surgery.

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TRANSLATIONAL OPTICAL IMAGING IN ONCOLOGY: FACT OR FICTION

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In recent years, significant progress has been made in both optical imaging systems and fluorescent contrast agents for clinical applications. NIRF imaging with a free-floating imaging device mounted on the ceiling of the operating theatre or on a microscope articulating arm during surgery for cancer will enable visualization of tumor delineation, locoregional metastases, remnant disease as well as e.g. tumor-containing lymph nodes. Hereby, the surgeon can both detect (diagnostic) and excise (therapeutic) malignant tissue and possible residual disease at the same time. The use of NIRF optical imaging has a range of advantages. Most prominent among these is the fact that it is very safe technology, simple to operate, fast, high resolution (as low as 10 µm), relatively inexpensive and makes use of non-ionizing radiation. Based on the above, it is clear that intra-operative imaging is on the verge of entering standard clinical practice for surgery. Not only the imaging system but also the availability of clinical grade tumor-targeted probes is of the utmost importance for a succesful introduction into clinical practice. This talk will give an overview of the current concepts and future perspectives of intraoperative fluorescence image-guided surgery using non-targeted and targeted optical contrast agents for the first-time ever used in patients with ovarian cancer and the anticipated developments within the next 5 years. It will become clear that Europe will play a major role in bringing this technology into the clinic and the necessity of standardization of methodology when applied in multicenter studies.

NEAR-INFRARED FLUORESCENCE DETECTION OF SENTINEL LYMPH NODES: THE GREEN LIGHT STUDIES

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Background: Near-infrared fluorescence (NIRF) imaging is a technique that can provide real-time image guidance during surgery. Advantages of NIRF light (700-900nm) include high tissue penetration up to several centimeters deep and low autofluorescence. Therefore, NIRF imaging has the potential to improve the sentinel lymph node (SLN) procedure by facilitating percutaneous and intraoperative identification of lymphatic channels and sentinel lymph nodes. Indocyanine green (ICG) is currently the only FDA and EMEA approved NIRF lymphatic tracer. The fluorescence characteristics of ICG are improved when ICG is adsorbed to human serum albumine (ICG:HSA). The aims of our dose-finding studies were to test feasibility and to identify the optimal dose of ICG:HSA for intraoperative SLN identification using NIRF light.

Methods: Twenty-four consecutive breast cancer patients planned to undergo SLN procedure were included (mean age = 59.5, tumor size = 15 mm, body mass index = 25). Patients underwent standard of care SLN procedure: injection with 99Technetium-colloid and patent blue. In addition, 1.6 mL of ICG:HSA was injected directly after patent blue. Patients were allocated over 8 escalating ICG:HSA concentration groups (50-1000 µM) each containing 3 patients. For the NIRF SLN detection the Mini-FLARE™ camera system was used, which is capable of displaying NIR signal and visible image simultaneously and can superimpose the NIR signal over the color image. Similar studies were performed in, colorectal cancer, melanoma, bladder cancer, cervical cancer and vulvar cancer patients.

Results: In all patients (n = 24), NIRF imaging enabled visualization of lymphatic channels in the breast and SLN. A total of 35 sentinel lymph nodes (mean = 1.45, range = 1-3) were detected: 35 hot, 35 fluorescent, and 30 blue. In all patients, the NIRF signal in the SLN was detected through the axillary fat considerably earlier than patent blue. Optimal injection dose of ICG:HSA ranged from 400 -800 µM. In the 600 µM concentration group, the NIRF signal of the SLN was on average 17.4 ± 2.7 times higher than the surrounding axillary fat tissue. Similar results were obtained in the above mentioned cancer types.

Conclusion: This study demonstrated feasibility to detect the SLN in cancer patients using ICG:HSA and the Mini-FLARE™. Moreover, an ICG:HSA concentration window between 400 and 800 µM was found to be optimal for SLN mapping.

SURGICAL FLUORESCENCE IMAGING WITH TARGETED FLUOROCHROMES: ADVANCING TOWARDS 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.

Notes

day five: friday january 21, 2011

APPLICATION OF IMAGING IN THE DRUG DEVELOPMENT PROCESS: ADVANTAGES AND LIMITATIONS FROM AN INDUSTRIA

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Different imaging modalities are used to monitor disease progression and to analyze therapeutic efficacy in preclinical models. Each of these different methods have their advantages and limitations and no single imaging modality provides the tool to address all the questions arising in the drug development process. Near-infrared fluorescence (NIRF) based optical imaging is an excellent imaging technology for preclinical research because of its simplicity, fast scanning times, non hazardous radiation or radioactive isotopes and reasonable costs of equipment. NIR dyes have favourable photophysical properties, autofluorescence of tissue and scattering is low, and NIR wavelenghts penetrate effectively tissue. In addition, the application of different near-infrared labels enables multiplexing and probes specially designed facilitate the simultaneous observation of different biochemical and molecular events.

Excellent reviews describe the application of non-invasive imaging in the preclinical setting however, only limited data are available demonstrating how imaging (optical imaging) has improved the drug development process. We will summarize the advantages of optical imaging in preclinical cancer research and how this modality has supported project-related decisions. In addition we will demonstrate the pitfalls of this imaging modality which may lead to misinterpretations of data.

We have applied optical imaging (planar reflectance and tomographic reconstruction) without or in combination with micro-CT for pharmacokinetic and pharmacodynamic studies. Before the initiation of an in vivo imaging experiment we check if the labeled protein exerts the same binding characteristics and biological activity as the non-labeled and if the labeling efficacy has an impact on different parameters. After these quality controls we evaluate e.g. if a monoclonal antibody targeting a tumor-associated cell surface antigen exert specific binding to a s.c. or orthotopic implanted tumor. A strong fluorescence signal in the region of interest (tumor) may be generated by an unspecific accumulation of the labeled mab in necrotic areas, in cysts or by unspecific binding to stroma cells always present in the tumor. Such studies are mandatory to verify specific binding of a therapeutic mab to tumor tissue in vivo and only after confirmation are subsequent efficacy and kinetic studies justified. Optical imaging provides useful data in supporting a rationale for the combination of therapeutic antibodies. Tumor cells stably transfected with red fluorescence proteins are ideal to monitor therapeutic efficacy of new drugs when classical tumor volume measurements are difficult to perform (e.g. orthotopic models). In combination with µ-CT, optical imaging can successfully applied to analyze target expression of metastatic cells in secondary organs (e.g. lung). Regarding pharmacodynamic studies we will provide results of different approaches to monitor apoptosis and to monitor angiogenesis. Pharmacokinetic studies of mabs, F(ab)2, Fab or Fc fragments can be easily performed by optical imaging. Planar reflectance optical imaging – despite its limitations in penetration – provides meaningful information in pharmacodynamic and pharmacokinetic studies. Because of short acquisition times high-throughput and repeated measurements with high sensitivity are possible. Transillumination studies (FMT) require an appropriate reconstruction software and are more time-consuming, but quantification of fluorescence signal intensities is feasible.

INVESTIGATING CHRONIC LIVER DISEASES AND CANCER BY MULTIMODAL SPECTROSCOPY

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Alcoholic or metabolic fatty liver diseases, viral chronic hepatitis progressively lead to cirrhosis and hepatocellular carcinoma. The physiopathology of these liver diseases is highly complex. Multimodal spectroscopic analyses were performed on tissue sections from human normal livers, steatotic livers, cirrhotic livers and hepatocellular carcinoma to image the distributions of the biochemical components in these different pathologies. Serial tissue sections of 5-6 µm thick were alternatively stained by H&E for histological evaluation and used for spectroscopy experiments. Synchrotron microspectroscopy was performed using the beamlines SMIS (IR), DISCO (UV) and LUCIA (X-ray) at SOLEIL.

Infrared (IR) spectroscopy on normal liver was able to reveal metabolic liver zonation. IR spectra were acquired in both periportal and centrilobular areas and then analyzed using statistical method based on principal component analysis (PCA). Interestingly, data analysis of IR spectra allowed distinguishing a class of spectra specific to centrilobular hepatocytes. Moreover, major differences observed by this approach on centrilobular and periportal hepatocytes were focused on lipid content. Imaging the distribution of lipids on a large map on lobular hepatic led to visualize a gradient of distribution of lipids.

Spectroscopic studies were further applied to pathological conditions such as non alcoholic steatosis, cirrhosis and hepatocellular carcinoma. IR microspectroscopy on steatotic livers detected the presence of unsaturated lipids into steatotic vesicles in a particular lipid environment. The concentration of unsaturated lipids into steatotic vesicles which has not been hitherto demonstrated constitutes a potential highly reactive trigger for lipid peroxidation which is known to produce important molecular and cellular damages through iteratively propagated radical reaction. The molecular mechanism underlying the formation of lipid droplets has never been described and will have to be addressed in further studies. In addition, IR microspectroscopy investigations performed on the non steatotic areas of the fatty liver using has revealed important changes when compared to the normal liver; these results suggest interesting diagnostic applications.

Synchrotron IR microspectroscopy of cirrhotic livers and hepatocellular carcinoma followed by data analysis using PCA has allowed distinguishing a discriminating spectral signature for cirrhotic and tumor hepatocytes. Synchrotron-FTIR and -UV imaging can share the same tissue sample holder. Indeed, a tissue section from a cirrhotic liver was deposited on a gold coated glass slide and was firstly analyzed by FTIR microspectroscopy in order to image the distribution of lipids, proteins, sugars and nucleic acids. This technique has identified collagen enrichment in fibrosis whereas esters were mostly distributed into the cirrhotic nodules. The same section was subsequently investigated using synchrotron-UV microspectroscopy that allowed visualizing high autofluorescence confirming the presence of collagen in fibrous septa. Finally, X-ray microspectroscopy analysis of the same sample was performed that allowed observing a high level of iron in hepatocytes and calcium in fibrous septa.

Altogether, these results demonstrated that multimodal spectroscopy is a powerful approach for characterizing subtle biochemical changes associated with various liver pathologies.

INTRAOPERATIVE IMMUNOPHOTODETECTION TO IMPROVE PERITONEAL SURFACE MALIGNANCY DIAGNOSIS AND TREATMENT

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Twenty to thirty percent of the patients suffering from digestive cancer will develop locoregional recurrence as a unique relapse. It was shown that resection of the totality of tumour nodules and their disseminations is the most important factor influencing both post-surgery prognosis and patient survival. Nevertheless, the identification of tumor tissue is extremely difficult, especially when patients have been following neo-adjuvant treatments prior to surgery. Surgeons are then only guided by their visual and tactile senses, and by their experience; there is no intraoperative technique available at the moment to help them visualise the extensions of the tumor. The aim of our project is to provide oncologist surgeons with an innovative real-time intraoperative detection and diagnosis solution, based on the technique of immunophotodetection (IPD) that combines the specificity of monoclonal antibodies with the sensitivity of fluorescent detection. Based on our original data, (Pèlegrin A, et al Cancer 1991 67(10):2529-37), a further evaluation of the technique demonstrated its utility in LS174T human colon carcinomatosis bearing nude mice (Gutowski M, et al Clin Cancer Res 2001 7:1142-1148).

The fluorescent status of 333 biopsies was compared with their histological analysis. Sensitivity was 90.7%, specificity was 97.2%, the positive predictive value was 94.7%, and the negative predictive value was 94.9%. Detection of very small nodules (<1 mg in weight or <1 mm in diameter) was possible. The sensitivity being 100% for nodules >10 mg was still high (78%) for nodules <1 mg. This study demonstrated that intraoperative IPD is easy to use and associated with high sensitivity and specificity, even for low tumor masses. Recent developments of the technique allowed us to produce optimized conjugates that are extremely specific towards tumor cells, and that display physicochemical characteristics allowing their use in a clinical setting. By offering a genuine “optical biopsy” to surgeons, we aim to assist them for the resection of cancer cells, and allow a better grading of the disease: sub-clinical size nodules, invisible to the expert eyes of the surgeon, indeed light up and can be resected. This technique will also assist surgeons in the identification a tumor’s precise delimitation, allowing them to precisely orient the resection and to limit it to tumor tissue. Our first clinical grade MAb-dye conjugate was designed for colorectal tumors visualization. We are currently developing the cGMP production process and assessing the absence of toxicity of this molecule that should get to the clinic very shortly. Phase I assays shall take place in Monpellier Comprehensive Cancer Research Center (CRLC). Using a newly designed antibody, a second conjugate is under development for ovarian cancers and should be used in the clinic shortly after the colorectal conjugate.

NEW MULTISPECTRAL IMAGING METHOD FOR FLUORESCENCE MOLECULAR DIAGNOSTICS

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Introduction: Optical imaging is an ideal modality for in-vivo diagnostics since it correlates perfectly with the human view and perception (field of view, coordinate system) and is using non-ionizing radiation. Fluorescence molecular probes are increasingly being developed in the last years and open new gateways in disease detection and therapeutics. Specific and unspecific markers gradually become popular for intra-operative use as they offer good localization, high contrast and allow real time feedback to the surgeon. Multispectral imaging has the potential to further increase the information provided by the fluorescent agents (auto-fluorescence subtraction, multiple probes’ detection, etc.) and thus exhibits superior performance compared to single spectral band measurements. The performance of the existing multispectral technologies[1, 2] is mainly limited by a) the number of spectral bands they can capture, which is derived by the number of imaging channels they possess, and b) the complexity and size of their optics which usually results in big and bulky devices.

Methods: The algorithm that we used for the spectral decomposition is a generalization of a method presented before [3]. The central idea is that color CCD cameras when combined with optical filters (multiple band pass filters in this case) are able to capture multiple spectral images, whose content is defined by the spectral characteristics of those filters. Our setup consists of a CCD camera, a filter wheel and a zoom objective lens. A white light source is used for visible light illumination and diode lasers for fluorophore excitation. In this occasion we chose to focus on 2 applications of this technology, namely the simultaneous capturing of a) multiple spectral images and b) reflectance and fluorescence images using a single camera.

Results: We describe the implementation of these methods combined with specific molecular probes. Our preliminary results demonstrate the capability of our imaging system to simultaneously capture multiple spectral images with a single CCD camera. With this technique image registration and merging becomes a straightforward procedure without the use of complicated algorithms. Furthermore, we report concurrent capturing of fluorescence and reflectance (color) video streams in an animal disease model using specific fluorescence markers. In this way our system can be set to provide both anatomical and functional information using a single CCD camera.

Conclusions: We present the development of a novel multispectral imaging method that captures multispectral information using only a single CCD camera. The key advantage of the approach is the simple implementation in terms of hardware complexity, form factor and weight that can correspondingly lead to low-cost applications.

References:[1] Themelis G et al (2009), J Biomed Opt;14(6):064012.[2] Troyan SL et al (2009), Ann Surg Oncol 16:2943–2952.[3] Themelis G et al (2008), Opt Let Vol. 33, No. 9.

NEAR-INFRARED FLUORESCENCE IMAGING OF COLORECTAL LIVER METASTASES USING INDOCYANINE GREEN

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Background: 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 (1). The current study aims to optimize dosage and timing of ICG administration.

Materials and 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. Guided by these results, intraoperative identification of liver metastases was performed in 12 patients undergoing liver resection. NIR fluorescence imaging was performed 24 or 48 hours after administration of 10 or 20 mg ICG. 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 = 18). Average TLR was 11.1 ± 5.1 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 two patients, additional small (2 – 8 mm) metastases were identified that were missed preoperatively and intraoperatively using visual inspection and ultrasound. In all patients, a fluorescent rim around the tumor could be identified during ex vivo sliced resection specimen measurements, 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.

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. As described above, no differences in time of imaging and doses were found. Therefore, due to logistic motives, administering 10 mg ICG 24 hours preoperative would be preferable to perform NIR fluorescence guided liver surgery.

APPLICATION OF FLUORESCENCE IN SURGERY; EXPECTATIONS AND REALISTIC APPROACHES

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Intra operative fluorescence imaging is on its way to clinic and few systems are already available for clinical practice. . The first clinical applications of intra operative imaging relies on already known injectables fluorescent molecules: Indocyanine green, 5-aminolevulinic acid-induced (ALA), methylene blue and fluoreceine. The main applications are based on indocyanine green (ICG) because it is the only near infrared fluorescent molecule that is authorized for injection to human and it present a negligible level of adverse reaction to IV injection. Some targeting molecules are undergoing regulatory tests and are expected to be on the market by 3 to 5 years. The first clinical trials with these targeting molecules will address mainly tumor margin delineation and small metastasis detection.

Balancing the potential benefits for the patient against the potential risks is the first step in developing sound clinical applications for intra operative fluorescence. Nethertheless some issues such as the integration of the technique in the surgical room workflow, challenging the fluorescence approach with existing and validated intra operative techniques and evaluation of the number of relevant clinical case that will potentially be handled by intra operative fluorescence imaging must be addressed.

Taking into account this context and the fact that in continuous wave fluorescence reflectance imaging the penetration remains low, about 4mm for tumor imaging and 1cm for vascular or lymphatic drainage, we selected applications for real-time intra-operative fluorescence imaging and performed it with the Fluobeam®800 in classical surgical condition on large animals. Using ICG we present analysis of stenosis and anastomosis in vascular surgery and show that fluorescence can provide accute hemodynamic measurements. We present application of lymph node mapping on mastocytomes on dogs and tumor margin delineation obtained on rats.

These results confirm the pertinence of the fluorescence approach in the surgical environment and show that it can address molecular imaging application as well as classical imaging problem usually addressed by other cumbersome techniques.

HIGH RESOLUTION REAL-TIME IMAGING OF SMALL ANIMALS BY MULTISPECTRAL OPTOACOUSTIC;; OMOGRAPHY (MSOT)

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Small animal molecular imaging plays an important role in basic research of disease and development of therapeutic strategies. Multispectral Optoacoustic Tomography (MSOT) is an emerging technique for high resolution imaging of optical absorption and molecular contrast at depths of several centimetres in-vivo. The technique has an intrinsic sensitivity to hemoglobin, enabling high contrast visualization of the vascular tissues of interest in studies of cardiovascular disease and cancer. Additionally, the multispectral detection of chromophoric agents, including commonly available fluorescent dyes and emerging light absorbing nanoparticles, based on their spectral absorption signatures, enables molecular contrast. We present results from an MSOT system recently developed for small animal imaging studies. Based on multispectral near-infrared excitation and 64-element ultrasound detection, it is capable of generating tomographic transverse slices at a frame-rate of 10 images per second, attaining a resolution of approximately 150 microns in the image plane. Anatomical features relevant to cardiovascular disease, such as the carotid arteries, the aorta and the heart, have been imaged in mice, proving the ability to resolve features in the order of hundreds of microns deep below the skin surface. For imaging of the heart, the system's fast acquisition time (tens of microseconds for a single slice) allows images virtually free of motion artifacts from heartbeat and respiration, allowing the heart wall to be distinguished from surrounding tissues. In studies of tumor xenografts, we were able to not only visualize newly formed tumor vascularization, but also spatially distinguish blood oxygenation differences and contrast agent distribution throughout entire tumor volumes. We present in-vivo results of studies demonstrating these unique contrast capabilities at high spatial and temporal resolution, thus paving the way for molecular imaging applications.

LISTENING TO LIGHT AND SEEING BETTER

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The ability to optically interrogate and visualize intact organisms is beneficial due to the great variety of intrinsic optical contrast and exogenous molecular probes available in the visible and near-infrared spectra. With the recent introduction of a new hybrid imaging technology, termed multispectral optoacoustic tomography (MSOT), it has become possible to visualize, with high resolution, optically diffusive organisms whose sizes may vary from sub-millimeter up to a centimeter range and more. The size of many relevant biological samples and model organisms, e.g. developing insects, fishes, embryos, and adult small animals, lie in this range. By applying multispectral imaging approaches, it has been demonstrated that other molecularly-relevant information related to biodistribution of optical reporter agents and gene expression could now be visualized in whole bodies of opaque living objects with high sensitivity and spatial resolution close to a single cell dimensions. The talk will cover both algorithmic and technical challenges of the MSOT method as well as its current and potential applications in biological and medical research.

My Notes

EMIM2011 Do not forget: 6th annual meeting of the ESMI
Leiden from June 19-21,2011 WWW.ESMI2011.EU

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