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# Abstracts of Scientific Papers

## 2010 AISAL Symposium

### Imaging and Translational Medicine

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#### Main Lectures

##### Advanced Neuroimaging Techniques to Investigate Neurodegenerative Dementias: Clinical and Preclinical Data

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Thanks to its ability to image soft tissue in vivo noninvasively with detailed anatomic resolution, MRI has shown high sensitivity in detecting macroscopic abnormalities in several neurologic conditions. However, when patients present with cognitive decline, conventional MRI does not provide any substantial support in the diagnosis of degenerative dementias. Over the last few years, several advanced MRI techniques have been introduced to assess brain tissue modifications at both a macro- and microscopic level. Voxel-based morphometry (VBM) is a spatially-specific and unbiased method of analysis of MR images reflecting regional grey (GM) and white matter (WM) volumes at a voxel scale. Specific areas of decreased GM density were demonstrated in the brain of patients with Alzheimer disease (AD) at different stages, and different patterns of GM loss have been associated with the risk of a more rapid evolution to dementia. Diffusion tensor MRI (DT-MRI), by providing rotationally invariant measurements of both magnitude and directionality of water diffusion (mean diffusivity and anisotropic indexes), has clarified the critical role of WM damage in neurodegenerative dementias, introducing the concept of brain disconnection. Specific patterns of WM damage have been reported in the 2 main causes of dementia, AD and dementia with Lewy bodies, whose differential diagnosis still remains a challenging issue for clinicians. Functional MRI allows the brain activation to be investigated in vivo while performing neuropsychologic tasks. A recent investigation in patients with amnesic mild cognitive impairment, which is considered as a prodromal stage of AD, has demonstrated the ability to detect changes of brain functioning preceding the appearance of cognitive deficits. Another interesting fMRI approach is the so-called 'resting-state' fMRI, which provides measures of functional brain connectivity. The combination of VBM and 'resting state' fMRI applied to a group of patients with AD at different clinical stages, has recently shown how functional disconnection in the posterior cingulate gyrus precedes the occurrence of GM loss in the same anatomic area. This evidence supports the idea that the primary involvement of GM tissue is only one aspect of AD pathology, and that brain disconnection is likely to be responsible for some critical steps of AD evolution. Indeed, it is conceivable that GM involvement of the medial temporal lobes causes the memory

deficits observed in AD at early stages. With the progressive worsening of the disease, this regional GM loss in temporal areas may cause (in its turn) a deafferentation of the posterior cingulate gyrus, which will eventually become secondarily atrophic.

##### Biomarkers in Neuroimaging of Brain Disorders: from Mice to Men

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Imaging techniques can be used to noninvasively assess different aspects of neuroanatomy, neurochemistry, physiology, and pathology, providing indicators correlated to activity, amount, and characteristics of degenerative, neoplastic, and inflammatory processes and to neurotransmission. Imaging biomarkers could also be used to improve the selection of innovative therapeutic approaches by demonstrating early structural and functional changes associated with drug administration. Imaging biomarkers are, therefore, being extensively tested in the assessment of many degenerative brain disorders, such as Alzheimer and Parkinson disease and inflammatory diseases, such as multiple sclerosis, brain tumors, and neurodevelopmental abnormalities. Diagnostic imaging modalities can be classified in 2 groups: 1) those providing predominantly structural information, such as magnetic resonance imaging (MRI), computed tomography (CT), and ultrasound (US), and 2) those providing mainly functional information, such as positron emission tomography (PET), single photon emission computed tomography (SPECT), and optical imaging. However, in addition to structural information, MRI can also provide in vivo functional data regarding tissue perfusion, water diffusion, and tissue chemistry, using magnetic resonance spectroscopy (MRS) applications. MRI can provide volumetric indicators of global and local brain degeneration (atrophy rates), as well as the amount of damaged white matter. PET, SPECT, and optical imaging can provide measurements of biologic functions, such as glucose and oxygen utilization, protein and nucleic acid synthesis, detection of cell or tissue specific molecules and receptors, and allow the study of neurotransmission process. In brain tumors, size is measured with MRI, tumor metabolism and proliferation with PET, angiogenesis and blood flow with contrast-enhanced MRI or PET; while MRS can provide information concerning specific metabolite patterns and their modifications following treatment. Imaging biomarkers could be helpful in addressing the inherent limitations associated with conventional

endpoints in both preclinical and clinical trials, increasing the possibility of detecting small effects and reducing sample size. The use of imaging biomarkers in the study of animal models of CNS diseases can be particularly helpful when conducting longitudinal studies by reducing research costs, saving time, and providing valuable follow up data in a relatively short time frame. However, imaging of small animals, in particular mice, requires dedicated equipment, given the smaller size and the need of high spatial resolution to obtain results comparable to those achievable in humans. A multiparametric structural and functional biomarker approach, with the assistance of translational studies, seems to be the most promising methodology to improve diagnosis and prognosis, and to develop innovative and patient-tailored therapies in brain disorders.

### **Molecular Imaging for Drug Development in Translational Cancer Research**

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The identification of molecular pathways playing a key role in tumor growth and progression and the unraveling of human genome provided a plethora of new targets for drug development in oncology. In addition to conventional anticancer drugs, new compounds directed against molecular targets, such as growth factors, receptors, enzymes, and other mediators of cell growth and apoptosis, are now available and many others are being developed. Proving the clinical efficacy of these new drugs interacting with molecular targets has 2 important implications. The first is that not all patients are eligible for targeted therapy, but only those in whom the target is expressed. The second is that drug-target interaction results in the inhibition of target function, which in turn causes a cytostatic effect rather than a cytotoxic effect. Therefore, the classic criteria of tumor response based on reduction of tumor burden may not be appropriate for the evaluation of efficacy of targeted therapy. Recently, the development of imaging technologies, including hybrid systems such as PET/CT, allowed the visualization of biochemical, molecular, and physiologic pathways in tumors and organs of patients and animal models. In vivo evaluation of complex biologic processes such as proliferation, apoptosis, angiogenesis, metastasis, gene expression, receptor/ligand interactions, transport of substrates, and metabolism of nutrients in human cancers is feasible by using PET/CT and radiolabeled molecular probes. These imaging technologies can be employed in preclinical and clinical experimental settings for establishing drug efficacy, thus accelerating the drug development process. Here we provide prominent examples of how molecular imaging can be used for drug screening, how patients are selected for given treatments, real-time monitoring of therapy, early detection of drug resistance, and, finally, for tailoring therapy.

### **Preclinical Molecular Imaging**

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Positron emission tomography (PET) allows quantification of different metabolic/biologic functions and acquired a relevant role in the diagnosis of many forms of human tumors. It provides functional data on disease extent, response to therapy, and early identification of recurrence, contributing, in many cases, to changes in patients' clinical management. Animal models of human tumors are useful tools to study mechanisms underlying human disorders. Small animal PET (SA-PET) presents several advantages: it is whole-body and noninvasive, provides functional data of biologic processes in all body tissues and organs, and can be repeated in the same animal over time, allowing a reduction of the number of animals employed and limiting inter-animal variability. Moreover, SA-PET provides an accurate characterization of different biologic processes, such as metabolism, cell surface receptor expression status, apoptosis, angiogenesis, and gene expression; possesses high sensitivity; and allows the detection of viable tumor cells at early stage. SA-PET images allow a direct comparison of the metabolic changes in a given lesion over time or in response to a potentially curative drug, representing a major advantage over other imaging technique. Actually, SA-PET early tumor engraftment in experimental models is crucial for the selection of tumor-bearing candidates to be treated with new drugs since small and well-vascularized tumor lesions represent the best setting in which to test a potentially curative drug and to assess metabolic changes in response to treatment, reducing the overall time between development and commercialization of new compounds. Many positron-emitting tracers are currently available. The most common for clinical and preclinical metabolic studies is 18F-FDG (fluorine-18-fluorodeoxyglucose), an analogue of glucose fluorine-labeled considered a marker of increased cells metabolism, since highly metabolic cells demonstrate increased glucose metabolism. The formation of new vessels is a hallmark of cancer cells: angiogenesis is essential to tumor growth and is involved in tumor progression. PET probes specifically targeting angiogenesis have been proposed to study tumor angiogenesis. Particularly, antibodies directed against VEGF have been used in animal models using different labeling isotopes. Surface cells receptors have also been employed as potential targets for PET imaging: PET probes binding to specific receptors on tumor cells allow the detection of disease sites and the selection of receptor-bearing cases that may take advantage of receptor-targeted therapies. EGFR, for example, has been extensively studied for its direct implication in cancer development and progression. In conclusion, current evidence supports the role of SA-PET to study metabolic pathways of cancer in animal models of human tumors. SA-PET allows the performance of longitudinal studies in the same experimental animal with a reduction of animals employed, provides in vivo functional data on tissue and organ biologic processes, and represents a useful tool for drug efficacy studies. Finally, the employment of specific probes targeting gene expression may represent a new field of preclinical SA-PET applications.

## Preclinical Validation of Therapeutic and Diagnostic Nanoparticles Using In Vivo Small Animal Imaging

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Developments in nanotechnology have allowed us to create new probes combining multimodality detection properties and therapeutic properties, for example, lipidic structures going from liposomes to nanoemulsions, dendrimers, or inorganic particles. In vivo imaging has become an interesting tool in the validation process of these objects, especially for evaluation of the biodistribution analysis, targeting, and efficacy. Since there are multiple properties in these probes, and since each imaging modality has their own advantages and limitations, it is interesting to cross validate these probes using a multimodality strategy.

## Role of Tissue Niche on Muscle Regeneration

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One of the most exciting aspirations of current medical science is the regeneration of damaged body parts. The capacity of adult tissues to regenerate in response to injury stimuli represents an important homeostatic process. It is now generally accepted that each tissue type contains a reserve of undifferentiated progenitor cells, or stem cells, that participate in tissue regeneration and repair. However, tissue regeneration is affected in several pathologic conditions. One of the crucial parameters of tissue regeneration is the microenvironment in which the stem cells should operate. Stem cell microenvironment, or niche, provides essential cues that regulates stem cell proliferation and that directs cell fate decisions and survival. It is, therefore, plausible that loss of control over these cell-fate decisions might lead to a pathologic transdifferentiation or cellular transformation. Thus, while stem cells represent an important determinant for tissue regeneration, a "qualified" environment is necessary to guarantee and achieve functional results. Muscle regeneration is a coordinated process in which several factors are sequentially activated to maintain and preserve muscle structure and function upon injured stimuli. The major role in the growth, remodeling, and regeneration is played by satellite cells, a quiescent population of myogenic cells that reside between the basal lamina and plasmalemma and that are rapidly activated in response to appropriate stimuli. RT-PCR analysis, gene targeting strategies, and molecular imaging revealed that satellite cells present a heterogeneous profile of gene expression depending on the functional stage of the myogenic program. More recently, it has been suggested that other "nonmuscle" stem cell populations can participate in muscle regeneration and in some way contribute to maintain the pool of satellite cells. These stem cell populations could either reside within muscle, or be recruited via the circulation in response to homing signals emanating from injured muscle.

Nevertheless, if skeletal muscle possesses a stem cell compartment it is not clear why muscle fails to regenerate under pathologic conditions. Either the resident muscle stem cells drastically decrease or perhaps the injured/pathologic muscle is a prohibitive environment for stem cell activation and function. Several evidences suggested that a hostile microenvironment might prevent the activation of resident stem cells and thus might also reduce the success of exogenous cell therapies. We recently demonstrated that the modulation of the inflammatory response, by mIGF-1 expression, accelerates muscle regeneration. These results suggest that while stem cells represent an important determinant for tissue regeneration, a "qualified" environment is necessary to guarantee an efficient regeneration. In this context, therapeutic applications of adult stem cells to pathologic tissue repair in the context of regenerative medicine will require an increased understanding of stem cell biology, the environment of the aged/pathologic tissue, and the interaction between the two.

## Oral Presentations

### Regenerative Stem Cell Therapy in an Animal Model of Amyotrophic Lateral Sclerosis Disease: MRI Longitudinal Study

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The ability to track stem cell transplants in the brain by in vivo neuroimaging will aid our understanding of how these cells mediate functional recovery after transplantation. One major challenge for the development and refinement of stem cell transplantation is to map the spatial distribution and rate of migration in situ. Here we report the MRI monitoring of human skeletal muscle stem cells (hSkMSCs) transplanted into the brain of Wobbler (Wb) mice, an amyotrophic lateral sclerosis (ALS) mouse model. The homozygous mice develop progressive motor dysfunction with loss of motor neurons and astrogliosis, especially in the cervical tract of the spinal cord. In order to monitor hSkMSCs homing and engraftment, through longitudinal studies, we labeled cells before injection with an MRI contrast agent, an aqueous colloid of superparamagnetic iron oxide associated with dextran (SPIO). SPIO-labeled SkMSCs survive long-term in vivo and differentiate in a manner identical to that seen for unlabeled cells. Since motor neuron degeneration affects several areas of the central nervous system (CNS), in order to make the transplanted cells migrate and home throughout the CNS, we injected SPIO-labeled hSkMSCs into the lateral ventricles. Our study demonstrates that hSkMSCs labeled with SPIO survive in large numbers, are able to differentiate into neuronal and glial lineages, express neural markers, and appropriately respond to microenvironmental cues after transplantation to the Wb brain. Using MRI, we are able to detect the SPIO-related signal into the Wb brain until sacrifice. Moreover, using histology, we show that upon transplantation,

SPIO-labeled hSkMSCs migrate and integrate in a manner appropriate for their location. In order to identify the colocalization between SPIO signal and hSkMSCs engraftment, we performed histology with antidextran and human-specific neural marker antibodies. Sixteen weeks after transplantation, we detected hSkMSCs migrated into the parenchyma of the brain and along the ependyma of the spinal cord. This migration remained visible for up to 18 wk. Data are confirmed by histologic analyses. Knowledge of SkMSCs migration patterns and information obtained from diffusion-tensor imaging and MR spectroscopy might help to characterize the Wb model and improve the design of future clinical transplantation efforts.

### High-Frequency Ultrasound Evaluation of Subcutaneous Tumors in CD1 Nude Mice

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In the last decade high-frequency ultrasound (US) became an important preclinical tool for the study of complex pathologic models such as tumors. The possibility of repeating tests in the same animal, the large amount of information supplied, and the high procedural safety are the main advantages of this technique. These features make US compliant with the 3Rs. A431 (human epithelial carcinoma) and PC3 (human prostate carcinoma) tumors implanted subcutaneously in CD1 nude mice were analyzed. Anaesthetized animals (isoflurane 1.0%) were examined by a high-frequency US equipped by a 40 MHz scanhead. Both 2D and 3D images of the tumors were taken. Moreover, a contrast agent specific for ultrasound procedures was administered intravenously (50  $\mu$ L/animal) to assess the percentage of tumor microcirculation (PA%). 3D reconstruction images showed a volume range between 110 and 165 mm<sup>3</sup> for A431 and between 30 and 80 mm<sup>3</sup> (low growth) for PC3 tumors. The values of PA% were 5% and 8%, respectively. Single exams required about 30 min and there were no animal welfare issues during the experimental phase and further recovery period. The results showed that this procedure can be used in the development of new anti-tumor compounds. More specifically, the quantification of the microcirculation of the tumor is mandatory to assess the processes of angiogenic modulation as the main marker of the efficacy of new drugs. High frequency US, among other techniques (for example, MRI, PET, and MicroTC), is a helpful tool in modern translational medicine, enabling us to transfer biomedical knowledge from the preclinical to the clinical field, and subsequently speeding up the approval of new therapeutic compounds.

### Target Therapy and Cardiosafety in Breast Cancer: Our Experience

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The development of cardiac dysfunction during the treatment of breast cancer is not a recent observation. Ventricular dysfunction induced by anthracyclines has a clinical evolution worse than the cardiac dysfunction induced by other causes. Anthracyclines, effective in breast cancer in all clinical settings, have a toxicity profile that may limit their use. Ventricular remodeling is a progressive phenomenon, whose course is facilitated by sequential stress, until the heart fails to compensate and the heart failure, clinically evident, occurs. There is recent evidence that the damage of cardiomyocytes occurs at doses much lower than those commonly considered to be fairly safe. Unfortunately when the reduction of ejection fraction (LVEF) occurs, myocardial damage has already occurred. Regarding the study of ventricular remodeling, the most widely used diagnostic method is color Doppler echocardiography, which studies left ventricular systolic and diastolic function. We are implementing techniques of tissue Doppler, strain, strain rate, and speckle tracking in our daily practice and also in the study of the cardiotoxicity of Trastuzumab. In a mouse model of doxorubicin toxicity, it was possible to identify a reduction of 25% in the systolic endocardial velocity and a reduction of 33% in the strain rate to predict left ventricular systolic dysfunction and mortality. Further studies showed a significant reduction of the strain rate, even after low doses of anthracyclines, in patients with normal ejection fraction. The use of tissue Doppler in target therapy is an area of active research. The challenge in the field of prevention of cardiotoxicity of anthracyclines and target therapy is to also identify minimal and initial myocardial damage before the reduction of ejection fraction. We believe that expanding the use of tissue Doppler will provide a successful tool for diagnosis of initial cardiotoxicity in order to modify the dosage and the schedule of the medication.

### Evaluation of Tumoral Progression with Bioluminescence and MicroPET Analysis in MYCN-Amplified Neuroblastoma Murine Models

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Neuroblastoma, which originates from neural crest, is the most common pediatric extracranial tumor. Twenty-five percent of cases present amplification of MYCN oncogene, a factor associated with a poor prognosis and responsible for tumor progression and drug resistance control. It is, therefore, important to create preclinical murine models that accurately represent the pathology in order to evaluate the disease progression and its response to pharmacological treatment with noninvasive methods. In this study 2 MYCN-amplified neuroblastoma (MA-NB) murine models were set up, the xenograft orthotopic and the TH-MYCN, and subsequently analyzed with MicroPET and bioluminescence imaging. The orthotopic model was obtained by injection of 4

luciferase competent MA-NB cell lines, in the adrenal medulla of NOD/SCID mice. The tumor progression was analyzed weekly with bioluminescent imaging (BLI) starting from the day of injection. MicroPET analyses were conducted on TH-MYCN mice with 18F-FDG and 18F-DOPA radiotracers. The 18F-FDG uptake in the tumor of homozygous mice was analyzed every 4 d by standardized uptake value (SUV) index, starting from the fourth week of age. All animals were euthanized and each sample was used for histology, immunohistochemical, and molecular analysis of MYCN and N-Myc levels. The orthotopic model shows a 100% incidence; IMR-5 and BE2(c) showed a shorter progression and latency period, respectively. The comparison of 2 radiotracers has highlighted the higher informativity of the 18F-FDG. Homozygous TH-MYCN mice show a 100% incidence, 4-wk latency and 5-wk progression periods. Histologic analysis confirmed the accordance between imaging results and the presence of disease. All the collected tumor samples show amplification and over-expression of MYCN oncogene. The real-time monitoring by noninvasive imaging technique allows the early detection of MA-NB onset and progression in 2 complementary tumoral models. Moreover, the identification of a specific trend offers the possibility to define an optimal temporal window useful to evaluate the effectiveness of new therapies against MA-NB.

#### Dual Energy X-ray Absorptiometry and CT Applications for Bone Analysis in Mice Models

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Quantitative bone analysis is the reference technique to study age- or disease-related bone loss, to evaluate mineralization in fracture repair, and to test new therapies for bone mineralization. Several methods for evaluating bone mineral density and concentration are available, such as dual energy X-ray absorptiometry (DEXA) and quantitative computed tomography (QCT). The mouse is the primary laboratory model in the bone research field, and bone metabolism and diseases can be accurately evaluated, in vivo and in a longitudinal and noninvasive way, using dedicated small animal DEXA or high-resolution microCT ( $\mu$ CT) scanners. We describe our experience with DEXA and  $\mu$ CT applications in the laboratory mouse for performing bone analysis. We used a densitometer and a  $\mu$ CT scanner at 45  $\mu$ m spatial resolution. Mice were anesthetized with isoflurane 1.2% + oxygen 0.8 L/min or by intraperitoneal injection of ketamine 100 mg/kg + xylazine 10 mg/kg and placed in a prone position with fully extended limbs. DEXA and  $\mu$ CT dataset were analyzed by proprietary software to obtain 2D or 3D reconstructions of bone and serial measurements of bone mineral density (BMD) and content (BMC) in whole body or selected regions of interest (ROI). WE PHENOTYPED 28 C57BL/6J MICE, WILDTYPE, AND KNOCKOUT WITH DEXA FOR THE MOKA GENE. BMD (G/CM2) AND BMC (g) were measured in the femur. The rule of BMP4 protein in bone regeneration was characterized by DEXA and  $\mu$ CT (BMD, mg/cm<sup>3</sup>; BMC, mg)

in 25 athymic nude mice, injected intramuscularly with FG-Ad BMP-4 or FgAdBMP-4TK and treated with ganciclovir. Whole body vibration (WBV) effects on bone in growing mice were evaluated with DEXA in 35 C57BL/6J mice. Student *t* test revealed BMD < 10% in knockout compared to wildtype female mice ( $P = 0.0013$ ) for MoKA gene. BMD (mg/cm<sup>3</sup>) and BMC (mg) changes over time were measured to demonstrate ectopic bone formation into the quadriceps femoris induced by BMP4 expression. Statistical analysis of BMD and BMC with a Mann-Whitney test showed a significant increase of these parameters for total body ( $P = 0.002$  and  $P = 0.02$ , respectively) and femur ( $P = 0.001$ ) in mice treated with WBV. DEXA and  $\mu$ CT are feasible and noninvasive techniques to accurately perform in vivo quantitative bone analysis and to monitor changes of bone parameters over time in laboratory mice.

#### Ultrasound Contrast Agents for Imaging and Gene Therapy

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Ultrasound contrast agents, also known as microbubbles (MB), are a powerful research tool for diagnosis and therapy of animal model of human diseases. MB enhances ultrasound (US) applications, allowing for quantitative analysis of molecular biomarkers, perfusion studies, microvasculature targeting, and gene and drug delivery. We illustrate the importance of ultrasound contrast agent for gene therapy in a mice model of prostate adenocarcinomas in small animal research. Ultrasound contrast agents consisting of small, stabilized lipid microbubbles (< 10  $\mu$ m), which were prepared to contain adenoviruses expressing the melanoma differentiation-associated gene-7/interleukin-24 (Ad.mda-7), were injected intravenously in the tail vein of nude mice to enhance contrast of the blood pool signal and to target the adenoviruses to xenografted prostate adenocarcinomas. Viral targeting was achieved through the intrinsic characteristics of sonoporation and cavitation of microbubbles exposed to the appropriate US physics parameters, such as acoustic power ( $M_i > 0.3$  to 0.6). Prostate tumor xenografts were established on both flanks of 420 nude mice. Tail vein injections of the MB/adenoviral complexes and sonoporation of prostate tumors started 10 wk after tumor xenograft establishment when tumors reached an approximate volume of 150 to 200 mm<sup>3</sup> and were performed for 4 wk. US MB are viable candidates for gene delivery/therapy. MB/Ad.mda-7 complexes targeted to tumor prostate cells using US dramatically reduced tumor burden in xenografted nude mice. In conclusion, US with MB is a noninvasive and relatively inexpensive modality that can be quickly performed. Moreover, this technique could be applied on humans. For example, molecular imaging with US targeted MB could be potentially useful in the early diagnosis and treatment of tumor formations enhancing conventional treatment protocols.

## Generation of Bioluminescent MLL-Positive Acute Leukemia Mice Reveals Different MLL-Related Tumor Progressions

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The mixed-lineage leukemia (MLL) gene is a common target for chromosomal aberration in human acute leukemias (AL), frequent in infants, and associated with poor prognosis. Currently more than 60 fusion gene partners are known, and the most frequent translocations are t(9;11) MLL-AF9, t(4;11) MLL-AF4 and t(11;19) MLL-ENL. To evaluate the leukemic progression of MLL-related AL, we developed bioluminescent (BL) xenograft mouse models of MLL-AF9, MLL-AF4, and MLL-ENL AL. Bioluminescent imaging (BLI) is based on the reaction of the enzyme luciferase with its substrate, D-luciferin, in the presence of ATP, molecular oxygen and Mg<sup>2+</sup>, and the result is the emission of light proportional to the expression of the enzyme luciferase. In vivo BLI is simple, sensitive, with virtually no background noise, and is well tolerated by the laboratory animals. Acute myeloid leukemia (AML) MLL-AF9 cell lines (THP-1 from infant patient and MOLM-13 from adult patient), and acute lymphoblastic leukemia (ALL) MLL-AF4 (SEM) and MLL-ENL (KOPN-8) cell lines were stably transduced with *Photinus pyralis* firefly luciferase expression plasmid pMMP-LucNeo. Five to 10 × 10<sup>10</sup> luciferase-expressing cells were intravenously injected in 6-wk-old NOD/SCID immunodeficient mice. Mice were monitored once a week after intraperitoneal injection of 150 mg/kg D-luciferin with a charge-coupled device camera system under isoflurane anesthesia, both in prone and supine position. The BL curves were computed by the average of at least 10 mice signals, as the sum of both prone and supine acquisitions for each mouse (photon flux (ph/s) with proprietary software). BLI revealed to be a non-invasive, sensitive, rapid, and affordable method that enables the early detection of tumors, either superficially or in deep tissues, preceding the appearance of evident symptoms and blood dissemination. Our MLL-related AL mouse models recapitulate the different course of the infant and adult human MLL-AF9 AML, and the rapid aggressiveness of the human MLL-ENL and MLL-AF4 ALL. The parallelism between the results of BLI and the clinical course of MLL-related AL supports our proposed bioluminescent MLL-related acute leukemia mouse models as suitable tools for the study of MLL-related AL, and moreover for the bioluminescent evaluation of therapeutics in drug development and preclinical studies.

### Burkitt Lymphoma Therapy Monitored by In Vivo Bioluminescence Imaging

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In human Burkitt lymphoma (BL) BRG cells, a t(8;14) translocation, placing *c-myc* near the E $\mu$  enhancer of the H chain locus, causes tumor expansion. Earlier, we showed that a peptide nucleic acid complementary to the E $\mu$  sequence (PNAE $\mu$ ) specifically inhibited the expression of translocated *c-myc* and impaired the growth of BRG cells-induced subcutaneous tumors in CB17 SCID mice. In this study, the therapeutic potential of PNAE $\mu$  was evaluated in a systemic mouse model. BRG-BL cells transfected with the firefly luciferase gene (BRG-BL-Luc cells) were inoculated intravenously into SCID mice, resulting in a preferential expansion, similar to the one of human adult patients, in the abdominal cavity, central nervous system, and bone marrow. The mice were chronically injected intraperitoneally either with PNAE $\mu$ wt or with control PNAE $\mu$ mut (both 0.2mM in 100 mL of phosphate buffered saline) starting 1 wk after the inoculation. Once a week both groups of mice were injected intraperitoneally with D-luciferin (15 mg/kg), and anesthetized with continuous exposure to 1% to 3% isoflurane, inducing a linear response in photons/second for exposure times ranging from 1 to 5 min. A colorimetric-coded reference bar, from purple = minimum to red = maximum, allows a 'first glance' intensity quantization of the luminescence. Bioluminescent signals from BRG-BL-Luc cells, as detected by the camera system were recorded, integrated, digitalized, displayed, and quantified (in photons/second) using proprietary software. The treatment was stopped when the control animals developed severe neurologic symptoms. As detected both by inspection at necropsy and imaging, overall tumor growth in PNAE $\mu$ -treated mice decreased by less than 80%. Tumor cells growth in the PNAE $\mu$  or control PNAE $\mu$  treated groups was calculated (photons/seconds  $\pm$  SD) by measuring the luminescence emitted in each mouse by the BRG-Luc cells, during tumorigenesis at increasing times and number of treatments, with 2.5 min of exposure and with a homogeneous sensitivity setting. These data were confirmed by histologic and immunohistochemical studies that showed, only in PNAE $\mu$ -treated mice, a substantially reduced BL cell growth at the major sites of invasion and vast areas of necrosis in the lymphomatous tissues, with concomitant *c-myc* expression downregulation. Altogether, the data support the therapeutic potential of PNAE $\mu$  in human adult BL.

### Development of a Noninvasive Gastric Emptying Rate Measurement Method in Mice Using Bioluminescence Molecular Imaging

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Bioluminescence in vivo imaging (BLI) allows real-time monitoring of different physiologic and pathologic conditions in living, intact animals using bioluminescent (BL) reporter gene technology. The development of new drug acting on gastrointestinal motility requires the use of predictive animal models

suitable for preclinical structure activity studies. Gastric emptying (GE) in mice is usually measured with invasive techniques requiring animals be euthanized; alternatively, expensive and complex technologies such as scintigraphy, MRI, and  $^{13}\text{C}$ -acetic acid breath test can be used. A noninvasive, highly sensitive new test for gastric emptying time measurement has been developed using luciferase-expressing bacterial cells as a biomarker of the liquid content of the stomach. A new thermostable red-emitting luciferase was chosen as reporter gene to transform *E. coli* cells. Bioluminescent bacteria were administered to fasting mice, after a solid meal, and in response to different doses of metoclopramide and hyoscine butylbromide. BL imaging allowed to evaluate the real time 2D spatial and temporal distribution of bacteria along the gastrointestinal tract in whole animal and to calculate GE rate in basal conditions and following pharmacological stimulation. The gastric emptying has been monitored with a low-light imaging system using an ultrasensitive charge-coupled device camera by collecting an image every minute for up to 30 min. The administered BL bacteria were easily imaged and located in the stomach and subsequently followed in the duodenum and upper intestine allowing us to accurately calculate GE. GE after the test meal was significantly slower ( $t_{1/2} = 16 \pm 3$  min) than that obtained in fasting conditions ( $t_{1/2} = 2 \pm 1$  min); administration of hyoscine butylbromide (1 mg/kg body weight) significantly ( $P < 0.05$ ) increased half-life, which was delayed for up to  $25 \pm 4$  min; metoclopramide (1 mg/kg body weight) significantly ( $P < 0.05$ ) accelerated half-life, which was achieved within  $8 \pm 2$  min. A new method involving the use of a suspension of bacterial luminescent cells acting as floating nanobeads markers of gastric liquid content to monitor gastric emptying has been developed. Bioluminescence in vivo imaging technique gave results comparable with the other imaging techniques employed until now and can be applied for pharmacological studies and drug discovery.

#### Microtomographic Evaluation in Preclinical Orthopedic Studies

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Computerized microCT is a nondestructive technique that allows the tridimensional study of bone and biomaterials. In microCT images it is possible to see the internal structure of a small object with a high resolution and without any preparation or chemical fixation. Specialized software and hardware implemented the evolution of image analyses techniques, enabled a large amount of applicability, high-calculation intensity, and rigorous statistical approach. The sections obtained from a microCT acquisition are used in the analysis and the measurement of microstructural parameters. In preclinical orthopedic studies, microCT technique can be used in several different kinds of evaluations. Analysis on biomaterials before the implantation is done to evaluate porosity or mechanical characteristics; analysis on metallic implants, often utilized in orthopedics and dentistry, where the study of bone growth through bone regeneration and the integration with the surrounding bone; analysis on polymeric or ceramic implants to study principally the bone regeneration in the defects or the loss of bone substance; analysis of bone morphology that is

important because a lot of pathologies alter the bone microarchitecture (for example, osteoporosis or pathologies and therapy that affect bone tissue). After biomaterial implantation it is important to evaluate not only the quantity of bone growth, but also the quality of regenerated bone. The possibility to create virtual 3D models of samples based on microCT sections allows realistic visualizations of them and the complete understanding of structures that normally are observed only in a 2-dimensional mode typical of classic histologic sections. These models can be used to assemble filmed sequences of the analyzed sample in movement. Therefore, there is the belief that computerized microtomography can be a valid and nondestructive method, especially for preclinical evaluation of implant materials destined for the musculoskeletal system. This technique is useful in characterizing devices in preimplant phase and in the explant phase to evaluate possible deformations and/or degradations.

#### Usefulness of Small Animal Positron Emission Tomography/Computed Tomography to Noninvasively Analyze Insulin Sensitivity in Diet-Induced Obese Mice

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We analyzed the usefulness of small animal positron emission tomography/computed tomography (PET/CT) combined technique, as a new tool for in vivo study of insulin sensitivity in peripheral organs of mice. Standard techniques available to study insulin sensitivity of individual organs require animals be euthanized and complicated biochemical analysis of glucose uptake on explanted tissues. Using a glucose radioactive analogue (18F-FDG) we propose PET/CT as a new method to acquire metabolic information of peripheral organs by a simple image analysis approach. A software-based method has been used to overlap PET and CT images in the 3 spatial dimensions. CT images provide the anatomic reference of the metabolic active structures detected by PET, thus allowing us to correctly quantify PET signal. Three groups of mice were analyzed after administration of different diet regimens leading to progressive obesity levels: standard chow diet (SD), high-fat diet (HFD, 40 % energy from fat), and super high-fat diet (SHFD, 60 % energy from fat). Each group of animal underwent 2 repeated PET/CT scans, the first in fasting state (basal state), the second after insulin administration (0.7 U/k). Glucose levels were measured during PET/CT procedures with a glucometer. Standardized uptake values (SUV) of brown adipose tissue (BAT), white adipose tissue (WAT), skeletal muscle, and myocardium were calculated on PET/CT images. Analysis of glucose levels revealed a slight and a severely compromising of insulin sensitivity in HFD and SHFD groups, respectively. SUV analysis was in agreement with glucometric test; insulin treatment in normal SD mice induced a statistically significant increase of 18F-FDG uptake in myocardium, WAT, and BAT when compared to the basal fasting condition, whereas insulin effect on 18F-FDG uptake was lower in the HFD group,

and completely absent in the SHFD group. Interestingly, 18F-FDG uptake in skeletal muscle was not affected by insulin treatment in the 3 groups of animals, probably as a consequence of a counter-regulatory hormonal mechanism induced by hypoglycemia. Even if future studies are needed to further validate imaging data with gold standard methods, these data highlight the usefulness of PET/CT technique to noninvasively monitor insulin sensitivity in mice model of diet-induced obesity. The opportunity to evaluate the same animals longitudinally makes PET-CT an advantageous approach for the screening of new drugs against obesity-related metabolic dysregulation.

### **A Rat Model of Intestinal Infarction Due to Venous Occlusion: Usefulness of 7T MicroMR Imaging**

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Acute mesenteric ischemia (AMI) is a potentially fatal vascular emergency with overall mortality of 60% to 80% and an increasing incidence. The poor prognosis of gut ischemia is partially due to the lack of specific findings, either clinical or radiologic, that leads to delayed diagnosis and ineffective treatment. Early diagnosis seems to be the shortest way to reduce mortality rate. Therefore, the aim of this study is to validate a rat model of acute intestinal ischemia due to venous occlusion, in which MR imaging patterns are related to the evolution of intestinal morphodynamics and histologic analysis. The study was conducted on 32 Sprague-Dawley rats. After anesthesia, a laparotomy was performed and the superior mesenteric vein (SMV) isolated. Then the rats were randomly divided into 2 groups: in the first ( $n = 15$ ), control animals underwent the SMV occlusion by a tight ligation and, after macroscopical monitoring, rats were euthanized at different times and the bowel removed for histologic analysis; in the latter ( $n = 17$ ), a loop (3-0 gut) was tied loosely around the vessel and the tips tunneled from the abdominal cavity through a tube to the posterior cervical area without occluding the vessel. Three days after surgery, basal MR abdominal scans were collected for each rat using a 7T microMR; then the loop was squeezed pulling the external tips in order to occlude the vessel and MR sessions were repeated after 5 min, 4, and 8 h. The macroscopical monitoring of rats belonging to the first group showed a clear mesenteric vascular congestion at the first time point (5 min after ligation of SMV), erosion and frank ulcers with segmental changing in color and diameter of intestinal loops (spastic and hypotonic reflex ileus) at the second time-point (4 h), and worsening of these findings at the third (8 h). Instead, rats in the second group were scanned using a microMR with RARE T2 sequences: no evidence of pathologic patterns was detected at the first time point, while significant bowel wall thickening ( $> 1.5$  mm) and mesenteric hyperintensity were found at following time points (4 and 8 h). After experimentation, rats were euthanized and the entire bowel removed for histologic analysis on hematoxylin and eosin stained sections: vascular congestion in the submucose lamina with no sign of lysis or inflammation was present 5 min after ligation (first

time point), while destruction of the free portion of the villi, presence of dilated capillaries, and inflammatory cells were found at 4 h; eventually structural destruction of the villi with sparing of glandular cryptae, hemorrhage, presence of inflammatory cell and necrotic material was detected at the third time point. Compared to histologic analysis and macroscopical evidences, MR imaging can correctly detect morphofunctional alterations of ischemic gut. MR succeeded in early identification of the signs of venous mesenteric ischemia 4 h after SMV occlusion. Its future application in early diagnosis of mesenteric venous ischemia is highly reasonable.

### **MRI Quantitative Microvessels Characterization Based on Protein-Binding Contrast Agent**

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The potential of the protein-binding contrast medium B22956/1 in the assessment of tumor microvasculature development during antiangiogenic therapy has been investigated by dynamic contrast-enhanced MRI (DCE-MRI) in an experimental cancer model. PC-3 cells, a human prostate cancer line known to be hypovascularized, were implanted in 16 NCR athymic nude mice. Animals were assigned randomly to a control (vehicle) or drug-treated (AG013736) group. AG013736 is a potent receptor tyrosine kinase inhibitor that targets VEGFRs at subnanomolar concentrations with known activity on PC-3 xenografts. Tumor growth was monitored by means of caliper measurements. MRI was performed at baseline (T0) and after 7 d of treatment (T1). The transendothelial permeability (kTrans) to B22956/1 (injected at the dose of 0.1 mmol/kg) and the fractional plasma volume (fPV) were estimated from the kinetic analysis of dynamic MR data using a 2-compartment model; the initial area under the curve (IAUC) was calculated in several time windows after contrast agent injection ranging from 1 to 30 min. Tumors grew more slowly ( $P < 0.05$  Mann-Whitney U test) in the AG013736-treated group. The kTrans determined with B22956/1 decreased significantly in the treated group compared to baseline ( $P < 0.05$  Mann-Whitney U test), while no significant differences were observed in the control group. Significant differences were also observed for kTrans between treated and control group at T1 while no differences were observed at T0. The value of IAUC decreased significantly in the treated group compared to baseline ( $P < 0.05$  Mann-Whitney U test) provided that the MRI dynamic acquisition is extended for at least 5 min after contrast agent injection. Significant differences were also observed in the distribution of the IAUC parameters between treated and control groups at T1. In summary with the use of B22956/1 the therapeutic effects of a VEGFRs inhibitor in a hypovascularized tumor can be monitored by DCE-MRI. B22956/1's sensitivity to microvascular changes, granted by its high relaxivity and by its distinctive pharmacokinetic, suggests several potential clinical applications particularly in the therapy follow-up.

## Poster Session

### Imaging Application for Preclinical Cancer Studies

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Heterothopic and orthotopic injection of human tumor cells into immunodeficient animals such as nude mice have been used to create experimental models of human cancers. We show a heterothopic model of kidney cancer and lung cancer by fibrosarcoma and orthotopic model of pancreatic cancer using 3 different imaging techniques. In the heterothopic model we injected SN12C (human kidney cancer cell line) subcutaneously in nude mice transfected with pEGFP to monitor tumor growth and lymph node metastasis. In the other model we injected HT1080 transfected with pEGFP in the tail vein of nude mice to establish a model of lung cancer of metastasis. The evaluation of tumor growth and lymph node metastasis was done with macrocope fluorescence and confirmed with histologic analysis. However, spontaneous metastasis is rarely observed when tumors are placed in subcutaneous sites. Orthotopic models are now regarded as more likely to duplicate the process of local tumor growth and metastatic spread in patients. So in order to overcome this problem we performed an orthotopic model of pancreas injecting MiaPaca-2 into the pancreas. The tumor growth and progression was monitored by MRI and high-frequency ultrasound. We show that through imaging it is possible to monitor tumor progression and therapeutic efficacy of drugs during preclinical studies.

### Molecular Imaging by MicroPET to Evaluate the Antitumoral Activity of Natural Molecules for the Treatment of Alveolar Rhabdomyosarcoma

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Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma. Of the 2 main subtypes of RMS, alveolar (ARMS) and embryonal (ERMS), the alveolar type is associated with a poorer prognosis and is characterized by translocations involving fusion proteins PAX3-FKHR and PAX7-FKHR. Sulforaphane (SFN), initially discovered as an activator of phase 2 enzymes, is an isothiocyanate that plays an important role as an anticancer agent that interacts and modulates several critical cellular targets in the tumorigenic process. In this study, a mouse model of ARMS was set up for the preclinical evaluation of SFN, tumor necrosis

factor-related apoptosis-inducing ligand (TRAIL), and their combination effect. The microPET was used to monitor in real time the tumor growth and the response to treatment by the use of tumor to background activity ratio (TBR). The in vivo analysis was conducted on xenograft mice inoculated subcutaneously with 10 million rhabdomyosarcoma alveolar cells and treated intraperitoneally for 21 consecutive days with saline (control group), with SFN (treated group), and SFN + TRAIL at 2 scalar concentrations (group treated in combination). The TBR was calculated at regular intervals of 7 d for the duration of treatment. The positivity of the inoculated cells was seen by the microPET after only 2 d postinjection. The SFN alone resulted in a reduction of tumor growth, as well as TRAIL at the highest concentration but the combination of the same concentration of TRAIL with SFN showed instead the disappearance of the signal in microPET, leading the value of TBR under the threshold of positivity. The microPET is a useful tool through which it is possible to study the ongoing growth of tumors in mouse models, to decide the time of treatment, and to monitor, in real time, the effect of potential drugs or the antitumor activity of molecules corresponding to the decrease of TBR. The promising results obtained by the combination of SFN with TRAIL represent the bases for future association studies in which the SFN could be used not only as an anticancer agent but also as a potential chemopreventive agent.

### Use of MicroMR 7T in the Suspect of Acute Arterial Mesenteric Ischemia: Evaluation of an Animal Model

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Mesenteric ischemia is an uncommon but often underestimated cause of nontraumatic acute abdomen, and its global prevalence is around 0.1% of all hospital recovers. Despite our knowledge and improved diagnostic techniques, bowel infarction is still frequently a fatal disease, with reported mortality rates between 50% and 90%. This poor outcome has remained static for many decades and is associated with a variety of contributing factors. Of particular relevance are late presentation, late or incorrect diagnosis, and wrong therapies. Thanks to a simple, versatile, and inexpensive animal model, we reproduced this pathology, inducing intestinal ischemia without laparotomy. The aim of the study was to identify MR imaging patterns of lesions due to acute occlusion of the superior mesenteric artery (SMA), relating these radiologic findings to macroscopical monitoring and histology. Thirty Sprague-Dawley rats were anesthetized, a laparotomy was performed, and cranial mesenteric artery isolated. In a first group of control animals ( $n = 15$ ), the artery was directly occluded by a tight ligation, and during the following 8 h macroscopical lesions were monitored by photocamera. Eventually the bowel was excised for histologic analysis. In a second group ( $n = 15$ ), a loop (3-0 gut) was tied loosely around the vessel and the tips exposed on the rat back without occluding the artery. After 3 d from surgery, basal MR abdominal scans were collected for each rat of this group. Afterwards, the loop was tied by external tips to occlude the vessel and MR session was repeated 4 and 8 h later.

The animals were then euthanized and the entire intestinal package processed for histologic analysis on hematoxylin and eosin-stained sections. No animals died before the end of the study. One rat in the second group was excluded from analysis because SMA showed stenosis but not complete occlusion at 8 h after ligation in the angio-MR sequences. Abdominal MR scans showed no gas in the abdominal cavity and no signs of bowel or mesentery irritation. T2 MR sequences identified several injury signs of vascular occlusion such as loops dilatation, decrease of intestinal wall thickness, and peritoneal fluid. These changes were paralleled by histologic alterations as highlighted by *ex vivo* examination, with no significant differences in the histologic analysis of same intestinal tracts between the 2 groups. This animal model could represent a useful and highly reproducible tool to evaluate by imaging the evolution of intestinal ischemic lesion and assessing the effectiveness of new therapeutic models. Compared to histologic analysis, MR imaging can correctly identify morphofunctional alterations of intestinal ischemia due to acute occlusion of SMA.

### Management of Anesthesia in *Macaca fascicularis* Undergoing Medical Imaging Procedures

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Nonhuman primates (NHP) are important models for a wide variety of biomedical research. When medical imaging is needed in these animals, anesthesia is often a prerequisite to avoid gross movements. A large number of drug combinations is reported in the literature for NHP. However, there is little information on major and minor complications that can occur in the perianesthetic period. The aim of this study is to critically review the management of anesthesia for imaging in NHP and to analyze undesirable events encountered, in our experience, with standard anesthetic protocols. Twenty *Macaca fascicularis* have been anesthetized on 60 occasions to perform positron emission tomography (PET) or magnetic resonance imaging (MRI). Twenty-eight procedures were performed on healthy animals, 23 on parkinsonian subjects, and 9 in parkinsonian subjects after porcine embryonic neurons transplantation. A dissociative anesthetic combination (ketamine-xylazine or ketamine-medetomidine-midazolam) was used; all animals were intubated, connected to a breathing system, and allowed to spontaneously breathe oxygen 100%. Cardiovascular and respiratory functions were monitored during the procedure and in the postanesthetic period, and physiologic data and undesirable events were recorded. The most common events observed were: mild hypothermia (22 cases), prolonged recovery (over 30 min after extubation in 7 animals), stiffness (4 parkinsonian animals), postextubation hypersalivation (2 animals), and sporadic II degree AV blocks (2 animals). All events were considered minor complications with

no impact on patient's health. One animal showed postextubation retching and hiccup on the first anesthetic; on the second anesthetic, the same animal had a cardiorespiratory arrest after extubation that was promptly and successfully reversed by standard resuscitation technique with no long-term consequences. In 3 animals with nasal obstruction, anticipation and prevention of possible complications and modification of the perioperative management resulted in uneventful procedures. Observation and cautious monitoring before, during, and after general anesthesia enabled the prompt recognition of potential problems and rapid intervention before these could progress to serious life-threatening situations, preventing disastrous consequences. A continuous reassessment of the events occurred in the perianesthetic period provides important information enabling appropriate changes to improve animal welfare and scientific outcome.

### Generation of Medulloblastoma Bioluminescent Mouse Models and Identification of the Different Tumor Progression in Desmoplastic, Classic, and Anaplastic Variants

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Medulloblastoma (MB) is the most common pediatric primary tumor of the central nervous system. Currently only 50% to 60% of the patients are successfully cured and many of the patients who survive exhibit serious side effects due to the aggressive therapies they undergo. Our purpose is to develop a preclinical mouse model of MB to further investigate the mechanisms involved in the neoplasia and to evaluate new therapeutic agents. The mouse models were created using 3 MB cell lines derived from 3 different histologic variants: DAOY (desmoplastic variant), D341 (classic variant), and D556 (anaplastic variant). The tumor onset and progression were evaluated with a bioluminescence molecular imaging technique based on luciferase reporter gene extracted from the North American firefly (*Photinus pyralis*). In this regard, before being grafted in the mice's cerebellum, the tumor cells were transfected to permanently express luciferase gene. Afterwards, mice were monitored every 7 d by a charge-coupled device camera able to detect photons emission after intraperitoneal injection of D-luciferin. The mouse models have shown an incidence of 100% and different latency and tumor progression. Desmoplastic variant, characterized by the presence of nodules and low cellular density, correlates with a more favorable outcome. Mice were monitored for 3 mo with an endpoint at 108 d. Classic variant with small cells and low neuroblastic differentiation reveals an intermediate outcome with an endpoint at 66 d. Anaplastic variant with pleomorphic nuclei, prominent nucleoli, abundant cytoplasm, and intense mitotic activity, reveals a worse outcome with an endpoint at 17 d. At the end of the study, a histologic characterization of the tumor was performed. In vivo molecular bioluminescence imaging allowed us to follow tumor evolution from the first phases of tumor development, showing good sensibility and specificity. Thus, the created xenograft orthotopic bioluminescence mouse models, reflecting the same clinical

course of the histologic variants of medulloblastoma, represent a valid preclinical model to study new drugs for the treatment of this neoplasia.

### Feasibility of High-Resolution Ultrasound-Guided Microinjection in Mice Uterus

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The mouse is an important model for exploring the developmental consequences of altering gene expression using viral vectors, studying cell lineage or migration, and validating gene therapies. Accurate injection in specific sites currently requires surgical visualization of organs with many possible complications. This approach is impractical for repeated injections. High-resolution ultrasound (HRU) allows noninvasive visualization in real time of mouse embryo. Our first goal was to determine the progression of the needle as it traversed the uterus wall and if the position of the needle tip could be visualized. Microinjection was performed in 5 C57Bl/6J pregnant mice at embryonic day 14.5. Mice were anesthetized with isoflurane 2% and oxygen 0.8 L/min. Body temperature was monitored at 35 to 38 °C. To perform in uterus microinjection, a laparotomy was required. Abdominal skin was tricotomized and cleaned with 70% ethanol. A 2-cm midline incision was made along the linea alba, and the uterine horns were exteriorized to record implantation sites and then repositioned in the abdominal cavity, except a short segment containing 1 to 3 implantation sites. We positioned uterine horn on sterile gauze soaked with sterile PBS. Then HRU was performed with a 55-MHz probe to focus embryos' amniotic cavity. Microinjection was performed in the amniotic cavity with an automatic microinjector equipped with a capillary glass. The needle was advanced with the use of a micromanipulator under echo guidance until the needle tip was in the desired location. Then, 69 nL of contrast solution was injected and the accumulation of contrast was documented as a video clip. After microinjecting all sites, the maternal abdomen was closed with 8-0 silk using a continuous suture. HRU was performed for the following 2 d to test the fetuses' health status. In uterus microinjection was well-tolerated by both mother and fetus. In the process of externalization, an average of 8 fetuses was counted. After 21 d of pregnancy, an average of 8 healthy mice was born, confirming the absence of embryos' mortality after ultrasound guided in uterus microinjection. We were able to visualize and guide the needle into the amniotic cavity. The availability of real time in utero imaging of mouse embryos made it possible to perform ultrasound-guided injection of cells into a precise location in different stages of embryo development.

### Role of MRI in Detecting Gut Injury Induced by Ischemia-Reperfusion in a Rat Model

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Our purpose was to investigate the right evolution of damages in ischemia-reperfusion injury of rat ileum, relating it to MR imaging findings. Experiments were performed on 22 Sprague-Dawley male rats (250 to 300 g). After anesthesia, a median laparotomy was performed, and using an operating microscope, the superior mesenteric artery was carried out. The vessel was then clipped in a nontraumatic way for 1 h. After removing the clip, reperfusion was permitted for 6 h. MRI images were acquired at different times with a 7T microMRI scanner. MRI scans were performed at T0 (basal) at the end of ischemic period (T1, 1 h), after 3 (T2), and 6 (T3) h after reperfusion. Histologic analysis was performed on reperfused intestinal tissues removed at the different times from different rats. MRI showed the evolution of ischemia-reperfusion damages; during all the ischemic period, imaging findings were similar to pure arterial mesenteric ischemia. During the reperfusion period, MRI showed injury signs strictly associated with venous mesenteric ischemia. Histologic analysis on segments of ileum taken at different times confirmed the injury showed by MRI. MRI has proven to be a valuable tool, both in diagnosis of mesenteric ischemia and in the early detection of damage due to reperfusion. MRI shows clearly the broad range of lesions induced after ischemia-reperfusion, which were confirmed by pathologists.

### Small Animal PET Imaging of Brown Adipose Tissue Functional Activity with <sup>11</sup>C-Meta-Hydroxyephedrine

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Brown adipose tissue (BAT) is different from white adipose tissue because of its unique ability to burn energy as heat. The peripheral sympathetic nervous system (SNS) is the main regulator of BAT functions through norepinephrine (NE)-mediated signaling. In the past year, a large number of experiments on laboratory animals have shown that pharmacological stimulation of BAT functional activity decreases energy accumulation from diet, thus, counteracting diet-induced obesity. More recently, it has been shown that not only animals, but also human adults possess a functionally active BAT, highlighting BAT as a new target for antiobesity research. The experimental PET tracer <sup>11</sup>C-meta-hydroxyephedrine (HED) is an NE radioactive analogue widely used to study noradrenergic cardiac function with PET. In vitro analysis of HED uptake on explanted tissues pointed out this tracer as actively taken up also by other peripheral tissues as BAT. Based on this evidence we wondered if in vivo PET imaging of BAT with HED tracer was possible, and if it could be a reliable measure of peripheral sympathetic activity. To this aim, using PET/CT we analyzed HED biodistribution in the BAT of mice under different conditions: basal condition, cold exposure, and

chemical and surgical sympathectomy. Analysis of mice PET/CT fusion images under basal condition, demonstrated the presence of an active area of physiologic uptake of HED at the level of BAT. To analyze if this signal reliably reflects sympathetic activity, we exposed animals to a cold stimulus, known to induce an overactivation of NE signaling in the BAT. As expected, cold-exposed animals exhibit a drastic increase of HED uptake in BAT; moreover, when sympathetic function of the same animals was downregulated by chemical sympathectomy (6 OH-DOPAMINE), tracer uptake was reduced. Finally, when sympathetic activity was almost completely abolished by a procedure of surgical resection of sympathetic nerves surrounding BAT, PET signal in BAT area of cold-exposed animals was not different from those detected in animals analyzed in a basal state. To our knowledge, these are the first data demonstrating the possibility to noninvasively acquire direct information on SNS functional activity of BAT. The PET/CT imaging approach will allow repeated longitudinal evaluations of BAT SNS activity in the same animals after pharmacological or nutritional intervention.

### Identification of $\beta$ 3-Adrenoceptors in the Myenteric Plexus: Healthy versus Inflamed Gut

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Experimental models are useful to study inflammation-evoked neuroplasticity.  $\beta$ 3-adrenoceptor ( $\beta$ 3-AR) agonists showed protective effects in experimental models of intestinal inflammation, possibly by increasing blood flow and modulating gut motility. The aim of this work was to analyze: 1)  $\beta$ 3-AR distribution within the myenteric plexus of healthy rats; 2) the chemical coding (namely, ChAT for the cholinergic excitatory pathway, NOS for the nitrenergic inhibitory pathway and somatostatin for secretomotor neurons) of  $\beta$ 3-AR positive neurons; 3) the effect of DNBS-induced intestinal inflammation on  $\beta$ 3-AR distribution and chemical coding. Colitis was induced in Sprague-Dawley rats by a single intrarectal administration of DNBS (30 mg/rat). The intestine (the distal ileum and the whole proximal and distal colon) was removed after 6 d. Whole mount preparations of myenteric plexus were obtained by removing the mucosa, submucosa, and circular muscle layer. Myenteric ganglia were visualized by immunofluorescence: PGP-9.5 (1:50) was used to quantify the number of neurons/ganglia, PGP-9.5 and  $\beta$ 3-AR (1:50) to quantify the percentage of  $\beta$ 3-AR positive neurons/ganglia. ChAT (1:50), NOS (1:300), and somatostatin (1:100) were used to analyze the chemical coding of  $\beta$ 3-AR neurons. In healthy rats,  $\beta$ 3-AR positive neurons per ganglion were 28% in the ileum and 18% and 23% in the proximal and distal colon, respectively. Of these, approximately 90% were ChAT positive, 37% and 17% were NOS-positive in the distal and proximal colon, respectively, and approximately

2% to 3% were somatostatin positive. In the inflamed colon, a decreased number of neurons/ganglion and an upregulation of  $\beta$ 3-AR positive neurons only in the distal colon was observed, while the chemical coding of  $\beta$ 3-AR neurons was unchanged. DNBS-induced colitis represents a useful model to study neuroplastic changes occurring in IBD. Visualization of myenteric ganglia by immunofluorescence allowed characterization of  $\beta$ 3-AR positive neurons and analysis of their chemical coding in healthy and inflamed gut. Our results are consistent with previous studies indicating that activation of  $\beta$ 3-ARs can regulate gut motility through inhibition of cholinergic excitatory pathways and activation of nitrenergic inhibitory pathway and induce analgesia through somatostatin release. Finally, the presence of  $\beta$ 3-AR on myenteric neurons bears potential for therapeutic application in IBD.

### In Vivo Imaging of NF- $\kappa$ B Pathway in Acute Lung Inflammation Mouse Model

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NF- $\kappa$ B plays a central role in immunity, inflammation, development, and cell survival. Under normal conditions, NF- $\kappa$ B activation is subjected to several layers of regulation, check-points, and feedback controls and is rapid and transient in nature. Molecular events leading to the activation of NF- $\kappa$ B have been the subject of intense research for more than 20 y, largely because of the well-documented involvement of deregulated NF- $\kappa$ B activation in a variety of human disorders. Elevated NF- $\kappa$ B activity is a hallmark of various autoimmune and inflammatory diseases. It is easy to understand the importance of monitoring the NF- $\kappa$ B in a noninvasive way in lung inflammation mouse model. We used a simple system for in vivo gene delivery, in order to create a mouse expressing the gene in the lung (a plasmid containing human NF- $\kappa$ B reporter elements and luciferin gene as a reporter). The DNA was delivered intravenously to BALB/c female mice at the concentration of 40  $\mu$ g/mouse using an in vivo nucleic acid-delivery reagent as a transfectant agent. The transient transgenic mice were monitored using an in vivo imaging system to analyze NF- $\kappa$ B activation at different time points. All of the mice were imaged using bioluminescence in order to check the basal activity of the NF- $\kappa$ B. The mice were divided into 2 groups: the control, where they received the saline solution intraperitoneally and the treated group, where they got lipopolysaccharide (LPS) at 30  $\mu$ g/mouse IP. Each mouse was monitored at 2, 5, and 24 h after treatments. The LPS-treated mice showed a 5-fold increase of photons at 2 h compared to the saline group. These data show that it is feasible to monitor NF- $\kappa$ B in vivo in a noninvasive way using bioluminescence imaging and to create a new in vivo tool for drug discovery process.