MOLECULAR IMAGING AND BIOLOGY

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SCIENTIFIC PAPERS

Featured Speakers

General Plenary Sessions

No. 1

ADVANCES IN CLINICAL ONCOLOGY AND THE ROLE OF IMAGING
S. J. Horning
Stanford University School of Medicine, Stanford, CA.

Over the last three decades, the nation's investment in basic and clinical cancer research, screening and prevention has resulted in increased survival rates. Cancer care is a multidisciplinary effort, one in which imaging has an increasingly important role. This year, 1.4 million Americans will receive a cancer diagnosis with an estimated 64% five-year survival. Early detection and reliable assessment of limited disease is due to broad application of accurate imaging, facilitating major advances in the adjuvant therapy of common cancers. Targeted cancer therapies are proving effective in a wide range of tumors but their development has been lengthy and costly, target interaction and modulation is uncertain, and major questions remain as to optimal application for therapeutic success. Imaging is seen as a key technology for assessing, accelerating development and guiding the use of new therapeutics. 2-Deoxy-2-[F-18]fluoro-D-glucose (FDG)-positron emission tomography (PET) is widely used for staging and restaging in cancer management, guiding changes in therapy. However, glucose uptake as a validated surrogate marker for response or survival endpoints for specific tumors in the context of prospective clinical trials is mostly lacking, hindering optimal application in current trials. As we move from disease and anatomy-based approaches to more biologically-based cancer therapies, probes for fundamental neoplastic processes and prominent molecular targets in early clinical trials should reduce the time and resources to identify important therapeutics and discard unsuccessful candidates. Multidisciplinary efforts are required to realize the promise of molecular imaging in cancer management and oncologic drug development.

No. 2

APPLICATION OF BIOMARKERS TO CENTRAL NERVOUS SYSTEM DRUG DEVELOPMENT: NOW A REALITY
W. Z. Potter;
Merck Research Laboratories, Blue Bell, PA.

A major disappointment for the field is the limited translation to date of the molecular biology revolution to the clinic, especially with regard to drugs for central nervous system (CNS) disorders. Is this a failure of those novel targets discovered to date to mediate therapeutic processes or a failure to develop agents that really engage the target in human brain? We now know the latter to be the case for many compounds that have been tried in the clinic. Most of this knowledge is thanks to non-invasive imaging of drug effects (usually ligand displacement) in the CNS. In addition, cerebrospinal fluid (CSF) sampled before and after treatment can provide not only evidence of drug exposure but also, with the application of metabolomics and proteomics, evidence that an agent affects brain function. Thus brain imaging and CSF biochemistry provide the critical biomarkers to prove that drugs hit their targets. CNS drug development now depends on these biomarkers. The greatest challenge for the field is how to develop the appropriate biomarkers while carrying out the traditional work of bringing forward compounds suitable for human studies. How the availability of the relevant biomarker affects the prioritization and resources given to development of compounds for novel targets is a core issue. Since no single organization has the resources to develop all relevant biomarkers of CNS activity the field is exploring how to carry out this essential function in "pre-competitive space" through alliances of academia, industry and government. Progress in this arena should transform the way in which early development is carried out.

No. 3

MAGNETIC RESONANCE AND OPTICAL MOLECULAR IMAGING
R. Weissleder
Massachusetts General Hospital, Charlestown, MA.

The Center for Molecular Imaging Research at Harvard University and Massachusetts General Hospital brings together diverse disciplines such as chemical biology, genomics, molecular biology, bioengineering, computational sciences, imaging and medicine (http://cmir.mgh.harvard.edu) in an effort to develop novel imaging agents and devices. In this presentation I will first give a short overview of our generic strategies for developing new imaging probes. I will then review recent advances of two specific programs: a) novel nanomaterials for magnetic resonance imaging (MRI) and b) the development of new optical imaging agents and tomographic imaging systems. I will review the current clinical state of nanomaterials in MRI and then focus on the parallel synthesis of nanoparticle libraries decorated with multiple synthetic small molecules which can lead to amplified binding and new, unknown biological properties of such nanomaterials. Using a number of different fluorescent imaging probes I will show specific inroads that have recently been made in optical imaging. The method and described agents are expected to have important clinical applications in early disease detection, molecular medicine and drug discovery.

Institute for Clinical PET Sessions

No. 4

OVERVIEW OF CARDIAC STEM CELLS
R. Abraham;
Johns Hopkins Hospital, Baltimore, MD.

Background: Stem cells derived from a patient's own heart offer a potentially attractive source for cellular transplantation therapy with the goal of myocardial regeneration. *Methods:* Percutaneous endomyocardial biopsy specimens from 70 adult patients were grown and expanded in primary culture. Growth was tracked for each specimen for up to four months and correlated clinically. Human cardiac stem cells were then injected into the border zone of acute myocardial infarcts in immunodeficient mice, with histological myocardial regeneration and echocardiographic left ventricular function as endpoints. *Results:* Biopsy specimens from 69 of 70 patients typically yielded >100,000 stem cells within 20-30 days. These cells expressed antigenic markers of stem cells like ckit and CD105. Human stem cells injected into the border zone of myocardial infarctions engrafted, migrated into the infarct zone, partially replaced the scar and improved left ventricular function. Stem cell-injected mice had a higher fraction of viable fuchsin-positive tissue within the infarct zone (24.9±1.1%) compared to fibroblast-injected mice (17.7±1.8%, $p<0.01$) or PBS-injected mice (13.7±0.7%, $p<0.01$), but the overall total infarct area was similar to that in the two control groups (60.6±6.4 CDC, 76.9±7.0 fibroblast, 75.7±2.7 PBS, units in 10^4 pixels; p=NS). Echocardiograms performed on day 20 before harvesting the hearts revealed a higher ejection fraction in the stem cell-treated group (38.8±1.7%) compared to the fibroblast-treated (24.5±1.8%, $p<0.01$) and PBS-treated control groups (26.4±3.0%, $p<0.01$). *Conclusions:* Cardiac stem cells mediate cardiac regeneration and improve heart function

in a mouse infarct model, motivating further development for autologous therapeutic applications in patients.

No. 5

POSITRON EMISSION TOMOGRAPHY ONCOLOGY REVIEW: POSITRON EMISSION TOMOGRAPHY INSTRUMENTATION
R. Badawi
University of California, Davis, Sacramento, CA

This presentation will provide attendees with a detailed explanation of the operation of positron emission tomography (PET) Scanners. It will also list detector material that is used in PET/computed tomography (CT) imaging. 2-D and 3-D imaging, and the benefits and disadvantages of using each during acquisition will also be discussed.

No. 6

POSITRON EMISSION TOMOGRAPHY PHYSICS: IMAGE RECONSTRUCTION ALGORITHMS
R. Badawi
University of California, Davis, Sacramento, CA

This presentation will describe and delineate the difference in 2-D and 3-D reconstruction techniques. Also in this lecture, the importance of iterative reconstruction will be explained, and a description of the organization of positron emission tomography (PET) data will be included.

No. 7

4-D COMPUTED TOMOGRAPHY TECHNIQUES
P. Balter;
UT M.D. Anderson Cancer Center, Houston, TX.

Over the past 10 years 3-D-conformal radiation therapy (3-DXRT) and intensity modulated radiation therapy (IMRT) have become the standard-of-care for definitive radiotherapy in the United States. These treatment techniques are similar in that they are based on volumetric patient image datasets, usually computed tomography (CT), and that this data is used to create treatment plans with large doses (45 - 66 Gy) to a treatment volume while minimizing doses to normal tissues and critical structures. The ability to plan these treatments for thoracic and abdominal tumors has been somewhat limited by respiratory induced organ motion. Over the past two years respiratory correlated CT imaging has become commercially available from several vendors. The resulting datasets are volumetric datasets sampled at a number of points in the respiratory cycle and are referred to as 4-DCTs. The use of 4-DCT in radiotherapy allows the design of a treatment volume that accounts for the shape and position of the tumor and the surrounding normal tissue during normal breathing. 4-DCT combined with function imaging allows the design of 3-DXRT or IMRT plans that take into account the location, movement and biology of the tumors and normal tissue. Without all three pieces of information treatment plans cannot be effectively designed that maximize tumor control probability while minimizing normal tissue complications. This presentation will include the acquisition of 4-DCT datasets and their use in radiotherapy as well as providing a brief overview of the role of pre-treatment imaging in radiotherapy.

No. 8

POSITRON EMISSION TOMOGRAPHY ONCOLOGY REVIEW: PATIENT PREPARATION and PROTOCOLS
D. Bandy
Good Samaritan Medical Center, Phoenix, AZ

The first part of this presentation, Patient Preparation, will calculate standardized uptake value (SUV) and give examples of what causes variability in the value. The presentation will detail the historical and physical patient information necessary to perform positron emission tomography (PET) imaging in oncology. Another area of discussion will describe fluctuations in 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) uptake attributed to improper patient preparation. The second part of the presentation, Protocols, will list modes of PET acquisitions and their benefits. It will also discuss how modifications in protocols affect image quality and correlation, and will describe patient positioning specific to PET/computed tomography (CT) protocols.

No. 9

HYBRID POSITRON EMISSION TOMOGRAPHY /COMPUTED TOMOGRAPHY: WILL IT PLAY A ROLE FOR IMAGING OF ATHEROSCLEROSIS?
F. M. Bengel;
Johns Hopkins University, Baltimore, MD.

Integrated positron emission tomography/computed tomography (PET/CT) has been introduced to combine the assessment of morphology and biology, and has been considered a breakthrough in tumor imaging. The latest generation of PET/CT scanners now includes fast multi-slice CT. This has opened their application to cardiovascular imaging. While cardiac PET/CT at present holds promise as a clinical tool in the workup of patients with suspected or known coronary artery disease by combining coronary angiography and myocardial perfusion assessment, newest trends in imaging go towards targeting of atherosclerotic lesions. This can be achieved by CT (high resolution plaque composition) and/or PET (specific definition of plaque activity). Whether the integration of both techniques for atherosclerosis imaging really enters the clinical arena and provides benefit over use of two standalone systems will be dependent on the development of accurate algorithms for fusion of subsecond CT acquisitions with PET data which are usually acquired over multiple cardiac and breathing cycles.

No. 10

POSITRON EMISSION TOMOGRAPHY ONCOLOGY REVIEW: POSITRON EMISSION TOMOGRAPHY PHYSICS
P. Christian
Huntsman Cancer Institute, Salt Lake City, UT

Topics that will be discussed in this presentation include a description of Bremsstrahlung radiation, and annihilation reaction will be explained. The linear attenuation coefficient and its relationship to half value layer will also be discussed.

No. 11

POSITRON EMISSION TOMOGRAPHY ONCOLOGY REVIEW: CYCLOTRON ANATOMY AND RADIOPHARMACEUTICAL PRODUCTION
P. Christian
Huntsman Cancer Institute, Salt Lake City, UT

This presentation will provide attendees with a description of the properties of positron emission tomography (PET) radiopharmaceuticals. The mechanism for F-18 production in a cyclotron will be explained in detail. The presentation will also list the steps in 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) synthesis.

No. 12

POSITRON EMISSION TOMOGRAPHY ONCOLOGY REVIEW: SITE PLANNING
P. Christian
Huntsman Cancer Institute, Salt Lake City, UT

In this presentation, attendees will be provided with the design of a rough outline of a positron emission tomography (PET)/computed tomography (CT) suite. Other topics to be included in this presentation will be a listing of important considerations in planning a PET/CT facility, and details about how distance and shielding are incorporated into a site plan.

No. 13

BRAIN POSITRON EMISSION TOMOGRAPHY: CLINICAL CASE PRESENTATIONS - EPILEPSY CASES

H. Chugani
Children's Hospital of Michigan, Detroit, MI

Positron emission tomography (PET) scanning can provide valuable data in localizing epileptic foci or defining the true extent of the epileptogenic zone for surgical treatment of intractable epilepsy. In *temporal lobe epilepsy*, interictal 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) PET identifies areas of decreased glucose utilization corresponding to epileptogenic areas. In *nonlesional extratemporal lobe epilepsy*, FDG-PET abnormalities guide the placement of intracranial electrodes which, otherwise, may be subject to sampling error. In *infantile spasms*, resection of focal PET abnormalities corresponding to focal EEG abnormalities is associated with improved seizure and cognitive outcome. PET tracers other than FDG have been developed in order to provide more specific characterization of the seizure focus. For example, *C-11-flumazenil (FMZ)* labels central benzodiazepine receptors and is useful in showing: decreased receptor binding in medial temporal sclerosis, dual pathology, perilesional epileptogenic zones, more accurate localization of seizure onset zones compared to FDG, and potential secondary epileptic foci which often are responsible for surgical failures. Another PET tracer, *C-11-alpha-methyl-L-tryptophan (AMT),* is an analogue of tryptophan, and traces serotonin synthesis as well as tryptophan metabolism via the kynurenine pathway. AMT-PET in patients with epilepsy demonstrate focally increased uptake in cortical regions of epileptogenesis interictally. In children with tuberous sclerosis and intractable epilepsy, focal increase in AMT uptake is seen in the region of epileptogenic tubers, but not in nonepileptogenic tubers. The list of PET tracers for epilepsy evaluation continues to grow as efforts are being made to pinpoint precisely the cortical regions that should be resected in order to achieve the best postoperative outcome.

No. 14

COMPUTED TOMOGRAPHY RADIATION DOSE

D. Cody
U.T. MD Anderson Cancer Center, Houston, TX

This presentation will be aimed at hybrid-computed tomography (CT) technologists, and will cover issues specific to CT radiation dose. Topics will include how radiation dose is defined in CT, how radiation dose is measured in CT, which CT scan parameters affect radiation dose, what the dose values displayed on the console really mean, and how radiation dose variation affects image quality. To help put CT radiation dose levels into perspective, some routine diagnostic CT dose values, some routine hybrid-CT dose values, and some routine positron emission tomography (PET) dose values will all be compared. The concept of a 'BERT' will be introduced to help provide a more intuitive understanding of radiation risk. Finally, the contribution of the 'scout' scan to the total exam dose will be discussed, along with techniques to further decrease this component of CT dose.

No. 15

POSITRON EMISSION TOMOGRAPHY CLINICAL INDICATION AND APPLICATIONS: POSITRON EMISSION TOMOGRAPHY ILUSTRATIONS WITH RADIOISOTOPES OTHER THAN FDG

J. Czernin
David Geffen School of Medicine at UCLA, Los Angeles, CA

This presentation will describe the application of positron emission tomography (PET) tracers other than 2-deoxy-2-[F-18]fluoro-D-glucose (FDG), including detailing the characteristics of PET labeled isotopes other than FDG. The future direction of PET imaging will also be discussed.

No. 16

POSITRON EMISSION TOMOGRAPHY /COMPUTED TOMOGRAPHY IN OVARIAN AND UTERINE CANCER

F. Dehdashti
Mallinckrodt Institute of Radiology, St. Louis, MO.

Gynecologic malignancies are the second most common type of cancers in women in the United States. Computed tomography (CT) is the most widely used imaging method for staging and restaging of this group of patients. However, CT is limited in detection small metastatic foci and to differentiate metastasis from reactive hyperplasia in enlarged nodes. Ovarian cancer is the leading cause of death among gynecological malignancies. The majority of patients present with advanced disease at initial diagnosis. Majority of patients with advanced disease have elevated levels of the serum tumor marker CA-125. This tumor marker is widely used in the follow-up of patients after therapy. In a large number of patients with recurrent disease evident by an increase in CA-125, disease cannot be localized. While positron emission tomography (PET) is not very useful in early detection of ovarian cancer, it has been shown to be very useful in patients with elevated tumor markers and radiographically occult disease. PET and especially PET/CT has been shown to be of particular use in identifying potentially resectable macroscopic disease. PET/CT is a useful imaging technique in properly selecting patients with suspected recurrent ovarian carcinoma. Endometrial cancer is the most common gynecologic cancer in the United States. There are no reports of the use of FDG-PET for diagnosis of primary endometrial cancer. PET has been evaluated in the postoperative or posttherapy setting and was shown to be very useful in detection of asymptomatic recurrences and was shown to significantly alter treatment decisions by detecting otherwise unsuspected distant metastases.

No. 17

CLINICAL STAGING OF NON-SMALL CELL LUNG CANCER AND MALIGNANT PLEURAL MESOTHELIOMA: THE ROLE OF COMPUTED TOMOGRAPHY AND POSITRON EMISSION TOMOGRAPHY

J. Erasmus
MD Anderson Cancer Center, Houston, TX.

This talk reviews: (1), the staging of non-small cell lung cancer using the 1997 TNM International Staging System for Lung Cancer and will emphasize the appropriate use of imaging in patient management. The accuracy and limitations of computer tomography (CT) in staging the primary tumor (T), nodal metastasis (N) and extrathoracic metastasis (M) are compared and contrasted with positron emission tomography (PET) imaging and; (2), the staging of malignant pleural mesothelioma (MPM) using the International Mesothelioma Interest Group TNM staging system for MPM and will emphasize the current status of imaging in the management of patients with MPM. Specifically, the talk will address the importance of CT and PET in TNM staging of patients being considered for extrapleural pneumonectomy and will delineate the advantages and limitations of the different imaging modalities, including integrated CT-PET imaging, in the determination of resectability.

No. 18

POSITRON EMISSION TOMOGRAPHY/COMPUTED TOMOGRAPHY OF THE HEAD AND NECK

M. M. Graham
University of Iowa, Iowa City, IA.

Interpretation of 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) positron emission tomography (PET)/computed tomography (CT) images of the head and neck are particularly challenging because of the complex, closely packed anatomy and because many normal structures often show significant uptake of FDG. The goal in interpretation of these studies is to accurately distinguish malignant from benign uptake, accurately describe the level of nodal involvement, and to clearly communicate these findings to the referring physician. Normal structures showing significant, although

variable, uptake include the adenoid, palatine, and lingual tonsils, soft palate, base of tongue, floor of mouth, and larynx. Various muscles can show significant uptake, occasionally asymmetrically. The common nodal levels seen in PET/CT studies are level I: submandibular, level II: above the hyoid bone, level III: between the hyoid and the thyroid cartilage, level IV: between the thyroid cartilage and the clavicle, and level V: posterior to the sternocleidomastoid muscle. Common sites of tumors are nasopharyngeal, salivary glands, tongue, tonsils, epiglottis, vallecula, piriform sinus, and larynx. It is becoming very clear that PET/CT is useful in staging head and neck cancers and in the follow-up of these tumors, however it is also clear that interpretation in this region is more difficult than other parts of the body and requires significant thoughtfulness and experience.

No. 19

POSITRON EMISSION TOMOGRAPHY IMAGING OF CERVICAL CANCER

P. W. Grigsby
Washington University Mallinckrodt Institute of Radiology, St. Louis, MO.

When a clinician evaluates a patient with a new diagnosis of invasive cervical cancer the three primary tumor-related questions that need to be answered are the size and extent of the primary tumor, sites of metastatic disease, and the presence of hydronephrosis. Metastatic sites include the extent of lymph node metastases and sites of distant disease such as lung, liver, and bone. 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) - positron emission tomography (PET)/computed tomography (CT) is currently the best available imaging tool to answer these questions. PET does not give information concerning parametrial invasion in small cervical tumors. This is best determined with magnetic resonance imaging (MRI). Patients with parametrial invasion are not treated with surgery. The sensitivity and specificity of PET for the detection of lymph node metastases is much greater than either CT or MRI. This increased sensitivity of PET for lymph nodes mostly negates the issue of parametrial invasion. This is because standard treatment for patients with positive lymph nodes does not include surgery. In our population 450 patients with cervical cancer who had a pre-treatment PET, 80% of the positive lymph nodes were less than 1 cm in diameter. The pre-treatment evaluation of lymph node status with FDG-PET is critically important. Current research issues utilizing FDG-PET/CT data for cervical cancer patients include developing prognostic scoring systems, evaluating response to therapy, PET/CT radiation simulation for intensity modulated radiation therapy (IMRT), and the development of laboratory correlates with the FDG metabolic findings.

No. 20

IMAGING SUBSTRATE METABOLISM IN DIABETES

R. Gropler
Washington University School of Medicine, St. Louis, MO

Evidence is emerging that the excess cardiovascular morbidity and mortality that occurs in patients with diabetes mellitus is attributable, at least in part, to abnormalities in myocardial substrate use. The overdependence on the metabolism of fatty acid metabolism by the diabetic heart initiates a cascade of events leading to cardiac dysfunction including an increased susceptibility to ischemia, oxygen free radical injury, lipotoxocity and apoptosis. Cardiac positron emission tomography (PET) using well-validated compartmental models of the myocardial kinetics of appropriate radiotracers is the most common imaging technique to characterize these abnormalities in myocardial substrate use. Cardiac ^{31}P MRS is used to quantify impact of the metabolic derangements on myocardial energetics. In this lecture the application of these and other imaging techniques in the study of myocardial substrate use in the diabetic heart will be summarized. Specifically, correlation of findings in small animal models of diabetes mellitus to the human condition and the use of these imaging techniques to assess therapeutic responses will be discussed.

No. 21

IMAGING METABOLISM IN HEART FAILURE

R. Gropler
Washington University School of Medicine, St. Louis, MO

Alterations in myocardial substrate metabolism have been implicated in the pathogenesis of contractile dysfunction and heart failure. Animal models of heart failure have shown that in the progression from cardiac hypertrophy to ventricular dysfunction, the expression of genes encoding for myocardial fatty acid oxidation enzymes is coordinately decreased, resulting in a shift in myocardial substrate metabolism to primarily glucose use, similar to that seen in the fetal heart. These metabolic changes are paralleled by re-expression of fetal isoforms of a variety of contractile and calcium regulatory proteins program. The reactivation of the metabolic fetal gene program may have detrimental consequences on myocardial contractile function such as energy deprivation and in the setting of increased fatty acid delivery, myocardial lipid accumulation or lipotoxicity. Indeed, alterations in myocardial substrate use are now becoming attractive targets for novel treatments for heart failure, such as the administration of the insulin sensitizer glucagon-like peptide-1. Positron Emission Tomography (PET) has been used extensively to characterize these abnormalities in myocardial substrate metabolism. In contrast, P-31 MRS studies have provided important information on the myocardial energetics in heart failure. In this lecture the use of these and other imaging techniques in the study of myocardial substrate use in the failing heart will be summarized. Correlation of findings in animal models of heart failure to the human condition and the use of these imaging techniques to assess therapeutic responses will be discussed.

No. 22

INTERSTITIAL PULMONAY DISEASES: CLINICAL AND MOLECULAR BACKGROUND

J. C. Grutters;
Heart Lung Center Utrecht, loc. St. Antonius Hospital, Nieuwegein, The Netherlands

“Interstitial lung diseases” is an umbrella term that is characterized by varying types of inflammation and/or fibroproliferation in the lung parenchyma. Together they comprise ±15% of respiratory practice. The two most commonly seen forms are sarcoidosis and idiopathic pulmonary fibrosis (IPF). The pathobiology of sarcoidosis is characterized by granulomatous inflammation that occurs in genetically susceptible individuals upon contact with yet unknown antigens. Granulomas can be present in virtually any organ. Usually, the disease resolves spontaneously in one to two years, but in ±30% it persists and may lead to scarring, *i.e.* fibrosis. Current tools for evaluation of disease activity and extent are limited. For example, the ability to distinguish areas of inflammation from secundary fibrosis in pulmonary sarcoidosis, and detection of myocardial involvement are difficult. In addition, unraveling the genetic basis of sarcoidosis has shown the importance of precision of disease phenotyping, yet a diagnostic standard for such method is lacking. IPF is a lethal disease characterized by damage of the alveolar epithelium and interstitial fibroproliferation, with only minimal inflammation. Accurate tools for the evaluation of the underlying pathobiological processes are not yet available. Interestingly, ±15% of IPF patients have a familial background and in some families we and others have recently found evidence for a role of mutated surfactant protein C (SP-C). This may cause proprotein misfolding leading to alveolar cell apoptosis and/or abnormal concentrations alveolar SP-C. In conclusion, it remains a challenge for clinicians to accurately establish disease status in patients with ILD. It is intriguing to speculate if aforementioned insights may provide new uses for molecular imaging technology.

No. 23

TRANSLATIONAL MOLECULAR AND BIOMARKER DEVELOPMENT

J. M. Hoffman;
University of Utah School of Medicine, Salt Lake City, UT.

Molecular imaging agents and probes have great potential for use in basic research, clinical care of patients with numerous diseases, and in therapeutic drug development. Many challenges are encountered in the development and eventual translation of these molecular imaging agents and probes into clinical trials. This session at the 2006 Academy of Molecular Imaging (AMI) meeting will assist those individuals interested in molecular imaging agent and probe development and eventual performance of clinical trials to (1) better understand the importance of molecular imaging techniques as a biomarker in therapeutic drug trials; (2) to better understand the current regulatory process for new and existing molecular imaging agents and probes including the FDA guidance documents for Developing Medical Imaging Drug and Biological Products, the IND submission process, the newly published FDA Draft Guidance for an Exploratory IND, using the Radioactive Drug Research Committee (RDRC) process at your local university; (3) to gain a better understanding of the principles of design and conduct of high- quality molecular imaging clinical trials; (4) to gain a better understanding on getting a molecular imaging agent or probe clinical trial approved by the IRB, Radiation Safety Committee, and the FDA; (5) to gain a better understanding of the issues related to patient accrual, adverse event reporting, development of case report forms, etc and (6) to get an update on how to get funding for molecular imaging trials from federal and private sources.

No. 24

POSITRON EMISSION TOMOGRAPHY AND POSITRON EMISSION TOMOGRAPHY/COMPUTED TOMOGRAPHY FOR NMTS: SYSTEM COMPONENTS, OPERATION, IMAGE FORMATION, AND IMAGE QUALITY

K. Kanal and P. Kinahan
Department of Radiology, University of Washington

These presentations will provide attendees with an introduction to the physics and operational aspects of obtaining computed tomography (CT) and positron emission tomography (PET)/CT images. The four presentations are (1) CT and PET/CT Scanner Anatomy, (2) CT Image Formation, (3) CT Image Quality, and (4) CT and PET/CT Artifacts. Topics will include data acquisition and image reconstruction. The hardware, software and technical parameters, as well as imaging pitfalls will also be presented. Patient dosimetry and quality assurance will be briefly discussed. A synergy of PET/CT scanners is the use of the CT data for X-ray-based attenuation correction of the PET emission data. Proper interpretation of PET emission images corrected for attenuation using the CT image relies on an understanding of the potential artifacts. In cases where an artifact or bias is suspected, careful inspection of all three available images (CT, PET emission with and without attenuation correction) is recommended.

No. 25

MOLECULAR IMAGING OF SARCOIDOSIS

R. Keijsers;
St. Antonius Hospital, Nieuwegein, The Netherlands

Pulmonary and extra-pulmonary sarcoidosis activity can be assessed using Ga-67 planar/ single photon emission computed tomography (SPECT) imaging or 2-deoxy-2-[F-18]fluoro-D-glucose (FDG)- positron emission tomography (PET). Sensitivity of Ga-67 scans is high, but specificity is low. Therefore, Ga-67 imaging is of limited value in disease management. For FDG-PET, sensitivity and specificity have not been determined. In this study, we have compared FDG-PET and Ga-67 imaging in patients with biopsy proven sarcoidosis. A consecutive series of patients with suspected active sarcoidosis was studied. Both imaging modalities were analysed by three experienced nuclear physicians. Sarcoidosis activity was scored by three pulmonologists using a list of clinical, serological, radiological and physiological parameters. Preliminary results of the first 12 patients showed that sensitivity of Ga-67 imaging was 83%, while this was 92% for FDG-PET. This justified a larger study in which we have included 70 steroid-naïve patients until now. In general, FDG-PET was easier to interpret and significantly more lesions could be detected because of the higher target/non-target ratio. Several specific FDG uptake-patterns could be identified not only with regard to distribution in the lung parenchyma, but also in extrapulmonary sites. In conclusion, FDG-PET is a useful modality to assess sarcoidosis activity. Completion of this study will determine the exact sensitivity and specificity of FDG-PET versus Ga-67 imaging. We hypothesize that specific FDG uptake-patterns represent certain phenotypes that not only could be related to clinical outcome, but also to genotypes. Further investigation is needed to determine whether FDG-PET may contribute to individualization of clinical management, and may clarify the genetic basis of this disease.

No. 26

TIPS ON ACQUISITION, RECONSTRUCTION, AND DISPLAY OF BRAIN IMAGES

D. Kondas
Shared PET Imaging, Bloomfield Hills, MI.

Structural and functional imaging studies have been used in the evaluation of patients with dementia to determine the etiology of this disease. Positron emission tomography (PET) is a valuable tool due to the improvement in diagnostic accuracy in differentiating etiologies. PET can also be used in the diagnostic workup of other diseases such as seizure disorders, recurrent brain tumor, and cerebral perfusion deficits. As with any diagnostic technique the improvement in diagnostic accuracy is closely linked to the technical aspects of the study protocol. This will be a general overview of the use of PET in neurology focused on the technical aspects of the study. In addition there have been many improvements in the tools available for interpreting these cases and we will touch on the current options available.

No. 27

NEUROLOGY FOR THE IMAGING SPECIALIST: NORMAL BRAIN ANATOMY AND PHYSIOLOGY

D. Kondas
Shared PET Imaging, Bloomfield Hills, MI

Topics to be covered in this presentation include a description of the complex anatomy of the cranium and facial bones, and the major structures in the brain. The presentation will also provide attendees with a review of the functions of the different parts of the brain.

No. 28

MAGNETIC RESONANCE IMAGING OF STEM CELLS

D. L. Kraitchman;
The Johns Hopkins University, Baltimore, MD.

Exogenous cellular therapies are currently being explored to aid in the regeneration and to promote angiogenesis in many organs. The potential of these therapies to-date has been determined using models that require serially animal sacrifice for histological analysis. However, such techniques are not amenable to patient studies due to the limited ability to obtain tissue biopsies. Recently, magnetic labeling of stem cells using superparamagnetic iron oxide (SPIO) compounds followed by transplantation and transfusion has enabled non-invasive tracking of cellular distribution using magnetic resonance imaging (MRI) in animal models. Using modifications of electroporation techniques for DNA transfection, cells can be rapidly labeled with clinical approved MR contrast agents. MRI can then be used to determine the optimal timing and dosing of cellular therapies as well as cellular persistent. Because of the biocompatibility of these labeling techniques, clinical trials exploring the safety of SPIO-labeled cells have now been performed in Europe. When

combined with the ability to perform serial non-invasive MRI, these MR cellular labeling techniques appear well-poised to make the transition to the clinical realm for the evaluation of the efficacy of cellular therapies in human trials.

No. 29

F-18-PROLINE POSITRON EMISSION TOMOGRAPHY AND IDIOPATHIC PULMONARY FIBROSIS

J. Lavalaye;
St. Antonius Hospital, Nieuwegein, The Netherlands.

Diagnosis of idiopathic pulmonary fibrosis is made by multiple modalities, including lung function test, computed tomography (CT) and lung biopsy. However, no diagnostic test is yet able to assess actual activity of the fibrosis at the moment of diagnosis. If the clinician could assess disease activity at time point zero, he would be able to start the most proper patient management. In pulmonary fibrosis, an excess of collagen is found. While the amino acid proline represents a major constituent of collagen, labeling with F-18 would make it a dedicated positron emission tomography (PET) tracer. Previous studies found that Cis-4-fluoro-L-proline was incorporated in collagen. In a validated animal model, pulmonary fibrosis was induced in rabbits by inflating silica dust particles into one lung. Preliminary experiments demonstrated significant uptake of cis-4-fluoro-L-proline in the affected lung. In humans the radioligand cis-4-fluoro-L-proline has been found save, with an effective dose of 6.0 mSv for a dose of 400 MBq. We set up a proof of principle study, to determine cis-4-fluoro-L-proline uptake in the lungs of patients with severe pulmonary fibrosis. A dose of 400 MBq Cis-4-fluoro-L-proline was injected. PET was obtained two hours p.i. A standard 3-D RAMLA reconstruction algorithm was used and PET images were fused with recent spiral CT scans. We hope to show the first results of this pilot study at the AMI conference. As for now, cis-4-fluoro-L-proline is a promising ligand for PET imaging of idiopathic pulmonary fibrosis.

No. 30

POSITRON EMISSION TOMOGRAPHY CLINICAL INDICATIONS AND APPLICATIONS: CLINICAL INDICATIONS IN POSITRON EMISSION TOMOGRAPHY WITH 2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE ONCOLOGY

H. Macapinlac
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This presentation will provide attendees with an overview of the future directions of positron emission tomography (PET) imaging in oncology. Topics that will be covered include 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) biodistribution and the benefits of using FDG in PET oncology.

No. 31

BRAIN POSITRON EMISSION TOMOGRAPHY IN EVALUATION OF DEPRESSION AND ANTIDEPRESSANT THERAPY

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Columbia University / New York State Psychiatric Institute, New York, NY.

Positron emission tomography (PET) studies of mood disorders fall into three domains. The first examines regional blood flow or metabolism and has identified relationships of mood disorders and components of mood disorders to non-overlapping brain regions, suggesting that those brain regions have a role in the genesis of those components of mood disorders. Similarly, patterns of regional metabolic activity predict response to antidepressant treatment. The second domain targets neurotransmitter system components and has shown that serotonin transporter binding on serotonin nerve terminals and at the level of the serotonin raphe nuclei in the brainstem are altered in specific brain regions in major depressive disorder or bipolar disorders. Moreover, alterations in 5-HT1A autoreceptor and postsynaptic receptor binding have also been demonstrated in mood disorders. Serotonin receptor binding can also distinguish patients in terms of remission one year after antidepressant treatment. Studies combining PET imaging with relevant functional genetic variants for PET ligand targets offer great promise both in terms of a biochemical classification of mood disorders as well as in prediction of antidepressant treatment outcome. A third domain of PET research quantifies the relationship of receptor occupancy by antidepressants to therapeutic effects and side effects, as well as to plasma levels and oral doses of these drugs. Such methods allow for the most rapid estimation of likely therapeutic doses in order to guide the design of clinical trials testing the efficacy and safety of new medications.

No. 32

POSITRON EMISSION TOMOGRAPHY PHYSICS: QUANTITATIVE TECHNIQUES IN POSITRON EMISSION TOMOGRAPHY

O. Mawlawi
UT M.D. Anderson Cancer Center, Houston, TX

Tracer kinetic methodology /modeling will be explained in detail in this presentation, and the importance of attenuation correction will be discussed. Other topics that will be discussed include the factors that impact the accuracy and precision of positron emission tomography (PET) measurements.

No. 33

POSITRON EMISSION TOMOGRAPHY/COMPUTED TOMOGRAPHY IN ESOPHAGEAL CANCER

R. F. Munden;
MD Anderson Cancer Center, Houston, TX.

This presentation,"PET/CT in Esophageal Cancer" will focus on the role of positron emission tomography (PET)/ computed tomography (CT) in staging and evaluation of esophageal carcinoma. Emphasis will be on findings that differentiate surgical from nonsurgical candidates, especially in consideration of the nodal staging and detection of distant metastatic disease. At the end of this discussion, the attendee should be able to discuss the role of CT and PET/CT in the diagnosis and staging of esophageal carcinoma; discuss trends in treatment of esophageal carcinoma and the utility of imaging in re-staging; and understand the limitations of PET/CT imaging in esophageal carcinoma.

No. 34

USEFULNESS AND LIMITATIONS OF MAGNETIC RESONANCE IMAGING IN THE EVALUATION OF BRAIN TUMORS

W. Pope
David Geffen School of Medicine at UCLA, Los Angeles, CA

Some of the topics that will be discussed in this presentation are statistics, including prevalence and incidence of intracranial neoplasms and glioma subtypes; astrocytomas; survival for GBM vs. anaplastic astrocytoma and factors affecting survival; pathologic grading of astrocytomas, and low grade astrocytomas. The presentation will also discuss oligodendroglioma; intraventricular and pineal region tumors; DDX for peripherally enhancing lesions and tumor mimics including infection; the use of spectroscopy in differentiating tumors; Lhermitte-Duclos; extra-axial tumors including schwannoma, meningioma and other; and metasteses.

No. 35

NORMAL VARIANTS AND CEREBROVASCULAR DISEASE FINDING IN BRAIN COMPUTED TOMOGRAPHY

W. Pope
David Geffen School of Medicine at UCLA, Los Angeles, CA

This presentation will discuss the topics of basic intracranial vascular anatomy; CTA versus conventional angiography; ischemic and hemorrhagic stroke; and detecting acute stroke on computed tomography

(CT). Further topics will include cerebral cortical infarct including MCA, ACA and PCA; posterior fossa infarcts, watershed infarcts, and venous infarcts; and treatment for acute infarct including clot busting drugs and mechanical clot retrieval. The presentation will continue with the evolution of stroke, both acute (swelling, extension and hemorrhage) and chronic (encephalomalacia); small vessel ischemic disease (acute and chronic); and stroke mimics.

No. 36

2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE- POSITRON EMISSION TOMOGRAPHY AND POSITRON EMISSION TOMOGRAPHY/ COMPUTED TOMOGRAPHY IMAGING FOR LYMPHOMA AND MELANOMA

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The utilization of 2-deoxy-2-[F-18]fluoro-D-glucose (FDG)- positron emission tomography (PET) imaging is rapidly becoming the standard of care in the clinical management of patients with lymphoma and melanoma. In lymphoma, there are several clinical scenarios where PET has been proven to be particularly accurate and have an important impact on patient management: (1) Diagnosis, (2) initial staging, (3) restaging, and (4) treatment monitoring. All four of these clinical indications are covered by the Centers of Medicare Services (CMS) for reimbursement. In comparison to computed tomography (CT) and Gallium-67 imaging, FDG-PET is both more sensitive and specific (88% and 85% respectively) in identifying sites of disease at initial staging. This leads to a significant change in clinical management in approximately 10-20% of patients overall, most commonly through an upstaging of the patient's initial evaluation. Additionally, integrated PET/CT, which now comprise the vast majority of new PET scanner sales, appears to provide a further improvement in overall accuracy over standalone PET. In restaging and treatment monitoring, FDG-PET has a very high negative predictive value of 93-95% and is superior to conventional anatomical imaging for evaluating post-treatment response. This is not surprising given that residual masses are frequently present after therapy on CT imaging despite successful treatment. Importantly, a negative PET scan after therapy predicts longer progression free and overall survival than CT. FDG-PET is well suited for the evaluation of melanoma both because of the whole body coverage that PET imaging provides as well as because of the remarkable avidity of melanoma for FDG. Accordingly, CMS covers the usage of FDG-PET for the initial diagnosis, staging, and restaging of disease. It is specifically not covered for regional metastatic lymph node evaluation since PET is not able to detect micrometastases. Clinically, PET has the greatest potential impact on patient management in those patients with stage III disease with known/suspected regional lymph node disease and PET is used to detect distant metastases. PET may also be useful in the surveillance of those patients with high risk of recurrence.

No. 37

MOLECULAR IMAGING OF PROSTATE CANCER

H. Schöder
Memorial Sloan-Kettering Cancer Center, New York, NY

This presentation will categorize the clinical states of prostate cancer, and will name clinical scenarios in which positron emission tomography (PET) imaging of prostate cancer can affect patient management. Advantages and disadvantages of at least three PET tracers for imaging of prostate cancer will also be discussed.

No. 38

POSITRON EMISSION TOMOGRAPHY FOR CANCER SCREENING AND ASSESSMENT OF THE UNKNOWN PRIMARY. CLINICAL MANAGEMENT OF INCIDENTAL FINDINGS.

H. Schöder
Memorial Sloan-Kettering Cancer Center, New York, NY

This presentation will characterize, in statistical terms, a population in which cancer screening can be justified from a medical and economical point of view. The presentation will also define the term "carcinoma of unknown primary" and describe at least three common tests for evaluation of these patients. Other topics that will be covered include frequent misrepresentations of reported sensitivities and specificities of imaging studies with unknown primary.

No. 39

IMAGING OF VASCULAR DISEASE IN DIABETES: WHO AND HOW?

L. Shaw
Cedars-Sinai Medical Center, Los Angeles, CA

Diabetes mellitus continues to run rampant throughout the world. Recent estimates reveal that ~6% of the US population has diabetes (or 17 million Americans), with ~90% of the cases of diabetes being of type II. Diabetes has long been recognized to be an independent risk factor for cardiovascular diseases. Epidemiological studies have documented the excess cardiovascular disease risk in patients with diabetes from multiple racial and ethnic groups. Of particular importance for screening and diagnostic testing, the onset of microvascular and macrovascular complications start with the onset of hyperglycemia and in the prediabetic phase (i.e., "ticking clock" hypotheses).

Screening Asymptomatic Diabetics - Electron Beam Tomography (EBT) is increasingly being used to measure the extent of coronary artery calcium (CAC, by means of a calcium score) in asymptomatic and symptomatic patients with the intent of identifying subjects at increased risk of suffering a CAD event. Two larger prospective registries addressed the question of whether coronary calcium constitutes a risk for events in asymptomatic patients, but came to opposite conclusions. In the first, the South Bay Heart Watch (SBHW) was a prospective cohort study designed to determine the relation between radiographically detectable CAC and cardiovascular outcome in high-risk asymptomatic adults. In this study, 1,461 asymptomatic subjects $\geq$45 years old with cardiac risk factors were recruited via mass-mailing advertisement in the Los Angeles area; of these 19% were diabetics. In a sub-analysis of the main database after a mean follow-up of 6.3$\pm$1.4 years, Qu et al. found an increased risk of cardiovascular events (death, MI, stroke, or revascularization) in diabetic as compared to non-diabetic subjects in the presence of CAC. Raggi et al. utilized data from a database of 10,377 asymptomatic individuals (903 diabetic patients), followed for an average of five years after having been referred for an EBT screening for CAC. In this study, the risk of all-cause mortality was higher in diabetic patients for any degree of calcification than for non-diabetic subjects.

Diagnostic Testing Symptomatic Diabetic Patients - In symptomatic diabetics, a number of recent studies have noted an added predictive value of stress cardiac imaging. Of careful concern for the evaluation of risk is the fact that the ensuing event rates in diabetics are substantially higher than non-diabetic populations. As previously stated and recently noted in the National Cholesterol Education Program, diabetics are considered a risk equivalent with an expected 10-year rate of cardiovascular death or nonfatal myocardial infarction exceeding 20%. Thus, it is critical for physicians to understand that all event rates are shifted upwards with high complications noted for low, mildly abnormal, and moderate-severely abnormal studies.

Stress imaging is most commonly performed using echocardiography or gated single photon emission computed tomography (SPECT) imaging. An abundance of risk stratification evidence notes that the high degree of predictive accuracy of SPECT imaging in diabetic populations. In particular, diabetics have higher rates of cardiac death or myocardial infarction when compared to non-diabetics. Moreover, the rate of major adverse cardiac events is directly proportional to the extent and severity of perfusion abnormalities in female and male diabetics. Female diabetics with provocative ischemia, however, have worsening event-free survival rates that are nearly double that of their male counterparts. Other high risk subsets of diabetics include those requiring insulin to manage their diabetes.

Thus, a growing body of evidence is supportive of the utility of computed tomography (CT) and SPECT imaging strategies to identify risk in diabetic patients.

No. 40

BRAIN PET IN EVALUATION OF MILD COGNITIVE IMPAIRMENT AND DEMENTIA

D. H. Silverman;
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Several medical and neuroimaging advances are leading to a paradigmatic shift in evaluation and management of dementing illness. The last decade saw the emergence of the first drugs approved for treating neurodegenerative dementia; all of those indicated for mild to moderate Alzheimer's disease (AD) may actually worsen symptoms of patients with frontotemporal dementia (FTD), another neurodegenerative disease frequently clinically mistaken for AD. In 2004, Medicare coverage for 2-deoxy-2-[F-18]fluoro-D-glucose (FDG)- positron emission tomography (PET) was extended to assisting with the differential diagnosis of AD versus FTD. The same year, the FDA approved the first software package indicated for automatically regionally quantifying brain PET, and rapidly comparing activity in hundreds of standardized regions of interest with activity in normal subjects. OUTDATED PARADIGM: clinical diagnosis possessing limited accuracy, and rarely informed by metabolic neuroimaging - which, even when available, was often unaffordable and variably interpreted - to establish presence of a disease for which there was no specific treatment. UPDATED PARADIGM: more accurate diagnostic assessment, informed by metabolic cerebral imaging - available to the vast majority of affected Americans, as well as interpretable and quantifiable with a high degree of uniformity - to establish presence of a disease for which the standard of care includes specific pharmacotherapy. This new paradigm, which has a strong evidentiary basis for the evaluation of patients with dementia, is also being increasingly applied to the evaluation of patients with mild cognitive symptoms. The extent to which the current status of available evidence supports, or fails to support, the latter application will be examined.

No. 41

ROLE OF TARGETED MOLECULAR IMAGING FOR PREDICTION OF POST MYOCARDIAL LEFT VENTRICULAR REMODELING

A. J. Sinusas;
Yale University, New Haven, CT.

Changes in structure, geometry and function of the left ventricle (LV) occur following myocardial infarction (MI) and have been termed post-MI remodeling. It is now well recognized that the process of post-MI myocardial LV remodeling is associated with important changes within the myocardial extracellular matrix (ECM). The matrix metalloproteinases (MMPs) constitute a large family of proteolytic enzymes responsible for ECM degradation and remodeling under normal and pathological conditions. A clear cause/effect relationship between MMPs and the LV remodeling process has been demonstrated through the use of animal models of developing congestive heart failure, transgenic models, as well as through the use of pharmacological MMP inhibition studies. However, a non-invasive method for detecting and quantifying MMP activity in-vivo during the evolution of post-MI remodeling has yet to be developed and forms a critical component for translating these basic observations to clinical applicability. We have been developing high sensitivity MMP targeted molecular imaging approaches in order to quantify regional MMP activity post-MI. The studies to be discussed employ multi-modality single photon emission computed tomography (SPECT)/ computed tomography (CT) imaging and established murine and porcine models of post-MI remodeling, to relate temporal changes in regional MMP activation with changes in regional myocardial deformation. The targeted molecular imaging approach that will be discussed should provide unique insights into the role of MMP activation in the processes involved in LV remodeling, and translate to direct clinical applications that hold both prognostic and diagnostic potential.

No. 42

POSITRON EMISSION TOMOGRAPHY ONCOLOGY REVIEW: CLINICAL INDICATIONS

N. Swanston
UT M.D. Anderson Cancer Center, Houston, TX

This presentation will review the principles of oncology imaging, and will discuss positron emission tomography (PET) oncology application in a variety of cancers. The staging system and characteristics of cancers typically imaged in PET will also be described.

No. 43

POSITRON EMISSION TOMOGRAPHY ONCOLOGY REVIEW: RADIATION SAFETY

N. Swanston
UT M.D. Anderson Cancer Center, Houston, TX

This presentation will give a detailed description of exposure levels to positron emission tomography (PET) personnel, and will review the safe handling of PET radioisotopes. Techniques on how to maintain ALARA principles in PET will also be discussed.

No. 44

2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE-POSITRON EMISSION TOMOGRAPHY IMAGING OF NON-MALIGNANT PULMONARY DISEASES

F. Verzijlbergen
St. Antonius Hospital, The Netherlands

2-Deoxy-2-[F-18]fluoro-D-glucose (FDG)-uptake in benign pulmonary lesions may be as high and as variable as in malignant disease. Discrimination on the FDG- positron emission tomography (PET) images is difficult. The clinical findings and distribution pattern of FDG may be helpful, but in most cases only histologic verification will lead to the diagnosis. Data from Japan suggest that an increasing standardized uptake value (SUV) on early and late images is indicative of malignancy and a stable or decreasing SUV is found in patients with inflammation. In a prospective study in our hospital in 87 patients with pulmonary lesions, early (45 min. after injection) and late (90 min.) FDG-PET images were obtained. In 76% of the patients with a malignancy the SUV increased, but in 46% of the patients with an infection the SUV increased as well and as strong. In both groups the SUV decreases in 2% of the patients. Clearly, obtaining both early and late FDG-images has very limited value with regard to discrimination between malignancy and inflammation. It is very important to be able to recognize disease-specific patterns of FDG-distribution in the lungs, relate the images with the clinical findings and to point at areas where a biopsy may lead to the final diagnosis. During the presentation FDG-PET images of patients with sarcoidosis, idiopathic pulmonary fibrosis, vasculitis and specific pulmonary infections will be demonstrated. In most cases FDG-PET not only played an important role in the diagnostic strategy of these patients, but also in monitoring disease activity during therapy.

No. 45

QUANTIFICATION OF 2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE-POSITRON EMISSION TOMOGRAPHY/ COMPUTED TOMOGRAPHY IMAGING

W. Weber
David Geffen School of Medicine at UCLA, Los Angeles, CA.

For staging of malignant tumors 2-deoxy-2-[F-18]fluoro-D-glucose (FDG)-positron emission tomography (PET) scans are assessed visually, and focally increased FDG-uptake not explained by the normal biodistribution

of FDG is considered to be suspicious for metastatic disease. In a similar way PET scans may also be read *after completion* of chemo- or radiotherapy. FDG uptake should have normalized at this time and focal FDG-uptake generally indicates residual viable tumor tissue. However, quantitative assessment of tumor metabolism becomes necessary, when FDG-PET scans are performed during treatment in order to *predict* subsequent tumor response. At this time the metabolic activity of the tumor tissue has decreased in responders, but generally there will be still considerable residual FDG-uptake. This presentation focuses on practical aspects of FDG-PET imaging for treatment monitoring and discusses how to perform quantitative assessment of tumor FDG-uptake in clinical studies.

Quantification of tumor metabolic activity by FDG-PET is complicated by the fact that several factors other than tumor glucose use have an impact on the FDG-signal. Partial volume effects can cause a marked underestimation of the true activity concentration within a tumor. For a spherical lesion with a diameter equivalent to 1.5 times the spatial resolution of the PET scanner at full width half maximum (FWHM) the measured maximum activity concentration is only about 60% of the true activity concentration. The mean activity concentration is even lower, about 30% of the true activity concentration. In addition to these principal physical limitations of PET imaging, the processing of PET images and the definition of regions of interest affects the results of quantitative measurements of tumor FDG-uptake. Smoothing of images, for example by a Gauss filter, will decrease the measured FDG-uptake. Due to partial volume effects the mean measured tumor FDG-uptake will decrease, when the size of the region of interest used to define the tumor is increased. On the other hand, image noise will lead to larger random errors of the measured tracer uptake, when the size of the region of interest is decreased. Furthermore, it must be considered that FDG-uptake of malignant tumors is time dependent. In most tumors tracer uptake increases for at least 90 minutes after injection of FDG. Thus FDG-uptake will generally be considerably higher at later than at earlier time points.

Considering all these factors it becomes clear that it is very challenging to quantify tumor glucose utilization by FDG-PET in a clinical setting. However, this does not mean that it is equally difficult to measure relative *changes* in tumor glucose utilization over time. In this case only an intraindividual comparison of two studies is performed. This significantly reduces the number of factors that may confound the FDG-signal. Therefore, the accuracy of FDG-PET for measuring changes in tumor glucose utilization is considerably better than its accuracy to quantify tumor glucose utilization in absolute units. If patients are imaged according to a standardized protocol, the test-retest reproducibility of FDG-PET is high and even relatively small changes in metabolic activity (20%) can be detected by PET imaging. Several studies have now indicated that early quantitative changes in metabolic activity are significantly correlated with subsequent tumor response and patient survival.

No. 46

EARLY RESPONSE EVALUATION IN BREAST CANCER USING POSITRON EMISSION TOMOGRAPHY

W. Weber
David Geffen School of Medicine at UCLA, Los Angeles, CA.

Neoadjuvant chemotherapy is increasingly used to treat patients with locally advanced breast cancer in order increase the rate of curative resections. Additionally, patients with a histopathologic response have significantly higher disease-free and overall survival rates than nonresponders. Approximately 70% of the patients undergoing primary chemotherapy show clinical response, but only 20% to 30% have partial or complete regression in histopathologic tissue analysis. The therapeutic effectiveness of neoadjuvant chemotherapy cannot be determined accurately until definitive breast surgery is performed. Considering the side effects of chemotherapy, there is a need for early identification of nonresponding patients. Following the encouraging results from Wahl et al. published already in 1993, several groups have evaluated the use of 2-deoxy-2-[F-18]fluoro-D-glucose (FDG)- positron emission tomography (PET) for monitoring treatment of breast cancer.

Smith et al. have evaluated the accuracy of FDG-PET to predict histopathologic response in 30 patients with locally advanced breast cancer undergoing preoperative chemotherapy. After a single cycle of chemotherapy, PET predicted complete pathologic response with a sensitivity of 90% and specificity of 74%. In another study, Schelling et al compared results from PET imaging with pathologic response. As early as after the first course of therapy, responding and non- responding tumors could be differentiated by PET. In contrast, Mankoff et al found a large overlap between changes in metabolic activity in histopathologic responders and nonresponders. This discrepant finding may be explained by the different timing of the PET scans. In the study by Mankoff et al. the follow-up PET scans were performed after two months of chemotherapy. After that period of time histopathologic nonresponding tumors may demonstrate a relatively large decrease in tumor size and FDG-PET may be unable to differentiate between small absolute differences in the amount of viable tumor cells. Consistent with this explanation, Smith et al also observed a higher accuracy of FDG-PET for prediction of tumor response after the first cycle of chemotherapy than at later points in time.

A transient increase in glucose use has been found in responding tumors treated with hormonotherapy. An explanation for this metabolic flare effect is that antiestrogen therapy first has an agonist effect before the antagonist effect overrules. This agonist effect occurs within seven to 10 days after the beginning of a treatment and is usually followed by disease remission. There are no reports so far about metabolic flare in more aggressive chemotherapeutic regimes.

No. 47

RESPONSE EVALUATION IN LUNG CANCER

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2-deoxy-2-[F-18]fluoro-D-glucose (FDG) - positron emission tomography (PET) has been used in several study to monitor the effects of chemo- and chemoradiotherapy. Most of these studies have evaluated definitive chemoradiotherapy or preoperative (neoadjuvant) therapy in patients with locally advanced non-small cell lung cancer.

MacManus et al studied the use of FDG-PET after chemoradiotherapy in patients with locally advanced non-small cell lung cancer. Sevnety-three patients were prospectively evaluated for tumor response to chemoradiotherapy by computed tomography (CT) and FDG-PET. Complete response in FDG-PET was defined as normalization of all sites with abnormal FDG uptake and partial response as a significant reduction in FDG uptake of all known lesions without the appearance of new lesions. Tumor response assessed by FDG-PET predicted better patient survival than response by CT criteria, the pre-treatment tumor stage, or patient performance status. The correlation between tumor FDG-uptake after chemoradiotherapy and patient outcome was confirmed in a recent study by Hellwig et al. These investigators studied 47 patients after preoperative chemoradiotherapy. In several studies FDG-uptake after chemoradiotherapy and or changes during chemoradiotherapy have been correlated with histopathologic tumor regression. All these studies report a significant correlation between the findings in FDG-PET and histopathological tumor regression. The individual values for sensitivity and specificity, however, vary widely (58%-100%). This is likely due to the fact that different criteria for a histopathologic response were used in this study (no viable tumor cells, less than 10% viable tumor cells). Furthermore, the number of patients with a histopathological response was generally small in these studies; as a consequence the values for the specificity of FDG-PET to detect residual tumor tissue (non-responders) must be interpreted with caution.

Quantitative measurements of tumor FDG uptake *during* preoperative chemotherapy have also been shown to be predictive for patient outcome. In a group of 47 patients treated by preoperative chemoradiotherapy followed by surgical resection Residual metabolic rates after the first chemotherapy cycle and relative changes of tumor FDG-uptake were significantly correlated with overall survival. Similarly, changes of tumor FDG-uptake after the first cycle of palliative therapy have been shown to be a significant prognostic factor. These data suggest that FDG-PET may

be used to individualize the use of chemotherapy in patients with lung cancer.

No. 48

NURSING INTERVENTIONS AND CLINICAL SKILLS IN ONCOLOGY: ASSESSMENT SKILLS FOR THE ONCOLOGY PATIENT and MANAGING COMPLEX INTERVENTIONS AND SUPPORTIVE CARE

S. Worchesik
UT M.D. Anderson Cancer Center, Houston, TX

The first part of this presentation, Assessment Skills for the Oncology Patient, will include a review of how to obtain accurate respiration and pulse rate, describe normal and abnormal blood pressure and blood glucose measurements, and explain medications commonly seen in oncology settings. Part two of the presentation, Managing Complex Interventions and Supportive Care, will describe techniques for proper administration of radiopharmaceuticals and will outline actions taken in various medical emergencies. The presentation will also review management of diabetes and allergic reactions to contrast media.

No. 49

ANTIBODY IMAGING: PRESENT AND FUTURE

A. M. Wu;
David Geffen School of Medicine at UCLA, Los Angeles, CA.

Antibody-mediated delivery of radionuclides offers the potential for highly sensitive imaging of specific biological markers *in vivo*. Particularly in oncology, radioimmunoimaging can play an important role at many points including: detection of disease, identification based on the expression of cell-surface tumor markers, selection of targeted therapies, and monitoring response to therapy. Although currently approved antibody imaging agents have only achieved modest success in the marketplace, the field is poised to advance due to a number of recent developments. Advances in proteomics research are generating novel candidate markers, including cell surface proteins, that would be amenable to antibody targeting. Antibody engineering has solved the issue of immunogenicity, with routine humanization of murine monoclonal antibodies and direct generation of human antibodies from transgenic mice. Furthermore, protein engineering allows broad control over the biological properties of antibodies, including production of recombinant fragments (such as diabodies and minibodies) with pharmacokinetics tailored for *in vivo* imaging. Greater availability of positron-emitting radionuclides with longer half lives (such as Cu-64, Y-86, and I-124) has led to a resurgence of interest in immunoPET. This presentation will provide an update on promising antibody tracers that are progressing from pre-clinical to early clinical evaluation.

No. 50

POSITRON EMISSION TOMOGRAPHY/OPTICAL IMAGING OF STEM CELLS

J. C. Wu;
Stanford University School of Medicine, Stanford, CA.

In recent years, stem cell therapy has rivaled gene therapy as a promising treatment modality for ischemic heart disease. Several phase 1 clinical studies have shown that the implantation of skeletal myoblasts, endothelial progenitor cells, or bone marrow stem cells into the infarcted myocardium can result in improved function. The mechanisms by which stem cells achieve this effect are not completely known. Possible mechanisms include stem cells secreting paracrine factors, providing a mechanical scaffold, and/or recruiting other peripheral and resident cardiac stem cells. However, the analysis of stem cell therapy, like gene therapy, relies primarily on postmortem histology to identify their presence. For human studies, changes in clinical endpoints (e.g., contractility on echocardiography or perfusion on positron emission tomography (PET)/ single photon emission computed tomography (SPECT)) can be followed, but they are only indirect evidence of cell survival and function. Thus, the ability to study stem cell survival and proliferation in the context of the intact living body rather than postmortem histology would yield better insight into stem cell biology and physiology. In this talk, we will review recent advances in PET and optical imaging cardiac stem cell therapy.

No. 51

CHARACTERIZING PLAQUE WITH COMPUTED TOMOGRAPHY AND MAGNETIC RESONANCE IMAGING: IS IT POSSIBLE?

C. Yuan;
University of Washington, Seattle, WA.

Atherosclerotic disease is a major source of thrombo-embolism and subsequent heart attack and stroke. Non-invasive *in vivo* investigations of the relationship between atherosclerotic plaque tissue composition/morphology and the development of clinical symptoms may provide critical information for the detection of vulnerable plaque. Histological studies suggest that the vulnerable plaque is characterized by thinning and rupture of the fibrous cap that overlies the necrotic core. Exposure of the thrombogenic core following cap rupture is thought to lead to ischemic complications from thrombotic occlusion of the vessel or distal embolization of athero-thrombotic debris.
This lecture will discuss 1) the current status of magnetic resonance imaging (MRI) and computed tomography (CT) techniques in atherosclerosis imaging of various arterial beds; 2) the use of these techniques to measure plaque burden and identify tissue composition; 3) the use of contrast agents in plaque imaging; and 4) the potential clinical use of these techniques.

Institute for Molecular Imaging Sessions

No. 52

THE PINHOLE: GATEWAY TO ULTRA-HIGH RESOLUTION 3-D RADIO-NUCLIDE IMAGING

F. J. Beekman;
University Medical Centre Utrecht, Utrecht, THE NETHERLANDS.

Today the majority of clinical molecular imaging procedures are carried out with gamma cameras, both planar and in single photon emission computed tomography (SPECT) mode. SPECT with pinholes for the imaging of 3-D radio-molecule distributions in small experimental animals is rapidly gaining popularity. With pinhole SPECT very high spatial resolutions (< 0.35mm, < 0.05 micro-liter) are obtained, which allows to assess functions of much smaller structures than is possible with small animal positron emission tomography (PET) (volumetric resolution ~1 micro-liter). In this paper the history of image formation with pinholes will be summarized, and the principles of pinhole imaging and pinhole tomography including the basics of image reconstruction are explained. In addition recently introduced ultra-high resolution small animal SPECT imaging systems with focusing pinholes are presented as well as new avenues to further improve the system performance. It is expected that these systems will open complete new application fields. In the near future not only small animal SPECT systems but also clinical SPECT systems with focusing pinhole gamma cameras will have resolutions that improve over that of PET devices for an increasing number of clinical applications: A case study about a cardiac pinhole system shows that an order of magnitude faster SPECT imaging or a much more detailed molecular imaging of the human heart will be possible soon.

No. 53

IMAGING SIGNAL TRANSDUCTION

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Hormone-induced signaling by cell surface receptors is often studied in *in vitro*. This enables isolation of ligand-dependent effects under controlled

situations, but such models do not assess signaling in the context of the intact animal. Even assessment of signaling *in vivo* usually involves sacrifice of the animal and harvesting of the tissue in question for its analysis, not allowing serial measurement in the same animal. Growth hormone (GH) regulates postnatal growth and metabolism in humans and other vertebrates by interacting with cell surface GH receptor (GHR), which couples to the tyrosine kinase, JAK2, for signaling. Liver is a major GH target organ, responding with production of insulin-like growth factor-1 (IGF-1), a GH effector. This hepatic GH response is mediated by JAK2-dependent activation of a transcription factor called signal transducer and activator of transcription 5b (STAT5b). In this presentation, I will discuss development of an *in vivo* serial bioluminescence imaging system to noninvasively study hepatic GH signaling. We prepared adenoviruses encoding the rabbit GHR (Ad-GHR) and a luciferase reporter gene driven by STAT5-binding GH response elements (Ad-GHRE-luc). We first verified that Ad-GHR directs hepatic cell surface GHR expression *in vivo* by infecting nude mice and detecting liver GHR with Tc-99m-labeled antibodies to the extracellular domain and gamma camera imaging. Coexpression of Ad-GHR and Ad-GHRE-luc *in vitro* in a cell culture model system allowed detection of GH-induced signaling (luciferase activity), proving that the intactness of the receptor and reporter system. We pursued *in vivo* experiments in nude mice in which Ad-GHRE-luc and Ad-GHR (1 x 10^9 pfu each) were injected i.v. one day apart (vs. Ad-GHRE-luc alone or no virus). Two days later, baseline bioluminescence images were obtained and mice were injected with GH (1 mcg/gm iv). Thereafter, images were obtained 1, 3, 5, and 7 hours after GH. We observed substantial GH-induced liver bioluminescence in mice receiving both viruses, which was significantly more robust than in those not receiving Ad-GHR. Peak response was at three hours after GH. This is the first system of which we are aware that allows noninvasive monitoring of *in vivo* hormone signaling serially within the same animals. This should allow important studies of GHR, JAK2, and STAT5 mutants in intact animals.

No. 54

MULTIMODALITY SMALL ANIMAL IMAGING: RECONSTRUCTION AND REGISTRATION

R. M. Leahy;
University of Southern California, Los Angeles, CA.

I will describe our recent work in molecular imaging using radiolabelled, bioluminescent and fluorescent probes or markers, with an emphasis on the problem of reconstructing fully 3-D quantitative images. Using a common framework of accurate modeling of the physics and statistics of nuclear and optical imaging systems, we compute images designed to optimize resolution recovery. The factors limiting resolution and image quality in positron emission tomography (PET), bioluminescence and fluorescence imaging using these methods will also be discussed. As with clinical imaging, it is important to relate these functional images to the underyling anatomy. For this reason there is an increased emphasis on multimodality imaging, combing functional imaging with X-ray computed tomography (CT) or anatomical magnetic resonance imaging (MRI). This in turn requires the use of computational methods for image alignment. Co-registration of anatomical and functional images can use either rigid or elastic transformations guided either by a limited number of fiducial points or the intensities of the images themselves. I will illustrate these techniques in application to the coregistration of PET and X-ray CT volumes with a 3-D mouse atlas. Finally, I will discuss the use anatomical images directly in the formation of functional images, both for accurate modeling of photon scatter and attenuation, and as priors in which anatomical morphology influences the spatial structure of the reconstructed functional image.

No. 55

REPORTER PROTEIN-FRAGMENT -ASSISTED-COMPLEMENTATION FOR IMAGING CELLULAR PROTEIN-PROTEIN INTERACTIONS IN LIVING ANIMALS

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Reporter genes such as luciferases, □-galactosidase and fluorescent proteins have been used extensively for tracking different cellular processes. Before the development of yeast two-hybrid system and split optical reporters, the studies of protein-protein interactions were exclusively restricted to either in cell lysates or very rarely in intact cells. Recent technological advancement in detection of photons emitted from fluorescent proteins or enzyme-based reporter proteins in the presence of their corresponding substrates has further enhanced the use of these reporter genes in pre-clinical drug development as well as validation of developed drugs for many diseases in living subjects. In the split reporter protein-fragment-assisted-complementation strategy, the reporter genes were rationally split and fused with each of the interacting protein partners such that both these fusion proteins are inactive until they are brought to close proximity by the two interacting partners. We have utilized the split optical reporter genes like Firefly and Renilla luciferases, and fluorescent proteins like green fluorescent protein for studying different protein-protein interactions in intact cells and in living animals. Recently we used this split protein strategies for imaging different protein-protein interactions including positively interacting proteins (transcription factors Id/MyoD), homodimerization (Herpes Simplex Virus Thymidine Kinase, TK/TK) and small molecule-induced heterodimerization (FRB/FKBP12 by Rapamycin) in cells and in living animals. We have also extended this strategy for imaging intramolecular folding of estrogen receptor in response to different ligands in intact cells and living animals. The protein-fragment-assisted-complementation technology is very useful in studying real time protein-protein interactions in living subject that was not possible before.

Society of Non-Invasive Imaging in Drug Development Sessions

No. 56

MEASURING RECEPTOR DENSITY AND AFFINITY IN PHARMACOLOGICAL CHALLENGES: APPLICATION TO DRUG DEVELOPMENT

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The current method to evaluate the status of receptors or transporters in health and disease is to measure the binding potential, BP. Most positron emission tomography (PET) measurements yield a final value that can be likened to a BP. This BP is often assumed to represent mostly the density of the receptor but in fact is an estimate of the ratio of receptor density to affinity. *In vivo* scatchard studies differ from in vitro studies in many respect but can be used to provide an estimate of the "true" density and affinity in a live organism. The most interesting condition would be to evaluate the effect of a drug both acutely and chronically on a receptor system. *In vivo* evaluation of density and affinity after a challenge faces multiple challenges, one being the design and interpretation of the PET study. We will review some of the differences between *in vitro* and *in vivo* scatchard studies. We will describe our PET experience with *in vivo* evaluation of receptor density and affinity in a drug exposure condition and outline some of the pitfalls to avoid using as an example, the DA D2 receptors and drugs known to modify DA concentrations.

No. 57

POSITRON EMISSION TOMOGRAPHY IMAGING OF GENE THERAPY FOR PARKINSON'S DISEASE

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The ability to determine the level and duration of gene expression in-vivo is critical for the clinical use of gene therapy. Positron emission tomography (PET) can be used to monitor gene expression using tracers that image the spatial distribution of the therapeutic transgene product. We have used PET to monitor gene expression in a primate model of Parkinson's disease (PD). Our therapeutic strategy is aimed at increasing striatal aromatic L-amino acid decarboxylase (AADC) levels. Striatal

neurons infected with the AADC gene by an adeno-associated viral (AAV) vector can convert low doses of systemically administered L-dopa to dopamine resulting in clinical improvement without the side effects typically association with higher doses of L-dopa. We have shown that PET measures of striatal uptake of the AADC tracer, 6-[F-18]fluoro-L-m-tyrosine (FMT), is substantially increased following the AAV delivery of AADC gene in parkinsonian monkeys, and striatal FMT uptake correlates with histological measures of the extent of gene expression. We have also performed longitudinal PET studies and have demonstrated sustained gene expression and clinical improvement for over five years. These findings have lead to the development of a human clinical trial that began last year. Initial PET studies show an increase in the gene product, AADC, indicating successful gene transfer. These early findings show that this gene therapy approach appears to be both safe and effective. Future studies will determine the therapeutic efficacy and duration of gene expression in PD patients.

No. 58

3.0 TESLA MAGNETIC RESONANCE IMAGING OF CAROTID ATHEROSCLEROSIS

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In the carotid artery, the rupture of vulnerable plaques, characterized by thin fibrous caps overlying large lipid pools with abundant macrophages, is a common cause of stroke, the third leading cause of mortality and morbidity in the United States. Magnetic resonance imaging (MRI) of carotid atherosclerotic plaque composition although not clinically routine at 1.5 Tesla is now increasingly used for clinical trials and prospective epidemiological studies. With FDA approved 3.0 Tesla whole body MRI platforms making their way into the clinical arena, both neuro and body imaging applications have realized significant gains in spatial resolution and image acquisition speed. We have developed carotid artery imaging at 3.0T with the main motivation of applying the signal to noise gain for high spatial resolution and reduction of signal averaging requirements to characterize atherosclerotic plaque. We present the multi-contrast approach relying on the endogeneous contrast mechanisms of T1, T2 and proton density weighting and enhancement with the exogenous contrast agent GdDPTA to elucidate the major plaque components including calcium, lipid necrotic core, fibrous cap and hemorrhage at 3.0T. In comparison to our earlier 1.5T *in vivo* patient data acquired with a single loop surface coil, we can achieve comparable signal to noise (SNR) in images with 3.5 times higher spatial resolution at 3.0T using a phased array surface coil. With respect to scan time, the implementation of parallel methods such as generalized autocalibrating partially parallel acquisitions (GRAPPA) provide the opportunity to reduce total image acquisition times with only a slight penalty in SNR which is offset by the higher magnetic field strength. To assess feasibility of a longitudinal study of plaque progression and monitoring therapy response at 3.0T, we have investigated the test-retest reproducibility in patients with a spectrum of atherosclerotic disease states. Atherosclerosis in its earliest form of intimal-medial thickening can be readily measured with sub-millimeter isotropic resolution and characterization of an advanced plaque in an endarterectomy candidate has been validated with histology. The 3.0T carotid artery exam is stable and provides quantitatively better MR imaging endpoints than 1.5T for plaque progression analysis. Thus we conclude, “the shape of things to come” will be utilization of 3.0 Tesla MR in longitudinal assessment of atherosclerotic plaque for clinical management and therapeutic agent trials.

No. 59

CELL TRACKING WITH MAGNETIC RESONANCE IMAGING USING MICRO SIZED PARTICLES OF IRON OXIDE

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There is growing interest in using iron oxide based magnetic resonance imaging (MRI) contrast agents for targeting specific receptors and for imaging cell migration. Typically, dextran coated iron oxide particles ranging in size from 20-100 nm are used for these applications and typically it takes on the order of a few million particles (> 1 pg of iron) in a voxel for adequate detection. Over the past few years we have demonstrated that widely available, micron sized particles of iron oxide (MPIO) are advantageous for cell labeling and tracking studies. Indeed, individual micron sized particles can be detected in phantoms, in cultured cells, and in fixed mouse embryos. MPIOs readily label all dividing adherent cells that we have tested with labeling as high as 100 pg of iron per cell. This large amount of labeling enables detection of single cells *in vivo*. Due to the inert, divinyl benzene/polystyrene coating on these particles, they last for at least six months in cells. In non-adherent cells, the particles can be loaded into cells, such as lymphocytes by an antibody mediated delivery system. Finally, the need to accumulate only a few of these particles in a cell for detection enables inefficient labeling strategies. For example, injection of MPIOs into the ventricles of the rat brain enables labeling of the neural stem cells that migrate from the subventricular zone to the olfactory bulb. This talk will summarize these results and indicate future directions for integrating cellular imaging with MPIOs with other MRI based functional and molecular imaging.

IMI/SNIDD Joint Sessions

No. 60

IN VIVO IMAGING OF TRANSPLANTED PANCREATIC ISLETS: IS POSITRON EMISSION TOMOGRAPHY THE ANSWER?

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Type 1 diabetes mellitus (T1DM) results from the almost total destruction of insulin-producing pancreatic islet Î²-cells. Islet transplantation is an attractive approach for the maintenance of normal blood glucose levels in T1DM patients but, despite advances in transplantation protocols, there is a massive loss of islets during and after transplantation. At present it is only possible to estimate functioning islet mass indirectly, through the measurement of C-peptide and insulin. Additionally, the development of methods for tracking islets non-invasively in rodents is important for facilitating the development of *in vitro* and *in vivo* protocols for prolonging islet survival. *In vivo* islet imaging protocols utilizing magnetic resonance imaging (MRI), optical imaging and positron emission tomography (PET) exhibit varying degrees of sensitivity and specificity. We have established a quantitative *in vivo* microPET system for imaging transplanted mouse islets infected with a recombinant adenovirus expressing a mutant form of herpes simplex virus 1 thymidine kinase (HSV1-Sr39TK) (rAD-TK), with detection using the PET reporter ligand 9-(4-[18F]-Fluoro-3-hydroxymethylbutyl)-guanine ([18F]FHBG. The microPET signal was directly proportional to the number of transplanted islets and sufficiently sensitive to detect small changes in mass. Islet graft survival in diabetic NOD mice could be tracked for one month and the PET signal reflected the insulin secretory capacity of transplanted islets. This demonstrates the viability of quantitative *in vivo* PET imaging of islets and suggests that appropriate PET systems for human islet tracking may be an answer to the current lack of appropriate technology.

No. 61

UNDERSTANDING ANTIPSYCHOTIC ACTION VIA IMAGING - LESSONS FROM THE BENCH TO THE BEDSIDE

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For nearly half a century antipsychotics have been used in animals as well as humans. This talk will review the contributions of "receptor occupancy" imaging studies in humans, and parallel preclinical studies, in understanding antipsychotics. The talk will first explain the concept of receptor occupancy and show how for most antipsychotics haloperidol, olanzapine, risperidone and ziprasidone response is achieved when 60%-70% striatal D2 receptors are blocked and much higher blockade (>80%) leads to motor side-effects. Exceptions to these rules (clozapine,

aripiprazole) will be addressed. The talk will then focus on more recent studies that have examined the differential contributions of striatal ([C-11]-raclopride) and extrastriatal ([C-11]-FLB-457) receptors to antipsychotic response. The talk will then illustrate how the receptor occupancy studies help unite preclinical findings at the "bench" to their "bedside" counterparts. In particular, the models of catalepsy and conditioned avoidance response which are used as models of motor side-effects and antipsychotic efficacy, respectively, required occupancies in animals which reflect their clinical counterparts. Thus, D2 occupancy provides a mechanistic foundation for relating these preclinical findings to their clinical counterparts. Finally, it will be shown how this bench-bedside knowledge can be used to inform clinical practice and new drug development.

Oral Presentations

Basic Science Oral Presentations

No. 62

POSITRON EMISSION TOMOGRAPHY IMAGING OF UNANESTHETIZED ANIMALS USING A RADIOACTIVE FIDUCIAL MARKER-BASED TRACKING SYSTEM

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Anesthetics used to keep small animals motionless during positron emission tomography (PET) studies are a major confounding factor in the uptake and retention of radiotracers, particularly in the brain. This study proposes the use of three small (1mm) radioactive fiducial markers (10uCi Na-22) placed on the head of an awake animal to track the motion using only data from the PET scanner. Phantom studies were performed to measure the accuracy with which the fiducial markers could be tracked in the presence of a radioactive background. A hot-rod cylinder (3cm diameter) was filled with 1mCi F-18, and the three markers attached to the outside. The cylinder was translated through the field of view at a known speed, and list mode data acquired, which was subdivided into 100-500ms time frames, and reconstructed using OSEM. The fiducials were detected automatically based on a thresholding algorithm, and the translation parameters used to reorient the sinogram frames. To test the motion of an animal, a rat had fiducials taped to its head, and was allowed to move freely within the scanner. 200ms frames were reconstructed and the movement of the head of the rat was tracked. The fiducial markers on the phantom could be detected in frames as short as 100ms, and the mean absolute error in tracking was 0.34mm. Motion of the rat's head was tracked in all 200ms frames, without significant blurring of the markers. These studies suggest that motion tracking with radioactive fiducials is possible, and can accurately follow movement in an awake rat.

No. 63

F-18-LABELED ANTI-CEA DIABODY FOR POSITRON EMISSION TOMOGRAPHY IMAGING OF COLORECTAL CANCER

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Objective: In this study we investigated the F-18-labeled anti-carcinoembryonic antigen (CEA) T84.66 diabody, a genetically engineered non-covalent dimer of scFv, for microPET imaging of colon cancer xenografts. Methods: F-18 labeling was achieved by coupling of the CEA diabody (molecular weight 55 kDa) with *N*-succinimidyl-4-[F-18]fluorobenzoate ([F-18]SFB). Biodistribution of [F-18]FB-anti-CEA diabody was evaluated in athymic nude mice bearing subcutaneous human colon carcinoma LS174T tumor. Serial microPET imaging studies were carried out to further evaluate *in vivo* targeting and pharmacokinetics. Results: Radiolabeling required 150 ± 20 min and specific activities of 102.6 ± 17.5 Ci/mmol were obtained. [F-18]FB-anti-CEA diabody showed rapid and specific tumor uptake with fast clearance from the circulation in the mouse xenograft model as evidenced by both microPET imaging and biodistribution studies. High-contrast images were obtained as early as one hour post-injection of [F-18]FB-anti-CEA diabody. Biodistribution studies revealed that the tumor-to-muscle ratios for the LS174T xenograft model were 7.35 ± 1.50 at two hours, 11.53 ± 2.73 at four hours, and 19.71 ± 4.71 at six hours post-injection, respectively. Clearance was via the kidney, with some activity in liver and spleen at early times. Conclusion: F-18-labeled diabody represents a new class of tumor-specific probes for PET imaging based on cell-surface antigen expression. The relatively short half life of F-18 is suitable for diabody labeling as the diabody has similar antigen-binding affinity and specificity as intact antibody but with much faster blood clearance. The [F-18]FB-anti-CEA diabody may have great potential as a PET tracer for CEA-positive colorectal cancer imaging in clinic.

No. 64

IMAGING OF EMBRYONIC STEM CELL AND IMMUNOGENIC RESPONSE AFTER TRANSPLANTATION INTO THE MYOCARDIUM

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Aim: We use a fusion reporter gene construct to track the fate of mouse embryonic stem cells (ESC) in living mice with different immune response background. Methods and Results: Mouse 129/SvJ-derived embryonic stem cells (ESC; $1x10^6$) which carried eGFP and firefly luciferase reporter genes were injected into murine hearts of allogeneic (Swiss Webster; n=20), syngeneic (129/SvJ; n=12), or immunocompromised recipients (SCID; n=12). Longitudinal ES cell survival was monitored by bioluminescence imaging (BLI). Host immune cells were isolated at 1, 2, 3, and 4 weeks in the SW group to assess T cell activation by FACS. Results: BLI signals increased progressively in SCID and 129/SvJ mice ($8.7\square10^5\pm5.3\square10^4$ at day 7 vs. $1.17\square10^7\pm5.1\square10^6$ at day 21; $P<0.05$). After four weeks, ESC grafts evolved into intracardiac and extracardiac teratomas in both groups. In contrast, BLI signals varied among individual Swiss Webster mouse. At four weeks, vigorous CD_4^+ T cells infiltrates were found in SW recipients with absent BLI signals. FACS analysis showed that the number of CD_4^+ T cells correlated inversely with the BLI signals. The number of CD_4^+ T cells were higher (21.2±7.1%) in SW group compared with sham group (6.1±1.1%). In contrast, animals with teratoma formations at week four had significantly less activation of $CD4^+$ T cell response. Conclusion: ESC transplant causes teratomas and elicits variable immune response in allogeneic hosts which imply that careful monitoring is needed for clinical ESC transplantation.

No. 65

EVALUATION OF THE PLEIOTROPIC EFFECTS OF SYSTEMIC ANGIOPOIETIN-2 OVEREXPRESSION ON TUMOR GROWTH AND ANGIOGENESIS BY MULTIPLE IMAGING APPROACHES

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Destabilization of pre-existing vasculature is one key step for tumor angiogenesis. Angiopoietin-2 (Ang2) is a destabilizing factor that regulates vessel remodeling and regression depending on VEGF level. Although Ang2 and VEGF are both locally upregulated in many tumors, the effects of systemic overexpression of Ang2 on tumor growth and angiogenesis are unclear. Here we use adenoviral vector and *in vivo* fluorescent, *ex vivo* bioluminescent and *in vitro* immunohistochemical imaging technologies to study the effects of systematic overexpression of angiopoietin-2 (Ang2) with or without simultaneous downregulating VEGF on tumor growth and angiogenesis. Four adenoviral vectors (AdCtrl, AdAng2, AdsVEGFR-1 and AdAng2+AdsVEGFR-1) were administered *i.v.* to mice with HCT116 human colon cancer xenografts. Mouse dorsal skin-fold window chambers show that AdAng2 treatment induced significant regression of tumor neovasculature within 24 hours. AdAng2 alone treatment significantly

inhibited tumor angiogenesis (P<0.01) and growth (P<0.05) similar to the therapeutic effects of AdsVEGFR-1 alone and the combined AdAng2+AdsVEGFR-1 treatment. There was not decrease in APT level 48 hours after initial treatments. Immunohistochemical studies revealed that all three therapeutic regimens inhibited tumor angiogenesis (P<0.05) and enhanced tumor apoptosis (P<0.05) while not significantly affecting tumor proliferation (P>0.05) and hypoxia (P>0.05). Our data, for the first time, demonstrate a novel antiangiogenic antitumoral therapeutic strategy by systemic overexpression of Ang2. More importantly, the similar therapeutic effects by Ang-2 alone, sVEGFR-1 alone and the combination of both indicate that the vessel destabilizing and antiangiogenic effects of systematic overexpression Ang-2 does not require simultaneous inhibition of VEGF signaling.

No. 66

NOVEL DONOR MUTATION ENHANCES SENSITIVITY FOR NON-INVASIVE IMAGING OF BIOLUMINESCENCE RESONANCE ENERGY TRANSFER SIGNAL IN LIVING SUBJECTS

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Bioluminescence resonance energy transfer (BRET) is a sensitive detection assay used for studying various protein functions *in vitro* and *in vivo*. The current study demonstrates construction and validation of new improved BRET vectors by fusing novel *Renilla luciferase* (RLuc) mutants (donor), selected for increased quantum yield and stability to the GFP^2 acceptor. Three different mutations of RLuc, a single mutation C124A, a double mutation C124A/M185V, and a combination of eight mutations called RLuc8, were fused with GFP^2 and tested in HT1080 fibro-sarcoma cells by using CCD camera based spectral imaging. The new vectors were also tested in a small animal tumor model with implanted cells at various tissue depths. In comparison to the cells expressing GFP^2-RLUC, normalized luciferase signal shows markedly significant (P<0.01) increase of 35 fold for GFP^2-RLUC8 fusion, and 25 fold for GFP^2-RLUCM185V, whereas BRET signal shows 80 and 40 fold increase respectively. No significant improvements are noticed with the C124A mutation. By establishing HT1080 cells constitutively over-expressing GFP^2-RLUC and GFP^2-RLUC8 with equal transgene expression, we determined that each GFP^2-RLUC8 cell yields a BRET signal that is equivalent to approximately 30 GFP^2-RLUC expressing cells. Further, we tested the sensitivity of the new BRET vector by imaging individual stable cells as well as cells at subcutaneous and deeper tissues of animals. These new BRET vectors with improved BRET efficiency and sensitivity should accelerate the study of distance dependent processes such as protein-protein interaction and protein phosphorylation by measuring the events directly from live cells and from small animal models.

No. 67

CHARACTERISATION OF THE STRUCTURAL BASIS FOR ALPHAVBETA6-SPECIFIC IMAGING PEPTIDES

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The integrin alphaVbeta6 is expressed only on epithelia and then only during development, wound healing, inflammation and by several types of epithelial cancer. Thus the accessible, cell surface location of alphaVbeta6 makes it particularly suitable as a potential target for imaging. Using a series of 7-20mer peptides, based on the sequences of known ligands of alphaVbeta6, and analysed in inhibition of cell adhesion assays our data confirm the published finding from phage display that the RGDLXXL/I motif promotes anti-alphaVbeta6 activity. Furthermore longer peptides (20mers) have a greater potency than shorter peptides based on the same sequences. This is in contrast to alphaVbeta3 and alphaVbeta5 peptidic inhibitors which are usually 5-6mers. Thus we hypothesised that additional amino acids, outside of the RGDLXXL/I motif, were involved in formation of a conformational epitope. This hypothesis was supported by NMR analysis which revealed that the 20mer peptides have an intrinsic alpha-helical propensity in the LXXL region. Formation of a helix in this region causes the two leucine residues to be brought into juxtaposition, forming an interface that binds directly to alphaVbeta6. Moreover, NMR shows that the peptides A20 FMDV1, A20 LAP and A20 FMDV2 have increasing helix length which correlates with increased potency (IC50s of 87μM, 14μM and 1μM respectively for inhibition of alphaVbeta6-dependent cell adhesion); in addition, abolition of the helix using two D-amino acid substitutions significantly reduces anti-alphaVbeta6 activity, showing that the helix is important for potency. Thus these anti-alphaVbeta6 peptides form the prototype for the molecular imaging of alphaVbeta6-positive cancers.

No. 68

HYBRID MICROSPECT/ COMPUTED TOMOGRAPHY IMAGING PERMITS SERIAL QUANTITATIVE NON-INVASIVE EVALUATION OF ANGIOGENESIS AND ARTERIOGENESIS IN MURINE MODEL OF HINDLIMB ISCHEMIA

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Background: Previous studies by our group have demonstrated impaired angiogenesis and arteriogenesis in eNOS-deficient (eNOS-/-) mice sacrificed at selected times following hindlimb ischemia, using invasive methodology. Sequential hybrid micro single photon emission computed tomography (SPECT) imaging of the □v□3 integrin (a marker of angiogenesis) and contrast micro computed tomography (CT) imaging may allow serial non-invasive evaluation of both angiogenesis and arteriogenesis in mice. Methods: The femoral artery was surgically resected in eNOS-/- mice (n=5) and wild-type (WT, n=3) controls. Angiogenesis was evaluated before, and one week and four weeks after creation of hindlimb ischemia by serial pinhole microSPECT/CT imaging of mice 60min following injection of a technetium-99m-labeled RGD peptide, NC100692 (1.62±0.28mCi, i.v.), targeted at □v□3 integrin. We also performed adjunctive microCT imaging during administration of contrast (Omnipaque, 25ul/min) at each time point. Both images were reconstructed, fused, and 3-D regions-of-interest were drawn from CT images to quantify relative NC100692 uptake (% non-ischemic) in proximal (pHL) and distal (dHL) hindlimbs. Arteriogenesis was evaluated qualitatively. Muscles were also examined histologically using endothelial markers (CD31). Results: Relative NC100692 uptake peaked at 7d in both groups, in the ischemic dHL. NC100692 uptake was significantly reduced in dHL of eNOS-/- mice (eNOS-/-:294±45%; WT:381±60%; p=0.04). NC100692 uptake was not significantly different in pHL and dHL between the groups before and at four weeks post surgery. Arteriogenesis was qualitatively similar. Conclusions: Impaired angiogenesis was confirmed in eNOS-/- mice deficiency by non-invasive imaging of □v□3 integrin with NC100692, which selectively localized in regions of increased angiogenesis. Time dependent changes in angiogenesis and arteriogenesis were serially monitored in mice with microSPECT/CT.

No. 69

SIMPLIFIED PET DETECTORS FOR MOUSE IMAGING USING WAVELENGTH SHIFTING (WLS) FIBERS

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Advanced small-animal positron emission tomography (PET) scanners have been developed for preclinical applications. These PET systems generally are smaller versions of clinical scanners, have large numbers of channels and are designed to accommodate a range of species. Statistics show that more than 90% of the mammals used in research are mice. We therefore propose a compact PET scanner specific for mouse imaging that utilizes far fewer detectors whilst retaining state-of-the-art performance.

The detectors are based on LSO arrays, read out by WLS fibers placed on the top and bottom of the arrays. Depth of interaction information will be obtained from the ratio of the signals at either end of the array. Using depth-encoding detectors, the detector separation can be reduced to ~5cm for mouse imaging (most current systems use ~15cm). The WLS readout allows N^2 crystals to be decoded with 2N channels on a position-sensitive PMT. These two factors significantly reduce the number of detector channels required. While an attractive approach, the challenge is to collect sufficient scintillation light for high performance. To maximize light output, we experimentally tested coupling materials, reflectors, crystal size, fiber size, and fiber shape. 25% efficiency (light output from the WLS fiber versus direct coupling of a crystal) was achieved for a 2x2x30mm double-clad square fiber (Saint-Gobain BCF-91AMC) coupled to a 2x2x10mm LSO scintillator with epoxy adhesive (Epoxy Technology OG127-4) and with appropriate reflectors. We also characterized WLS properties, including attenuation coefficient, transverse absorption and light cross-talk between fibers. We are currently designing complete detector modules for testing.

No. 70

RIBOZYME MEDIATED IMAGING OF P53 MRNA IN LIVING ANIMALS

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Introduction: Group I introns of the ciliated protozoan, *Tetraymena thermophila*, constitute a class of catalytic RNA molecules or ribozymes, capable of catalyzing cis and trans-splicing reactions. We report here the use of the trans-splicing form of the *Tetrahymena* ribozyme in imaging the mRNA expression of the p53 gene in living mice. Methods: Plasmid constructs were designed with a 211nt p53-targeting sequence, the group I intron of the *Tetrahymena* ribozyme and the complete ORF of the firefly luciferase reporter. COS-7 cells were transfected with plasmid constructs expressing the p53 mRNAs, and the ribozyme-luciferase reporter and cell lysates prepared for RNA analysis by RT-PCR and luciferase activity assay *in vitro*. For the animal imaging study, $3x10^6$ COS-7 cells transiently transfected with the same plasmids were subcutaneously injected into nude mice (n=6) 48 hours after transfection. The mice were imaged every day in a CCD camera after D-Luciferin injections. Results: *In vitro* analysis revealed that although spliced products as seen from the RT-PCR were visible as early as 24 hours after transfection, detectable differences in splicing-dependant luciferase activity were seen only at 48 hours, with the peak at 72 hours. The animal experiments substantiated the *in vitro* data. A 9.5±1.9 fold difference was observed between tumors with and without the p53 mRNA 24 hours following the tumor implant (72 hours after transfection). Conclusion: We have shown here the first example of imaging ribozyme-mediated trans-splicing activity in living animals. This splicing-dependant reporter assay offers exciting opportunity to directly image endogenous mRNAs, especially over-expressed tumor-specific mRNAs in living subjects.

No. 71

DESIGN AND EVALUATION OF A NOVEL, HIGH-RESOLUTION SMALL-ANIMAL SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY SYSTEM

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Researchers in molecular imaging are continually striving to improve imaging systems. No matter how refined reconstruction and processing algorithms become, their effectiveness will always depend on the quality of the data given to them: the data gathered by the imaging system itself. Therefore, the optimization of imaging hardware must constitute one of the principal goals in improving an imaging system. Strict resolution and sensitivity requirements guide the design of molecular imaging systems. We recently applied task-based measures of image quality to the design of small-animal single photon emission computed tomography (SPECT) imaging systems. Task-based measures of image quality quantify the ability of an observer to perform a task relevant to molecular imaging. These measures of image quality implicitly account for both resolution and sensitivity, but also objectively scale the tradeoff between the two factors. However, different optimal system configurations will be returned for different tasks. For example, the optimal system design for an uptake-quantification task might differ greatly from that of a signal-detection task. To address these issues, we designed a novel, inexpensive, high-resolution small-animal SPECT system, dubbed \M3R, which can be easily modified for different tasks. This system serves a double purpose in that it will help validate our task-based hardware-optimization methods. Slots machined into the system shielding allow for the interchange of pinhole plates, enabling the system to operate over a wide range of magnification and with virtually any desired pinhole configuration. We will provide a brief overview of \M3R, focusing primarily on system design, construction, and initial performance.

No. 72

IMAGING PAI-1 TRANSCRIPTIONAL REPRESSION AND ACTIVATION IN INDIVIDUAL EPIDERMAL EPITHELIAL CELLS ENGINEERED TO EXPRESS PAI-1-GFP REPORTER CONSTRUCTS REGULATED BY PAI-1 SPECIFIC PROMOTER ELEMENTS

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Plasminogen activator inhibitor-1 (PAI-1) regulates urokinase PA activity and pericellular plasmin generation. This proteolytic cascade affects tumor cell invasion by controlling the extent and localization of stromal proteolysis. To directly image PAI-1 gene expression with regard to cellular migratory activity, 806 bp of the human PAI-1 promoter were cloned upstream of an insert encoding a PAI-1-GFP chimeric protein and induced PAI-1-GFP visualized *in situ* by fluorescence microscopy. PAI-1 gene activation, subsequent PAI-1-GFP expression and keratinocyte invasion through 3-D gels was assessed using confocal microscopy. Comparisons of PAI-1-GFP expression with that of the endogenous PAI-1 gene (at the mRNA/protein levels) revealed similar inductive kinetics indicating that the PAI-1-GFP construct provided an accessible "reporter" system to evaluate PAI-1 promoter function within the real-time of gene activation. PAI-1 synthesizing cells were highly motile in the 3-D model of induced migration. Chromatin immunoprecipitation indicated that induced PAI-1 transcription required replacement of the bHLH protein USF-1 with USF-2 at the PE2 E box (CACGTG) motif in the PAI-1 promoter. This chromatin-level isoform switch was monitored by visual analysis of nuclear transcription "domains." PAI-1 protein synthesis and 3-D migratory activity declined significantly in keratinocytes transfected with dominant-negative USF constructs, supporting a critical role of functionally-distinct USF isoforms in PAI-1 expression repression and activation. These findings illustrate the usefulness of this approach to image transcription and invasion in single epithelial cells using GFP reporters under control of promoter sequences from genes implicated in migratory activity. (Supported by NIH grant GM57242 and the Department of the Army)

No. 73

CHARACTERIZATION OF TC-99M-LABELED ANTI-DR5 ANTIBODY (MTRA-8) BY *IN VITRO* ANALYSES AND *IN VIVO* IMAGING IN HUMAN BREAST TUMOR XENOGRAFTS

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Purpose: The primary aim was to characterize Tc-99m-labeled mTRA-8, a murine, apoptosis-inducing monoclonal antibody targeting DR5, by detailed *in vitro* Scatchard analyses, and to subsequently image *in vivo* distribution within xenograft breast tumors. A secondary aim was to characterize a related breast cancer subclone that was resistant to mTRA-8 killing. Methods: The binding affinity (Kd) and the number of DR5 receptors were measured in MDA MB-231-derived 2LMP cell lines that

were sensitive or resistant to mTRA-8 killing. Single photon emission computed tomography (SPECT) evaluated the Tc-99m-mTRA-8 retention and distribution within xenograft tumors; biodistribution analyses confirmed the levels. Results: Scatchard assays showed specific and high affinity binding of Tc-99m-mTRA-8 to DR5. There was no significant difference between sensitive and resistant 2LMP cells for Kd values (1.2±0.1 nM=acid labile), or DR5 receptors (mean/cell = 9,900). SPECT/ computed tomography (CT) imaging at six and 20 hours after injection of Tc-99m-mTRA-8 revealed high retention at the tumor periphery. By imaging analyses, the 3rd 1-mm radial shell from the surface of the mammary fat pad tumors (n=6, 5634 mm3) retained 14.8±2.6 %ID/g, higher than the other shells. The Tc-99m-mTRA-8 distributions in the sensitive and resistant 2LMP tumors were not different. Entire tumors averaged 9.3±2.2 %ID/g. Conclusions: These studies demonstrate an *in vivo* SPECT imaging method to evaluate DR5 expression at high resolution within tumors, providing a mechanism to image the modulation of DR5 expression during therapy, and confirmed that the decreased efficacy of mTRA-8 in the resistant 2LMP tumors was not related to delivery.

No. 74

IN VIVO SMALL ANIMAL DEEP LUNG TUMORS EXAMINATION BY FLUORESCENCE DIFFUSE OPTICAL TOMOGRAPHY

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A fluorescent optical diffusion tomography instrument has been developed in our laboratory. It allows *in vivo* studies of tumor growth on small animals. This paper presents experimental results obtained with our system on several mice at different stages of tumor development. The experimental set-up defined for slab geometry, consists of a laser source (690nm), a CCD camera and a tank to receive the animal. The laser is coupled to a motorized stage in order to scan the animal. The excitation and emission wavelengths and the fluorescent probes are chosen to optimize transmission through the whole animal body and particularly the lungs. The slab thickness z varies from 10 to 15mm and the geometry is considered as infinite in (x, y) directions. This is achieved by immersing the animal in an index matching medium. In slab geometry, the diffusion equation admits an analytical solution, therefore reconstruction of fluorophore distribution can be linearized and solved throughout an iterative ART algorithm. Our approach is based upon a fine description of material-light interaction taking into account diffusion as well as absorption phenomena. Four healthy mice and 13 bearing lung metastasis are imaged at different stages of tumor development after the primary tumor implantation. Acquisitions are made three hours after intravenous injection of 150 mg Transferine/Alexa 750 or RaftcRGD/Alexa 750. Detection and localization of fluorophore fixations are presented according to the stage of the tumor development. Results show that the system is able to separate healthy from cancerous mice and point out influence of the marker.

No. 75

FASTING-INDUCED EXPRESSION OF MELANOCORTIN-4 RECEPTOR GENE: _IN VIVO_ PROMOTER ANALYSIS OF BIOLUMINESCENT MC4R TRANSGENIC MICE

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Obesity is a complex disease that has reached epidemic proportions. Advances in our understanding of energy homeostasis have identified a number of genes linking peripheral signals to CNS neuroendocrine pathways, including melanocortin peptides and their cognate G protein-coupled receptors (e.g. Melanocortin-4 Receptor; MC4R). MC4R is widely, yet weakly, expressed throughout the CNS, including putative satiety centers of the hypothalamus and brain stem. Genetic ablation in mice has shown that loss of one functional MC4R allele results in obesity. In linkage studies of obese cohorts, loss of function as well as hypomorphic alleles account for approximately 5% of morbidly obese patients. Alas, little is known about how MC4R gene expression is regulated *in vivo*. We created a series of MC4R promoter-luciferase reporter gene transgenic mice to identify key regions of the promoter involved in proper temporal, spatial, and regulated expression. *In vivo* bioluminescence imaging (IVIS-100 system, Xenogen) showed that transgenic mice with constructs containing 3300bp of 5'-flanking sequence (3300MC4Luc) displayed CNS preferential expression of the transgene (e.g. hypothalamus and brain stem); while 430bp of 5'-flanking sequence was sufficient to drive hypothalamic expression. Imaging revealed a 2.5-fold increase (249.4% ± 34 N=5, $p<0.01$) in luciferase activity in the CNS following a 24-hours fast in the same 3300MC4Luc transgenic mice, mirrored by a simultaneous increase in endogenous MC4R mRNA levels in the hypothalamus assessed by Real Time RT-PCR. We report the first model system to study regulated expression of the MC4R gene *in vivo*. (funded by the Whitehall Foundation, ADA and NIH-DK20593)

No. 76

MAGNETIC RESONANCE SPECTROSCOPY DETECTS METABOLIC CHANGES UPON CHEMOTHERAPY TO THE HUMAN NON-HODGKIN'S LYMPHOMA XENOGRAFT

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A preliminary multi-institutional study has recently demonstrated that ratios of the phosphomonoesters, phosphoethanolamine plus phosphocholine, to NTP of human non-Hodgkin's lymphoma (NHL) measured by P-31 MRS before initiation of therapy can identify about two out of three patients who will not exhibit a complete local clinical response. These patients should be encouraged to undergo more aggressive alternative therapy, which entails some risk of mortality but also offers hope of response, remission or cure. However, the limited sensitivity of P-31 MRS limits its application mostly to large, superficial tumors. We have, therefore, been developing much more sensitive H-1 MRS and MRI methods to examine smaller tumors. Here we report studies of mouse xenografts of the most common form of human NHL and the only form that exhibits cures (in about one out of three patients) -- diffuse large B-cell lymphoma (DLCL2). *In vivo* H-1 MRS using a selective multiple quantum coherence pulse sequence (Sel-MQC) detected decreases of lactate and total choline in DLCL2 tumors treated with five cycles of CHOP (cyclophospamide, doxorubicin, vincristine, prednisone) chemotherapy. Single voxel localized spectroscopy (STEAM) detected decreases in choline and lactate/lipid that accompanied response. These changes correlated with decreases in phosphomonesters detected by P-31 MRS and with tumor growth delay. *In vivo* data were correlated with data on tumor extracts and measurements of tumor histology.

No. 77

DISTINGUISHED PHOTONS: HARDWARE AND SOFTWARE ADVANCES IN MULTISPECTRAL APPROACHES FOR _IN VIVO_ FLUORESCENCE IMAGING

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New labeling technologies and increasingly sophisticated biological questions have increased the technical demands on *in vivo* fluorescence imaging systems, which must now handle a variety of simultaneous signals. In addition, autofluorescence signals from the skin and other structures need to be separated from those of labeled fluorophores. CRi's Maestro™ multispectral system, based on liquid crystal tunable filter (LCTF) technology, automatically acquires precise optical spectra at every pixel of an imaged scene, and is effective tool for multiplexing and quantitating fluorophores. Once spectrally characterized, the autofluorescence can be unmixed from other signals, revealing otherwise invisible labeled targets, which appear bright against a near-black background. The improved signal-to-noise can increase sensitivity by 1-2 orders of magnitude over

conventional approaches, and yields more reliable quantitation. In the near-infrared, as opposed to the visible range, autofluorescence, while present, is less troublesome. However, multiplexing multiple fluorophores is still desirable. The new Maestro Flex™ system, an extension of the LCTF design, allows the system's bandpass to be switched from broad, for maximum throughput, to relatively narrow, without affecting tuning precision (ability to position peak transmission) which remains at about 1-2 nm. The enhanced resolution allows increased multiplexing in the NIR, an optical region well-suited to deep tissue imaging. Finally, software approaches have been developed that automatically reveal multiple signals in situ, and help the user define the proper spectra required for precise spectral unmixing. These work even when no "pure" spectral signals are available from the imaged samples, and greatly improve ease-of-use as well as accuracy.

No. 78

MEASUREMENT OF CONTRAST-TO-NOISE RATIO AS A FUNCTION OF RADIATION DOSE FOR MICROCT

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A microCT scanner has been built and integrated with our microPET II scanner, forming a dual modality system for *in vivo* anatomic and molecular imaging of the mouse. Sample positron emission tomography (PET)/ computed tomography (CT) images will be presented along with the characterization of the CT system performance. One major design feature of our CT system was to achieve adequate image quality at the lowest radiation dose. The goal of this study was therefore to quantitatively understand the relationship between image quality represented by contrast-to-noise ratio and spatial resolution, and the radiation dose received by a mouse during a microCT scan, with different scanning parameters including kVp, X-ray tube current and the number of views used for image reconstruction. Contrast agents also are used to see if they can compensate for the reduction of X-ray exposure. Both phantom and animal experiments are analyzed. Radiation exposure was measured with an exposure meter placed at isocenter and converted into dose received by the subject using the conversion factors obtained from Monte-carlo simulations (Boone et al, Molecular Imaging, 2004; 3: 149-158). The results of this study will be used to guide the design of low dose microCT scanning protocols for microPET/CT studies.

No. 79

TRANSCRIPTIONAL PROFILING OF REPORTER GENES USED FOR MOLECULAR IMAGING OF EMBRYONIC STEM CELL TRANSPLANTATION

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Stem cell therapy offers exciting promise for treatment of ischemic heart diseases. Recent molecular imaging techniques allow investigators to monitor cell fate noninvasively and repetitively. Here we examined the effects of a triple fusion reporter gene on murine embryonic stem (mES) cell transcriptional profiles. mES cells were stably transduced with a lentiviral vector carrying a triple fusion (TF) construct consisting of fluorescence, bioluminescence, and positron emission tomography (PET) reporter genes. Microarray studies comparing gene expression in nontransduced control ES cells versus ES cells expressing triple fusion (ES-TF) revealed transcriptional variability. Using the criteria of expression fold change > 1.5, t-test p-value < 0.01, and TNoM score of 0, we identified 207 unique genes upregulated and 333 unique genes downregulated in ES-TF versus control ES cells. Annotation analysis showed that ES-TF cells down-regulated cell cycling, cell death, and protein and nucleic acid metabolism genes, while upregulating homeostatic and anti-apoptosis genes. Despite these transcriptional changes, expression of the TF reporter gene had no significant effects on ES cell viability, proliferation, and differentiation capability. Importantly, transplantation studies in murine myocardium demonstrated the feasibility of tracking ES-TF cells in living subjects using bioluminescence and PET imaging. The bioluminescence and PET signals were $1.51\times10^8\pm3.1\times10^7$ photons/sec/cm^2/sr and 0.63±0.09 %ID/g at day 7, respectively. Both signals increased significantly to $6.16\times10^8\pm1.04\times10^8$ photons/sec/cm^2/sr and 0.94±0.13 %ID/g by day 14 ($P<0.05$ vs. day 7). Taken together, this is the first detailed study to analyze the transcriptional profile of reporter genes used for imaging stem cells in living subjects.

No. 80

A NEW IMAGING APPROACH FOR CALCIUM DEPENDENT CALMODULIN ACTIVATION IN CELLS

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Background: Calmodulin (CaM), an important calcium sensor protein, is ubiquitously involved in a tremendous number of intracellular signaling events. By using fusion split hRL/eGFP reporter with calmodulin, we aim to develop bioluminescent and fluorescent imaging methods for studying calmodulin mediated calcium activation in intact living cells. Methods: Vector constitutively expressing a fusion calmodulin protein bounded on either end by a split renilla luciferase (CMV-Rluc-N-CaM-C-Rluc) or split enhanced green fluorescent protein (eGFP-N-CaM-C-eGFP) were constructed. Transient and stably transfected SK-N-SH neuroblastoma or 293T cells were prepared. Calcium influx into cells was induced with a mix of Ionomycin, L-glutamine and calcium ('induction mix'). To enhance calcium influx, cells were pretreated with a four hour incubation period of calcium-free media. Intact cells were imaged for bioluminescence using the IVIS 200 camera immediately after addition of the induction mix. Results: Intact transiently transfected 293T cells with the CaM-split Rluc fusion demonstrated higher bioluminescent signal after treatment with the induction mix (17.7x10^5 p/sec/cm^2/sr) than controls (7.2x10^5 p/sec/cm^2/sr) ($p<0.01$). This event could be detected soon after the addition of the mix (~1 minute). Similar results were obtained with stably transfected SK-N-SH cells and differentiated neuroblastoma cells. Fluorescence microscopy and quantitative fluorometry of GFP fluorescence showed that cells transient transfected with CaM-split eGFP construct had higher levels of fluorescence following treatment with induction mix. Conclusion: The developed system is useful to study calcium-dependent calmodulin activation in living systems. Optical cameras with optimal temporal resolution can potentially be used to study real-time calcium fluxes using this system.

No. 81

SIMULTANEOUS NON-INVASIVE IMAGING OF ESTROGEN RECEPTOR LIGAND INDUCED HOMODIMERIZATION AND GENE TRANSACTIVATION IN LIVING MICE

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Background: ER ligands influence the stability of the ER dimer as well as modulate the dimer's ability to transactivate target genes via estrogen response elements (ERE) in target genes. Using split and conventional optical reporter techniques, we have simultaneously shown dimerization of the estrogen receptor and transactivation of an ERE-containing gene in intact cells and tumor xenografts. Methods: To study ER dimerization, separate vectors expressing fusion proteins of ER with either N- or C-fragment of split Renilla luciferase (NhRluc-ER and ER-ChRluc) were created and co-transfected into 293T cells. To study ER-mediated transactivation, the human telomerase reverse transcriptase (hTERT) promoter, which contains an ERE, was used to drive the Firefly luciferase gene. Transfection and co-transfection of these vectors were studied in cells and cell implants in living animals in response to various ER-ligands. Results: Estradiol, tomoxifen, raloxifen and 4-hydroxytamoxifen (4-OHT) significantly increased ER homodimerization mediated RLUC complementation in transiently transfected 293T cells by 27, 11, 17 and 33-fold, respectively, compared to controls ($p<0.02$). E2 augmented hTERT promoter activity by 50%. The antagonists, tomoxifen and raloxifen, repressed hTERT promoter activity by 29% and 24%, respectively

($p<0.05$). No significant modulation of FLUC activity was seen when the ERE was removed from the hTERT promoter. In a tumor xenograft model, animals(N=5) who received Raloxifene demonstrated 5±2 fold increase in RLUC signal ($p<0.05$) and concomitantly showed a 50% decrease in FLUC activity than controls ($p<0.05$). Conclusion: This system will be useful for screening anti-estrogen drugs, studying ER homodimerization and ERE-mediated transactivation of target genes.

No. 82

A NOVEL STRATEGY FOR IMAGING TRANSACTIVATION OF GENES USING ESTROGEN MEDIATED INTRA-MOLECULAR FOLDING OF THE ESTROGEN RECEPTOR

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Background: Estrogens control many functional pathways through the estrogen receptor (ER). The estrogen receptor changes its structural confirmation in response to different ligands. In this study we developed a new estrogen receptor intramolecular-folding based transcriptional activation system to control gene expression, differentiate ligands and also to study ER biology. Materials and Methods: We constructed vectors expressing fusion chimera of ER with gal4 and vp16 domains (Gal4-ER-VP16). Co-transfection of this vector with the vector containing the reporter gene under gal4 binding DNA sequence flanking a minimal promoter was studied in different cell lines, and the cell implants were imaged in living mice (N=6). Results: The fusion chimera containing ER of domains D, E and F showed efficient transactivation by both agonists and antagonists (3000±200 fold more greater than without ligands ($P<0.001$)). Similarly the cells expressed the fusion chimera containing ER of only D and E domains showed similar transactivation with the property of distinguishing agonists and antagonists (agonists: 600±200 fold; antagonists: 3000±500 fold; partial agonist: 1500±200 fold greater than without ligand). The system in living mice with an identified mutant ER (lacking significant estradiol binding) showed efficient controlled activation by the antagonist Raloxifene that is 15±5 fold greater than with control animals ($6\pm2x10^6$ vs $2\pm1x\ 10^5$ p/sec/cm^2/sr) ($P<0.05$). Conclusion: The system developed from this study is very sensitive and useful for *in vitro* and *in vivo* application for controlled gene expression, screening and imaging ER-ligands with therapeutic properties, and also for studying the underlying mechanisms of ER biology through molecular imaging.

No. 83

AUGMENTATION OF GENE EXPRESSIONS FROM THE TUMOR-SPECIFIC SURVIVIN PROMOTER VIA A BI-DIRECTIONAL TWO-STEP TRANSCRIPTIONAL AMPLIFICATION (TSTA) SYSTEM FOR CANCER GENE THERAPY

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Objectives: Survivin, a cell-cycle regulator and an inhibitor of apoptosis, is highly expressed in different malignant cells and tissues while absent in normal tissues. Survivin promoter(pSurv) is hence a plausible choice for transcriptionally-targeted cancer gene therapy. However, the promoter is a weaker activator of transcription compared to CMV (<1%). In the current study a bi-directional promoter system based on the Two-Step Transcriptional Amplification (TSTA) strategy was employed to amplify and monitor transgene expression from the human pSurv. Methods: The pSurv-TSTA-reporter plasmid (pSurv-Gal4-VP_2-E4TATA-fluc-(Gal4-$BS)_8$-E4TATA-hrluc) was constructed using standard cloning procedures. Expression of the transactivator fusion protein, Gal4-VP2, was driven by pSurv. The bi-directional promoter-system, containing 8-Gal4 binding sites (BS) in the center flanked by firefly (fl)and renilla (hrl) luciferase genes in opposite orientations, was placed downstream to the Gal4-VP2 coding sequences. Cancer cells transfected with the plasmid were lysed and assayed for luciferase activities 24-hours after transfection. Cells transfected with pSurv-fl/hrl were used as positive controls to quantify amplification. The hrl gene was switched for the TRAIL gene to build pSurv-TSTA-TRAIL plasmid. Results: Cells transfected with pSurv-TSTA-reporter plasmid showed significantly high fl and hrl activities compared to control [2-4 fold for hrl and 8-10 fold for fl ($P<0.05$)]. Intratumoral injections of the plasmid in xenograft mouse melanoma model also showed high fl expression. Gene therapy studies with the Surv-TSTA-TRAIL plasmid are planned in the future. Conclusion: The bidirectional TSTA system helped in increasing transcription from the Survivin promoter. Such a system will be immensely useful in the amplification and monitoring expression of a delivered transgene in gene therapy applications.

No. 84

THE FIRST FOUR-HEADED NANOSPECT: A HIGH-SENSITIVITY MULTI-PINHOLE SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY SYSTEM FOR IMAGING SMALL-ANIMALS WITH SUBMILLIMETER (NANOLITER) SPATIAL RESOLUTION

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Multi-Pinhole single photon emission computed tomography (SPECT) has become a proven modality in small-animal molecular imaging. Although the spatial-resolution capabilities of SPECT are greater than those of positron emission tomography (PET) , the latter is generally considered the gold-standard nuclear imaging modality due to high-sensitivities. In this work, we present a high-throughput SPECT system that achieves submillimeter reconstructed resolutions while simultaneously approaching the sensitivity of PET. This increase in sensitivity combined with existing advantages of SPECT, e.g. tracer chemistry, cost and dual isotope capabilities, improves the standing of SPECT as a molecular imager. This camera, the NanoSPECT, consists of four detectors (215x230mm2 NaI, 33 PMTs, 2.1mm intrinsic resolution at 140keV) mounted on a high-precision gantry. Each detector is outfitted with an interchangeable 9-pinhole aperture for a total of 36 pinholes surrounding the field of view (FOV). Pinhole diameter and FOV are chosen in accordance with the prescribed application, e.g., mouse or rat imaging. The axial FOV is extended using helical scanning (user-selectable range from 20 to 290mm). Additionally, helical orbits provide an increase in the angular sampling. All told, this increase in sensitivity and sampling greatly improves image quality both for detection and estimation (quantification) as compared to standard SPECT acquisition techniques. We will present a detailed description of the NanoSPECT along with numerous phantom studies and small-animal scans performed with an array of Tc-99m, I-123 and In-111 tracers. The results will address resolution, sensitivity, imaging times, injected dose and quantification results as well as multi-isotope and dynamic SPECT capabilities.

No. 85

BIOLUMINESCENT QUANTUM DOT CONJUGATES FOR IMAGING IN LIVING SUBJECTS

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Introduction: Semiconductor quantum dots (QDs) have generated wide interest because of their potential use in imaging in live subjects. All existing QDs, however, require excitation from external illumination sources to fluoresce, which limits their application in imaging of living opaque subjects due to the resultant strong autofluorecence background and little excitation light at non-superficial locations. We developed a new type of QD conjugates that can emit long wavelength (from red to near-infrared wavelength) light without external illumination, thus avoided the issue of high fluorescent background. Methods: We coupled QDs to a mutant of the

bioluminescent protein *Renilla* luciferase (Rluc8) via EDC-mediated amide bond formation. This design is based on the principle of bioluminescence resonance energy transfer (BRET), which is an energy transfer phenomenon between *Rluc8* (as the donor) and QDs (as the acceptor). Results: We characterized the QD conjugates *in vitro* and *in vivo*; the BRET ratio was between 0.70-2.30. The QD conjugates injected into a nude mouse all gave BRET emissions. The long wavelength BRET emissions were more easily detected, especially in deep tissues. Cells labeled with bioluminescent QDs were readily imaged in the lungs after i.v. injection, but were not detectable with fluorescence imaging. We also examined the possibility of multiplex bioluminescence imaging *in vitro* and in the living mouse. Conclusion: These unique features of BRET-based QDs should open many new avenues for QD-based imaging in living subjects, especially for imaging biological events at deep tissues in small living animals.

No. 86

[GA-68/ LU-177]-DOTA-PEG$_4$-BOMBESIN (7-14) (DOTA-PESIN), A POTENTIAL PROBE FOR POSITRON EMISSION TOMOGRAPHY IMAGING AND TARGETED THERAPY OF GRP RECEPTOR EXPRESSING TUMORS

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Aim: Design and evaluation of [Ga-68/Lu-177]-DOTA-PESIN as a bombesin (BN) analog for positron emission tomography (PET) imaging and targeted radiotherapy of GRP receptor positive tumors, including prostate, GIST and breast cancers. Material and method: DOTA-PESIN was synthesized using solid phase peptide synthesis and labeled with $^{67/68}$Ga and Lu-177. The binding affinity to human cancer tissue expressing BN receptor was determined by receptor autoradiography. Internalization and efflux were studied with PC-3 cells. Biodistribution was performed with PC-3 tumor mouse xenografts. Siemens ECAT EXACT HR$^+$ scanner was used for PET imaging. Results: [Ga^{III}/Lu^{III}]-DOTA-PESIN have good affinities to GRP receptors (IC_{50} values: 6.1±3.0 nM vs 6.6±0.1 nM). [Ga-67/Lu-177]-DOTA-PESIN internalized rapidly into PC-3 cells; the efflux from PC-3 cells was relatively slow with 50% at 20 h. [Ga-67]-DOTA-PESIN showed a high uptake in tumor (14.8±2.5 %ID/g at 1h); PET imaging with [Ga-68]-DOTA-PESIN visualized the tumor and the receptor positive pancreas with clear contrast from the adjacent background at one hour post injection. High tumor-to-kidney and tumor-to-liver ratios were observed during all tested time points due to rapid release from blood and non-target organs. [Lu-177]-DOTA-PESIN showed similar in-vivo properties as [Ga-67]-DOTA-PESIN. The tumor accumulation of radiolabeled DOTA-PESIN was regulated by receptor density and dependent on the administered dose; kinetic blocking experiments showed that 84% GRP receptors recycled to the surface of tumor cells within one hour in PC-3 tumor mouse xenografts, which allowed fractional administration. Conclusion: The above-mentioned results show that DOTA-PESIN is of high potential for targeted tumor diagnosis and radionuclide therapy, and is currently used in patients.

Clinical Oral Presentations

No. 87

2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE- POSITRON EMISSION TOMOGRAPHY DETECTION OF OCCULT TUMOR RECURRENCES IN CASE OF ELEVATED TUMOR MARKERS

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Introduction: Elevated serum tumor markers during follow up of cancer patients may indicate recurrence. Clinical management of such patients is currently based on conventional diagnostic methods. We hypothesized that 2-deoxy-2-[F-18]fluoro-D-glucose (FDG)- positron emission tomography (PET)-imaging yields earlier recurrence detection, thus significantly alter clinical management. Material/Methods: Forty-four patients (median age 58 years) with a history of FDG-avid malignancy and elevated tumor markers underwent FDG-PET-imaging. The clinical data, the FDG-PET-outcome and its influence on the clinical management were retrospectively studied in a median follow up of 11 months. Two subgroups were made, elevated S100 (melanoma, n=15) and CEA (gastrointestinal tumors, n=21). Pathological and/or clinical confirmation of a recurrence was the golden standard. Results: 31/44 patients had FDG-PET-uptake suggestive for recurrence. In 25/31 the uptake was true positive. It led in 60% cases to an essentially altered clinical management, of which almost half was an intervention. In the S100 subgroup 13/15 were true positive and in 11/13 clinical management was altered based on the PET results. In the CEA subgroup 13/21 patients had true positive PET outcome with an altered clinical management in 43%. Most of the PET-positive lesions were confirmed by conventional imaging, however with a delay of up to two months. Conclusion: This study shows an important incremental value of FDG-PET in the early detection of occult tumor recurrences. The most striking benefit was achieved in melanoma patients with elevated S100. Our data suggest that FDG-PET should be implemented up front when occult tumor recurrence is suspected, especially in patients with melanoma.

No. 88

IMAGING AMYLOID DEPOSITIONS AND GLUCOSE UPTAKE CHANGES IN ALZHEIMER'S DISEASE: A FOLLOW UP-STUDY

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Background: In the first study using Positron Emission Tomography and *N*-methyl[C-11]2-(4´methylaminophenyl) -6-hydroxy-benzothiazole (PIB), to detect amyloid depositions in patients with Alzheimer's disease (AD), high PIB retention was observed in the cerebral cortex of most of 16 AD patients compared to nine controls. Methods: The 16 patients have been reexamined 1.5 - 2.5 (2 ±0.5) years later in order to detect changes in PIB retention and in regional cerebral glucose metabolic rate (rCMRglc) measured with 2-deoxy-2-[F-18]fluoro-D-glucose (FDG). In a separate test-retest protocol, another four Alzheimer patients were examined twice with PIB to study the intra-individual variations of the method to quantify amyloid depositions. Results: *Test-retest*: In the cerebral cortex the variation in PIB retention was low (3.2 -7.3%) while a higher difference was found in small regions as the striatum (13%). *Follow-up study:* There were no significant average differences in PIB retention between both scans but as in the previous study, significant decrease in rCMRglc was observed in the parietal cortex. Five patients, who showed the highest PIB retention and decrease in rCMRglc, deteriorated also in MMSE more than three points. Interpretation: It seems that in the patients with lower MMSE

at follow-up, the deposition of fibrillar amyloid, already high at baseline, did not increase, whereas the cognitive deterioration continued, possibly associated with neuronal dysfunction or degeneration. The low intra-individual small variations in PIB retention, suggest that this tracer can be useful for evaluation of anti-amyloid therapy and in combination with FDG allows a better understanding of the pathological mechanisms behind AD.

No. 89

I-131-THERAPY IN ADVANCED DIFFERENTIATED THYROID CANCER: THERAPEUTIC IMPLICATIONS OF I-124-PET-DOSIMETRY

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Purpose: The aim of this study is to evaluate the clinical significance of I-124 positron emission tomography (PET) dosimetry in patients with advanced differentiated thyroid cancer (DTC) compared to standardized treatment protocols. Material and Methods: Sixteen patients with advanced DTC prior to first or second radioiodine therapy were orally given 23-50 MBq I-124. The radiation dose assessment for I-131 therapy using I-124 involves the determination of the maximum tolerated activity (MTA) via blood counting and whole-body counting (2, 4, 24, 48, 72, 96 hours after capsule intake) and the absorbed lesion dose per administered I-131 activity (LDpA) by serial PET (4, 24, 48, 72 and 96 hours) and PET/CT imaging (25 hours). Results of individual radiation dose assesment were compared to standardized therapy regimes. Results: All patients had iodine-avid metastases. Overall *n*=128 lesions were evaluated. LDpA was calculated for each lesion showing an interindividual range between 1 and 630 Gy/GBq I-131 with a high intraindividual variation range. The radiation dose assessment for I-131 therapy resulted in sufficient radiation dose to a majority of tumour-lesions in 11 patients. The comparison between the individual treatment protocol using I-124 isotope and with the standardized protocol based one empirical observations exhibits significant deviations. Specifically, 63% (10/16) patients had a different therapy regime: maximum I-131 activities up to 15 GBq were administered and primary surgery as well as palliative treatment was recommended. Conclusion: I-124-PET is a efficient diagnostic tool in DTC and, its application reveal significant deviation from the standardized treatment protocol.

No. 90

OPTIMIZING WHOLE-BODY POSITRON EMISSION TOMOGRAPHY / COMPUTED TOMOGRAPHY STUDIES FOR MELANOMA PATIENTS

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Background: 2-Deoxy-2-[F-18]fluoro-D-glucose (FDG) studies in melanoma patients are routinely acquired from top of the head to feet and include dedicated studies of the brain. This adds 25-30 minutes for positron emission tomography (PET)/ computed tomography (CT), and 60-75 minutes for PET. The goal of this study was to analyze the clinical value of the brain and extremity studies in melanoma patients. Methods: 296 consecutive studies acquired in 137 patients were reviewed. All positive findings were identified, tabulated and correlated with available clinical and pathological data. Incidence of hypermetabolic lesions in the initially uninvolved extremities and/or brain was analyzed. Results: The primary sites of melanoma were the head and neck (n=54), upper extremities (n=18), trunk (n=29), lower extremities (n=27), and unknown (n=9). Of the 296 studies reviewed, only three (1%) revealed metastases in the initially uninvolved extremities and/or brain. However, all three patients had widely metastatic disease with extensive visceral involvement. Six studies showed new hypermetabolic foci in the same extremities involved by the primary melanoma. False negative PET (one extremity and four brain lesions) occurred in five patients, while 11 false positive extremity findings occurred. Conclusions: The routine inclusion of the brain and the initially uninvolved extremities is of no significant benefit, when compared to the information already obtained by the whole-body FDG study from base of the skull to mid thigh. Elimination of the extra scanning time may help to increase patient throughput and decrease radiation exposure for the patients. Melanoma patients should therefore be scanned in the same manner as other oncology patients.

No. 91

AMIFOSTINE IS MOST PROMISING IN PROTECTING RENAL FUNCTION DURING RADIONUCLIDE THERAPY WITH [LU-177-DOTA0,TYR3] OCTREOTATE

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Aim: To investigate the renal-protective effect of amifostine in rats, when treated with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate. Amifostin is a radical scavenger, used routinely in chemotherapies and external beam radiation to prevent side effects. Radionuclide therapy with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate is the treatment of choice for patients with somatostatin receptor-positive neuroendocrine tumors. High dose treatment with [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate may cause renal failure. An effective method to protect the kidneys is therefore mandatory. Methods: Sixty-seven male Lewis rats were treated with 0, 278 or 555MBq [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate with or without amifostine. 70mg amifostine was given i.v. 30 minutes before [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate and 10mg amifostine was given s.c. daily from day 2-7. 105-146 days after treatment, SPECT of the kidneys was obtained with the new four head multipinhole collimators □-camera NanoSPECT (Bioscan Europe Ltd., France) using 50MBq ^{99m}Tc-DMSA (Dimercaptosuccinic acid). The reduction of *in vivo* DMSA-uptake was taken as an indication for tubular damage. The uptake was quantified by HiSPECT and INTERVIEW software. After scanning, the rats were sacrificed and kidneys were analysed histologically. The results were further correlated with autoradiography, protein excretion in urine and hematological parameters. Results: A significant, dose-dependent reduction of DMSA uptake compared to the control rats was found after [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate-treatment. The amifostine groups showed a significantly higher DMSA uptake than the corresponding groups without kidney protection. The results correlated well with the histological findings, autoradiography and urine-parameters. Conclusion: Amifostin is a most promising agent to protect kidneys from radiation damage during peptide receptor radionuclide therapy.

No. 92

NEW POSITRON EMISSION TOMOGRAPHY IMAGING DRUGS FOR CANCER DIAGNOSTICS: THE ^{18}[F]-FXAU FAMILY NUCLEOSIDES & ^{18}[F]-FEAU

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Background: Labeled thymidine and thymidine closest analogs have become preferred radiopharmaceuticals as they easily incorporate into DNA through the enzyme catalyzed thymidine kinase-1-mono-phosporilation reaction and sensitively indicate the cellular proliferation.Objective: Our primary objective is to demonstrate a reliable method to synthesize ^{18}[F]-FXAU family analog nucleosides and apply them as positron emission tomography (PET) imaging drugs. Based on previous investigations, ^{18}[F]-FEAU is a transient representative of ^{18}[F]-FXAU family drugs and is expected to be a promising imaging radio-pharmaceutical, with high selectivity to compete with ^{3}[H]-FEAU and ^{18}[F]-FHBG as HSV1-sr39-tk mono-phosphorilation drug and with ^{18}[F]-FMAU as highly proliferated cells' tracking radio-tracer. Method: We have modified the ^{18}[F]-FMAU synthesis procedure reported by Mangner *et* al, developed a dynamic version and applied for the ^{18}[F]-FEAU/^{18}[F]-FXAU drugs' synthesis. The separated ^{18}[F]-FEAU □/□-isomers, as independent isoforms, were compared to ^{3}[H]-FEAU, ^{3}[H]-PCV (Pencyclovir) and ^{18}[F]-FHBG by their cytoplasmic accumulation & relative mono-phosphorilation constants in C6 & C6Sr39-tk rat cells' uptake studies. Results: The ^{18}[F]-

FEAU □-isomer did not show any specific accumulation either in C6 or in C6-sr39tk cells. At the same time ^{18}[F]-FEAU □-isomer demonstrated high accumulation in C6 HSV1-sr39-tk cells applied in different concentrations in cell uptake studies: about 166 fold more that in the C6 control cells. Conclusion The alternative-high accumulation of 1-(2'-deoxy-2'-[^{18}F]fluoro-□-D-arabinofuranosyl)-5-ethyluracyl (□-^{18}[F]-FEAU) in mutant C6 HSV1-sr39-tk cells proves ^{18}[F]-FXAU family drugs huge potential as PET imaging radio-tracers that will be applied for translational studies from cells to animal models and humans through the HSV1-sr39tk gene expression.

No. 93

IMPROVING THE CLINICAL PRACTICALITY OF 4-D RESPIRATORY GATED POSITRON EMISSION TOMOGRAPHY WITH SIMULTANEOUS WHOLE-BODY SINOGRAM AND LISTMODE ACQUISITION

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Purpose: There are potential benefits of respiratory gated positron emission tomography (PET) to reduce blurring artifacts that impact small lesion detectability and quantification. Previous positron emission tomography (PET)/ computed tomography (CT) scanners allowed clinical acquisition of respiratory gated data, but required a secondary acquisition constrained to a single 15 cm field-of-view (FOV). It is hard to predict if and where motion will impact nodule detection since not all patients have large respiratory patterns. Therefore the routine utilization of 4-D PET has been limited. Our goal was to assess the functionality of a clinically practical whole-body respiratory gated PET protocol. Methods: The GE Discovery STE PET/CT scanner allows for simultaneous acquisition of a traditional whole-body scan and a listmode coincidence file. All patients scanned for whole-body PET were acquired with pulmonary monitoring used to retrospectively process the listmode data to generate multi-FOV percentage gated data. Results: Simultaneous 4-D PET has successfully been acquired as part of the clinical routine and is currently conducted on all whole-body PET/CT exams. Respiratory motion of tumors can clearly be visualized. In addition, respiratory motion of the lung, liver and heart can be captured and assessed across the entire patient FOV. Conclusion: Routine whole-body respiratory gated PET imaging is now possible. Although not all patients have respiratory motion that significantly impacts the PET image, this procedure allows non-invasive acquisition of 4-D data for those cases where it might be clinically relevant. Optimization of the binning protocol and assessment of the clinical impact is ongoing.

No. 94

FLUOR CHOLINE (FCH) POSITRON EMISSION TOMOGRAPHY/ COMPUTED TOMOGRAPHY IN PREOPERATIVE STAGING AND FOLLOW UP OF PROSTATE CANCER

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Aim of this study was to evaluate FCH (F-18 CHOLINE)-positron emission tomography (PET)/ computed tomography (CT) in the preoperative management and follow up of prostate cancer patients. Methods: Between October 2003 and September 2005 more than 185 patients with different kinds of prostate cancer were investigated with FCH-PET/CT. From these, 88 patients (group 1) were evaluated for preoperative staging, 82 patients (group 2) with elevated and/or increasing PSA levels for follow-up reasons (diagnosis of recurrence, radiation therapy planning etc). Nine patients were preliminary non operated and only received different treatment modalities (eg hormone therapy, RT etc) and six patients were evaluated for other diagnostic reasons. PET/CT results were compared with postoperative histological findings, CIM (conventional imaging modalities) and clinical follow up. Results: In group 1 metastases (LN 6, bone 4, LN and bone 2) were diagnosed in 14 % (12/88 patients). In group 2, in eighty two patients diagnosed for follow-up reasons, FCH-PET/CT revealed in 21 % (17/82) lymph node metastases, in 16 % (13/82) bone metastases and in 10 % (8/82) lymph node as well as bone metastases. Conclusion: FCH-PET/CT was clearly able to diagnose prostate cancer, lymph node and/or bone metastases being also superior compared to conventional imaging modalities**.** Due to our results, FCH-PET/CT seems to be highly efficient in the preoperative management as well as follow up procedure in prostate cancer patients.

No. 95

POSITRON EMISSION TOMOGRAPHY USING C-11-METOMIDATE IN ADRENOCORTICAL TUMOURS, AND INVESTIGATION OF FUTURE ^{18}F-ANALOGUES

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The primary aim of this work was to evaluate C-11-Metomidate (MTO) as a specific tracer in positron-emission tomography (PET) for detection tumors of adrenocortical origin. A secondary aim was to develop and evaluate F-18-labelled MTO analogues. Two hundred and twelve consecutive MTO-PET examinations and 75 histopathological specimens from 73 patients after were analyzed. F-18-etomidate (FMEA) was prepared in the following two step procedure: [F-18]Fluoride was reacted with 1,2-bis(tosyloxy)ethane using the kryptofix/potassium carbonate in acetonitrile to yield [F-18]fluoroethyl tosylate followed by reacting with the tetrabutylammonium salt of (R)-1-(1-phenylethyl)-1H-imidazole-5-carboxylic acid. The F-18-compound was investigated in rats and cynomolgus monkey. Sensitivity was 0.89 and specificity was 0.96 for MTO-PET in proving adrenocortical origin of the lesions. Pheocromocytomas, metastases to the adrenal gland and non-adrenal masses were all MTO-negative. PET measurements using standardized uptake values (SUV) was higher in aldosterone hypersecreting adenomas compared to normal adrenals and the SUV ratio between tumour and the contralateral gland was significantly higher in all hormonally hypersecreting adenomas as well as in adrenocortical cancer. The uptake of FMEA in adrenal was at comparable levels as that of MTO. However, the uptake in lung, liver, pancreas, and kidney was significantly lower leading to an increased adrenal to liver/kidney ratio for FMEA. MTO-PET is a specific and sensitive method for diagnosing adrenocortical tumors and in the work-up of adrenal incidentalomas and for examination of patients with adrenocortical cancer. Trials with F-18-labeled analogues in rats and primate show an increased adrenal to liver ratio which is advantageous in the characterization of incidentalomas.

No. 96

POSITRON EMISSION TOMOGRAPHY/ COMPUTED TOMOGRAPHY IN THE STAGING PROCEDURE OF CERVICAL CANCER

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Aim: The most important prognostic factors in cervical cancer are paraaortical spreading and distant metastases. The aim of this study was to evaluate the usefulness of positron emission tomography (PET)/ computed tomography (CT) in staging of cervical cancer. Methods: From November 2002 - April 2005, we included 100 patients with newly diagnosed cervical cancer. After a clinical staging according to FIGO a whole-body PET/CT with i.v. and oral contrast media was performed. If pathological lymph nodes or distant metastases were detected, a histological specimen was obtained if possible. PET/CT scans were described jointly by specialists in radiology and nuclear medicine. Furthermore, the whole-body CT-scans were independently reviewed by another radiologist. Results: The patients included were staged as follows: stage1: 26, stage 2: 34, stage 3: 35, stage 4: 5. Metastases were seen in 37 patients, 22 were extra-pelvic. True

positive paraaortic lymph node metastases were found in 13 patients, true positive distant pathological foci were found in nine, of which one was found to be a new primary (lung cancer). In 11 cases, CT missed paraaortic, mediastinal or inguinal lymph nodes or distant metastases found by PET/CT. Seven false positive PET/CT scans were correctly read as normal on CT alone, of which four were bone lesions. PET/CT had an impact on treatment planning in 30/37 patients with positive PET/CT. Conclusion: PET/CT is a useful clinical tool in the staging procedure of cervical cancer. It will now be implemented as a routine staging method in our facility.

No. 97

[C-11]TOPOTECAN POSITRON EMISSION TOMOGRAPHY IMAGING PREDICTS NEGATIVE RESPONSE OF TOPOTECAN THERAPY

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Topotecan is administered as second-line treatment for ovarian and small cell lung cancer and has an overall response rate of approximately 15 to 35%. A method for predicting response in individual patients, before initiating therapy, would be an enormous advantage to patients and their oncologists. It could potentially spare 65 to 85% of patients from ineffective therapy and enable them to expediently consider alternative therapeutic regiments. We hypothesize that the biodistribution of radioactivity measured via positron emission tomography (PET) following administration of radiolabeled topotecan will enable us to predict therapeutic response. Methods We have created [C-11]topotecan by reacting [C-11]methyl iodide with des-methyl topotecan prepared from topotecan. We have administered sub-therapeutic quantities of [C-11]topotecan into 5 cancer patients and imaged its biodistribution with PET. On a separate day 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) biodistribution was also imaged. Results In all subjects evaluated to date, uptake of C-11activity did not correlate with FDG uptake. On this basis we predicted that topotecan therapy would be ineffective. Clinical follow-up confirmed that prediction to be correct in five of five patients. This project is being continued with the hope that positive response would match with C-11 localizing into metabolically active tumors.

No. 98

CARDIAC AND EXTRA-CARDIAC SYMPATHETIC DENERVATION IN PARKINSON DISEASE WITH ORTHOSTATIC HYPOTENSION AND IN PURE AUTONOMIC FAILURE

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Uptake of 6-[F-18]fluorodopamine by cardiac noradrenergic nerves enables visualization of the sympathetic innervation of the left ventricular myocardium by positron emission tomography (PET) scanning. Patients with Parkinson's disease and orthostatic hypotension (PD+OH) or with pure autonomic failure (PAF) have markedly decreased myocardial 6-[F-18]fluorodopamine-derived radioactivity, consistent with cardiac sympathetic denervation, a phenomenon that neurochemical, neuropharmacological, and most recently post-mortem neuropathological studies have confirmed. In this study we asked whether 6-[F-18]fluorodopamine can visualize sympathetic innervation in extra-cardiac organs and if so whether patients with PD+OH or PAF have neuroimaging evidence of extra-cardiac noradrenergic denervation. Methods: To validate the method, healthy volunteers underwent 6-[F-18]fluorodopamine scanning of the head, thorax, and abdomen, with or without treatment with desipramine to block sympathoneural uptake of catecholamines. N-13-Ammonia scanning was used to address possible group differences in 6-[F-18]fluorodopamine delivery by blood perfusion. Results: Desipramine treatment was associated with decreased 6-[F-18]fluorodopamine-derived radioactivity in the heart, renal cortex, and thyroid but not in the liver, spleen, renal pelvis, or salivary glands. Both the PD+OH and PAF groups had decreased 6-[F-18]fluorodopamine-derived radioactivity in the heart ($p<0.0001$) and renal cortex ($p=0.02$, $p=0.005$). The PD+OH group also had decreased thyroid radioactivity ($p=0.01$). Neither group had decreased radioactivity in the other organs, after correction for N-13-ammonia-derived radioactivity. Conclusion: 6-[F-18]fluorodopamine scanning visualizes sympathetic innervation in the heart, renal cortex, and thyroid gland. Both PD+OH and PAF entail decreased noradrenergic innervation that is most prominent in the heart but is also detectable in extra-cardiac organs.

Drug Development Oral Presentations

No. 99

SMALL ANIMAL PET STUDIES WITH THE NEW POSITRON EMISSION TOMOGRAPHY RADIOTRACER [F-18]MCL-322 FOR IMAGING THE DOPAMINE TRANSPORTER

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The fluoroalkyl-containing tropane derivative 3-(4-bromo-phenyl)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid 2-fluoroethyl ester (MCL-322, 1) was shown to be a highly potent and selective ligand for the dopamine transporter (DAT). The compound was labeled with the short-lived positron emitter F-18 in a single step by nucleophilic displacement of the corresponding tosylate precursor with n.c.a. [F-18]fluoride. The positron emission tomography (PET) radiotracer [F-18]MCL-322 was obtained in decay-corrected radiochemical yields of 30 to 40% at a specific radioactivity >60 GBq/micromol at the end-of-synthesis. MicroPET, *ex vivo* and *in vivo* biodistribution studies in Wistar rats demonstrated a high uptake of [F-18]MCL-322 in the striatum (3.2 % ID/g) five minutes after injection, which increased to 4.2 % ID/g after 60 minutes. Specific binding of [F-18]MCL-322 to the DAT was confirmed by blocking experiments using the high affinity DAT ligand GBR 12909. The F-18-activity in the striatum represented the original compound and only minor amounts of metabolites were found in this target region. Furthermore, also the autoradiographic investigations confirmed the highly selective uptake of [F-18]MCL-322 in the striatum. The two compartment analysis of the microPET studies (n=4) with [F-18]MCL-322 using the arterial input curve yielded a mean binding potential of BP=9.6 (BP=k3/k4). The corresponding parameter images were calculated from the coregistred images for movement correction. The simple single-step radiosynthesis of [F-18]MCL-322 and the promising radiopharmacological profile make [F-18]MCL-322 an attractive candidate for the further development of a potential PET radiotracer for clinical imaging of the DAT in human brain.

No. 100

BIODISTRIBUTION OF SMOKED NICOTINE AS MEASURED BY POSITRON EMISSION TOMOGRAPHY

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In order to measure the rate of rise of nicotine in the brain after inhalation in tobacco smoke, a positron emission tomography (PET) study was undertaken with [C-11]nicotine as the tracer. In an initial phase of the study techniques were developed to formulate the radiotracer onto a cigarette so that it would behave in a single inhalation in the same way as the endogenous nicotine. In the scan phase of the study, 12 healthy volunteers, all smokers making no effort to quit, were recruited. Following synthesis of the tracer, a cigarette of the chosen brand of each volunteer was dosed for single inhalation administration. The cigarette was placed in a holder from which the volunteer could inhale in the supine position in the PET scanner, with the brain in the field-of-view. The data acquisition started before

inhalation and continued for 10 minutes afterward. Simultaneous venous and arterial blood curves were obtained. In a second inhalation on the same day, the process was repeated with the scanner positioned over the lungs. Complete kinetic data of all relevant compartments (lung, arterial and venous blood, and brain) was therefore obtained. Tracer transfer between compartments was analyzed. The study showed that the rate of rise in the brain tissue was rapid enough that delivery kinetics may contribute to cigarette habituation and sensitization, according to recent theories of addiction.

No. 101

***IN VITRO* AND *IN VIVO* CHARACTERIZATION OF CU-64-LABELED MEDI-222, A HUMANIZED MONOCLONAL ANTIBODY AGAINST INTEGRIN $\alpha_V\beta_3$**

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Objective: MEDI-222, a humanized monoclonal antibody against human integrin $\alpha_V\beta_3$, is in clinical trial for cancer therapy. However, the pharmacokinetics remains unclear. *In vivo* imaging using MEDI-222-based probes is needed for better treatment monitoring and dose optimization. Methods: In this study we conjugated MEDI-222 with macrocyclic chelating agent DOTA at five different DOTA/MEDI-222 ratios. The conjugates were labeled with Cu-64 and tested in three human (U87MG, MDA-MB-435, and PC-3) as well as mouse (GL-26) and rat (RT-2) tumor models. The *in vitro* and *in vivo* effects of these DOTA-MEDI-222 conjugate were evaluated. Results: The conjugates have varied from 3 to 35 DOTA chelators per antibody and the radiolabeling yield varied from 10 to 90%. No significant difference in radioimmunoreactivity was found among these conjugates (70-80%). MicroPET imaging using the conjugate at DOTA/MEDI-222 ratio of 35:1 showed as high as 24.1±6.1%ID/g at 48h postinjection. The receptor specificity of Cu-64-DOTA-MEDI-222 was confirmed by effective blocking of MDA-MB-435 tumor uptake by co-administration of non-radioactive MEDI-222. Appreciable uptake in GL-26 mouse glioma and RT-2 rat glioma (7.94±2.11 and 7.01±1.34%ID/g at 24 hours post-injection, respectively) was likely attributed to passive targeting based upon enhanced permeability of tumor vessels and lack of lymphatic drain in the tumor as compared to normal tissues, as evidenced by declined tumor uptake after 24 hours post-injection. Conclusions: The success of integrin specific tumor targeting of Cu-64-DOTA-MEDI-222 may be translated into clinic to characterize the pharmacokinetics, tumor targeting efficacy, dose optimization and dose interval of MEDI-222 and/or MEDI-222 conjugates in integrin-positive patients.

No. 102

2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE- POSITRON EMISSION TOMOGRAPHY IMAGING ASSESSMENT OF INFLAMMATION IN A RHEUMATOID ARITHRITIS MODEL IN THE RAT

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Assessment of rheumatoid arthritis (RA) by imaging methods can aid in following disease extent, progression, and response to drug therapy non-invasively. 2-deoxy-2-[F-18]fluoro-D-glucose (FDG)- positron emission tomography (PET) has been shown to localize radiotracer to inflammation sites in clinical RA patients. The aim of this study was to evaluate FDG microPET in a preclinical model of RA in the rat as a clinically-translateable biomarker. A systemic model of RA was induced in female dark Agouti rats by immunization with bovine type II collagen. Five RA rats and two aged-matched controls were scanned 21 and 35 days after initial immunization. Rats were injected with FDG into tail vein under isoflurane anesthesia. After a 60-minute uptake period rats were re-anesthetized, placed supine on the imaging bed with hind legs and feet fixed in position, then imaged using a Concorde Microsystems Focus220 microPET. Images were reconstructed with attenuation and scatter correction using OSEM3D-MAP and scaled to standardized uptake value (SUV). At day 21, RA rats showed intense soft tissue, ankle, and foot uptake compared to age-matched controls (max SUV = 2.95 vs 1.14). At day 35, RA rats showed reduced soft tissue uptake compared to day 21, but persistent focal uptake in the ankle and foot (max SUV = 2.72 in RA rats vs 1.20 in normals). FDG microPET scanning in a rat model of RA showed a clear separation in tracer uptake distribution between control and RA rats and may prove to be a useful clinically-translateable biomarker to assess therapeutic drug response for RA.

No. 103

QUANTIFICATION OF INTRA-TUMOR PHYSIOLOGICAL HETEROGENEITY AND THERAPEUTIC RESPONSE IN XENOGRAFT MCF-7 BREAST CANCER MODEL BY DYNAMIC CONTRAST ENHANCED COMPUTED TOMOGRAPHY

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Purpose: The objective is to evaluate the ability of dynamic contrast enhanced computed tomography (DCE-CT) to assess intra-tumor physiological heterogeneity and monitor physiological response to anti-angiogenesis therapy in xenograft tumor models. Methods: DCE-CT imaging was performed on athymic nude mice bearing xenograft wildtype and VEGF-transfected MCF-7 tumors by using a clinical multi-slice CT. Parametrical maps of tumor physiology of perfusion, permeability-surface area, fractional intravascular (fiv) and interstitial space (fis) were obtained by fitting voxel-wise contrast curves to a two-compartmental kinetic model. One group of mice with VEGF-transfected tumor was treated with VEGFR2 antibody ligand by intravascular or intraperitoneal administration and the other group served as a control. DCE-CT scans were followed 1-2 days and 7-10 days after treatment. Results: MCF-7 tumors showed larger variation of physiological parameters compared with muscle. The wildtype tumor showed a radial heterogeneity pattern with low values in the core region and increased values near the periphery. The VEGF-transfected tumor demonstrated a highly saccular heterogeneity. A significant decrease in perfusion (9-50%) and fiv (13-25%) averaged over the entire slice was found immediately after the treatment followed by a recovery of fiv about 5-7 days after the treatment. Tumor receiving intravascular administration showed more global reduction while intraperitoneal administration had localized effects. Continuous progression of perfusion and fiv was found in control tumors. Conclusion: This study demonstrated the feasibility of DCE-CT to quantify spatial and temporal physiology changes of tumor in small animal models, which offers an *in-vivo* tool for evaluation and optimization of anti-angiogenesis therapy.

No. 104

REAL-TIME IMAGING OF DISRUPTION OF HUMAN HEAT SHOCK PROTEIN 90/P23 INTERACTIONS BY GELDANAMYCIN-BASED HSP90 INHIBITORS IN LIVING SUBJECTS

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Background: Hsp90/co-chaperone p23 interactions are important for protein folding in cancer. Objectives: To evaluate the efficacies of Hsp90 inhibitors in disrupting Hsp90/p23 interactions in cell culture and in living mice using the split Renilla luciferase (RL) protein-fragment-assisted complementation technology (Anal. Chem. 75: 1584-9, 2003). Methods: Human Hsp90 and p23 split RL reporters were transiently transfected into 293T human embryonic kidney cancer cells (24hrs) and treated with geldanamycin-based Hsp90 inhibitors (12 hrs) prior to luminometer assay. Animal studies: 10 million 293T cells were transiently transfected with Nhrluc-p23/Hsp90-Chrluc (36 hrs), implanted subcutaneously at the back of nude mice and imaged using Xenogen IVIS system, and treated with 75 mg/kg of 17-(Dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG) or carrier control prior to re-imaging at 18 hours. Results: In cell culture studies, geldanamycin-based Hsp90 inhibitors led to dose dependent inhibition of RL activity from Hsp90/p23 complementation, with maximum inhibition ~ 60% relative to carrier control ($p < 0.05$). In

living mice implanted with 293T cells transiently transfected with Nhrluc-p23/Hsp90-Chrluc, a signal to background (pcDNA transfected cells) ratio of 5 was observed ($p < 0.001$). Treatment of these mice with 17-DMAG for 18 hrs led to a 84% decrease in signal to background ratio, compared to a 44% decrease in carrier control treated mice ($p < 0.05$). Conclusion: Molecular imaging of Hsp90/p23 interactions in living subjects including pharmacological modulation has been validated with a novel split reporter strategy. Molecular imaging with this new assay will be a powerful tool for accelerating drug development and validation in pre-clinical models.

No. 105

2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE ASSESSMENT OF GABAB RECEPTOR MEDIATED CHANGES IN REGIONAL BRAIN GLUCOSE METABOLISM IN THE RAT

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Introduction: GABAB receptors regulate brain metabolism and neural transcription, and are a target for new medications for drug abuse and depression. This study explores the hypothesis that drug mediated GABAB receptor activation influences brain glucose metabolism in a way that can be monitored via 2-deoxy-2-[F-18]fluoro-D-glucose (FDG)- positron emission tomography (PET) imaging. Methods: Regional brain FDG distribution and blocking studies were conducted in male rats (250g-350g). The following drugs were given (2.5 mg/kg, n = 4-10) 50 minutes prior to FDG administration: baclofen (GABAB agonist), CGP-54626 (GABAB antagonist), SR-95531 (GABAA antagonist), baclofen and CGP-54626, baclofen and SR-95531, baclofen and CGP-54626 plus SR-95531, or saline. Forty-five minutes after FDG administration, rats were killed and eight brain regions were dissected, weighed and assayed for radioactivity. The percent-injected dose/gram (ID%/g) was determined. Results: Baclofen treated animals exhibited lower FDG uptake in all regions examined (eg striatum: 0.93 +/- 0.08 %ID/g vs. 1.22 +/- 0.07 %ID/g, $p < 0.03$). Coinjection of CGP-545262 and SR-95531 before baclofen prevented this effect, whereas either one alone proved insufficient, suggesting that both GABA subtypes are involved. Both CGP-545262 and SR-95531 increased regional FDG uptake relative to control (eg striatum: 1.88 +/- 0.11 %ID/g and 1.50 +/- 0.11 %ID/g, vs. 1.22 +/- 0.08 %ID/g, ps < 0.05). Conclusion: Administration of the GABAB receptor agonist baclofen resulted in a decrease in regional brain glucose metabolism as measured with FDG, whereas GABA receptor antagonists produced the opposite response. These data support a paradigm for monitoring the effects of GABAB receptor targeted drugs by FDG-PET.

No. 106

THE POTENTIAL ROLE OF DYNAMIC CONTRAST-ENHANCED MAGNETIC RESONANCE IMAGING IN THE DEVELOPMENT OF BPH DRUGS SUCH AS 5□-REDUCTASE INHIBITORS

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Objective: The role of 5□-reductase inhibitors such as finasteride (Merck & Co., Inc.) has expanded over recent years to include the prophylaxis of benign prostatic hyperplasia-associated hematuria and the reduction of blood loss at surgical resection of the prostate. The underlying mechanism includes the decrease in vascular endothelial growth factor (VEGF) and the decrease in prostatic suburethral microvessel density. The purpose of this study is to apply dynamic contrast-enhanced magnetic resonance imaging (MRI) (DCE-MRI) to non-invasively assess the prostatic microcirculation changes under finasteride pharmacotherapy. Methods: Twelve male beagles were randomly allocated to one finasteride-treatment group and one control group with treatment duration of three months. Five MRI scans were performed as two baseline and three follow-up scans. DCE-MRI was performed using a 3-D spoiled gradient echo (3-D-SPGR) imaging sequence. A Gd-chelate was intravenously injected at a rate of 0.2 ml/s. Regions of interests were drawn on prostatic suburethral areas and the pharmacokinetic parameters were evaluated. Results: After completion of the therapeutic regiment, the time to maximum signal enhancement T_{max} was significantly longer in the finasteride group compared to controls ($p < 0.01$). Amplitude A and rate constant k_{ep} decreased in the finasteride group at the end of the trial, which significantly differed from the control group ($p < 0.05$). The changes in these parameters correspond to the reduction in prostatic suburethral microcirculation. Conclusion: DCE-MRI is highly effective in monitoring the molecular biologic drug effects on the prostatic microcirculation and could be used for effective therapeutic management.

No. 107

SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY IMAGING AND QUANTIFICATION OF TC-99M-LABELED HUMANIZED TRA-8 ANTI-DR5 ANTIBODY IN HUMAN BREAST AND PANCREATIC TUMOR XENOGRAFTS

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Purpose: To characterize binding and localization of Tc-99m-labeled humanized TRA-8 (hTRA-8), an apoptosis-inducing, monomeric monoclonal antibody targeting human DR5 on cancer cells. Methods: Scatchard assays were conducted with Tc-99m-labeled hTRA-8 using a subclone (2LMP) of MDA MB-231 human breast and pancreatic (MIA PaCa-2) tumor cell lines. High-resolution single photon emission computed tomography and X-ray computed tomography (SPECT/CT) imaging and biodistribution analyses were performed in nude mice with these xenografted s.c. tumors to measure *in vivo* tumor retention of Tc-99m-labeled hTRA-8. Planar imaging was conducted in cynomolgus monkeys. Results: Specific and high affinity binding to the DR5 receptor was demonstrated; DR5 receptors averaged 8110 and 10635 per cell, for MIA PaCa-2 and 2LMP cells, respectively. MicroSPECT images revealed higher uptake at the periphery of large tumors; 80% of Tc-99m-labeled hTRA-8 in 2LMP tumors (2234 mm3) was distributed within 2.8±0.2-mm of the tumor surface at 6h. For smaller MIA PaCa-2 tumors (427 mm3), the distribution of Tc-99m-labeled hTRA-8 was uniform throughout the tumors; 80% was distributed within 1.9±0.1-mm of the tumor surface at 6h. The planar monkey images revealed a blood-pool distribution of Tc-99m-labeled hTRA-8, which was confirmed by blood analyses. Conclusions: The *in vivo* distribution of Tc-99m-labeled hTRA-8 was analyzed at high resolution, with %ID/g determined in 1-mm radial shells within the tumors. These studies demonstrate effective delivery and retention of hTRA-8 in human tumor xenografts, with uniform distribution achieved in MIA PaCa-2 tumors. These preclinical data support further human studies for cancer therapy.

No. 108

HUMAN BIODISTRIBUTION OF F-18 PACLITAXEL (FPAC): A POTENTIAL PET TRACER FOR EVALUATING MULTIDRUG RESISTANCE (MDR)

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The expression of the multidrug resistance gene (mdr-1), which codes for a P-gp efflux pump, has been shown to play a major role in the resistance of cancer to paclitaxel (PAC) treatment. An effective *in vivo* assay, having an accurate positive predictive value for determining MDR prior to treatment, is needed. The biodistribution of [F-18] labeled PAC (FPAC) has been studied in rats, mice and mouse xenographs and found to correleate with PAC. We report the first use of FPAC in human subjects to determine biodistribution for the estimation of dosimetry. Method: Dynamic whole-body positron emission tomography (PET)/ computed tomography (CT) was obtained after IV administration of 3-7 mCi FPAC. Data were acquired for a total of three hours. Results: No untoward effects were observed in study subjects following dosage administration. The biodistribution was found similar to that reported in non-human primates (see table). These data show that quantitative PET imaging in human with FPAC is feasible. We have initiated FPAC imaging in breast cancer patients to evaluate the potential ability of FPAC to predict MDR prior to therapy.

Biodistribution of F-18 paclitaxel in humans

Organ	SUV(2-8min)	SUV(~3hrs)
Liver	39.0	3.1
Spleen	26.2	3.1
Kidney	18.9	1.1
Brain	0.22	0.12
Lungs	2.7	0.41
Vertebra	3.9	1.4
Breast	0.32	0.35
Heart	3.1	0.91
LV Cavity	3	0.7
Gall Bladder	424(max)	
Bowel	683(max)	
Urinary Bladder	37.2(max)	

No. 109

EVALUATION OF EMBRYONIC STEM CELLS FOR TREATMENT OF MYOCARDIAL INFARCTION

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Various types of stem cells and progenitor cells (e.g., mesenchymal stem cells and muscle progenitor cells) have been proposed for the treatment of myocardial infarction. However, only the embryonic stem cells (ESCs) have the potential of undergoing cardiac differentiation to regenerate cardiomyocytes that are lost as the result of an ischemia episode (infarction). We induced cardiac differentiation in murine ESCs: these cells were undergoing synchronized contraction and were stained positive for sarcomeric alpha-Actinin. In order for *in vivo* tracking of the survival status of ESCs that are transplanted in the rat heart, we did molecular manipulation to stably express HSV1-tk in these cells; therefore, surviving cells can specifically retain radio-labeled PET tracers including [F-18]FHBG and [F-18]FIAU. We demonstrated that in ESCs stably expressing HSV1-tk, accumulation of FIAU was over 40-fold higher than the untransfected control cells, whereas accumulation of FHBG was only about 5-8-fold higher than the control. In contrast, FHBG was preferably accumulated in HSV1-sr39tk expressed ESCs. *In vivo* imaging of sr39tk expressed cells grafted in the rat heart using A-PET system demonstrated reasonable contrast. We further performed autoradiographic studies to quantify the contrast between injection and remote site: [I-125]FIAU (1.2 mCi) was injected two-hour post surgery and the hearts were harvested and cryo-sectioned. The ARG film was exposed (4d) and digitized: a ratio of 4.3 (±0.6) was achieved between hot spots (injection sites) and the remote region of the heart, suggesting that the achieved contrast is favorable for *in vivo* detection.

No. 110

COPPER-64 POSITRON EMISSION TOMOGRAPHY IN BIODISTRIBUTION STUDIES OF THERAPEUTIC ANTIBODY CANDIDATES IN NON-HUMAN PRIMATES

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We have developed robust antibody labeling and positron emission tomography (PET) methods to image the organ-level biodistribution of therapeutic antibody candidates in non-human primates. Antibody development typically involves engineering to refine the affinity, effector function, or serum half-life, and sometimes requires substantial sequence substitutions for antibody humanization. The engineered changes can have unintended consequences that worsen the therapeutic profile, for example, by exacerbating bone marrow or liver uptake. When antibodies are used to deliver radioisotopes or potent toxins these changes in biodistribution can have profound effects. We have developed Cu-64 DOTA-conjugated monoclonal antibodies as a screening tool to evaluate biodistribution of antibody candidates in the first 48 hours post-injection using PET. Cynomolgous monkeys (n=36) received intravenously a trace antibody dose of 0.5 mg/kg (typically 1mg total) containing approximately 1 mCi of Cu-64-DOTA-antibody. Images were obtained using the Concorde Microsystems P4 system. Whole-body images were obtained from three bed positions at one hour post injection and again at 24 hours, then a single bed position covering tissues of interest was collected at 48 hours p.i. MAP-reconstructed images were rendered and quantified using Analyze. In the series of antibodies studied to date (n=9) we have seen both expected (blood pool plus target tissue) and unexpected biodistributions. Some of the off-target distributions seen have explained toxicities observed at therapeutic doses, in the marrow for example; while others have given the first indication that certain organs accumulate the antibody. Subsequently, some unfavorable distributions have been manipulated by image-guided pre-dosing strategies.

No. 111

IMAGING ANIMAL MODELS OF STROKE WITH MICROSPECT/CT

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Objectives: We evaluated methods of imaging rat models of stroke *in vivo* using the X-SPECT™ micro single photon emission computed tomography (SPECT)/ computed tomography (CT) system (Gamma Medica, Inc., Northridge, CA) with the general goal of simultaneous imaging of radiolabeled cerebral blood flow and of delivery of therapeutic agents. Specific goals included: a) development of a method to image two radionuclides (^{99m}Tc and ^{125}I) simultaneously, and b) demonstration of an *in vivo* imaging capability to assess intracranial infarct induced by a middle cerebral artery occlusion (MCAO) technique. Methods: We performed phantom studies to show the capability of simultaneous dual-isotope imaging (^{99m}Tc and ^{125}I) using a list-mode acquisition technique. We then performed an animal study; a) we established an MCAO technique to induce ischemic stroke in rats, b) validated the MCAO model using triphenyltetrazolium chloride (TTC) staining and histologic analysis, and c) performed *in vivo* radionuclide imaging of cerebral perfusion with both ^{99m}Tc-ethyl cysteinate dimer (ECD) and ^{99m}Tc-exametazime (HMPAO) using 1-mm pinhole SPECT. Correlated microCT imaging was performed to localize radiopharmaceutical uptake. Results: Our phantom studies demonstrated that the X-SPECT™ microSPECT/CT can identify ^{125}I uptake in the presence of ^{99m}Tc background structures using energy discrimination method. Both TTC staining and histology revealed sizable infarcts in successful MCAO rat brains. ^{99m}Tc-HMPAO was preferred to ^{99m}Tc-ECD because the excretion of ECD metabolite from the rat brain was too rapid to be imaged with SPECT. Conclusion: We demonstrated microSPECT/CT techniques of imaging animal models of stroke *in vivo* with the goal of performing simultaneous dual-radiopharmaceutical imaging of ^{99m}Tc-exametazime and ^{125}I labeled therapeutic agents.

No. 112

EVALUATING THE THERAPEUTIC RESPONSE OF PS-341 IN XENOGRAFT SKOV3X OVARIAN MOUSE MODEL BY DYNAMIC CONTRAST-ENHANCED CT

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Purpose: Dynamic contrast-enhanced CT was used to quantify the heterogeneous physiological response to PS-341 chemotherapy in ovarian (SKOV3x) tumor. Methods and Procedures: Approximately 10^6 SKOV3x ovarian cancer cells were s.c. injected into the flanks of athymic mice and allowed to grow for four to six weeks. Two of the mice were given an i.p.

injection of 1 mg/kg of PS-341 and the third was given an i.v. injection. DCE-CT imaging was performed prior to treatment (0-2 days) and during the week after treatment (four to seven days). Parametric maps of tumor physiology - perfusion, permeability-surface area, fractional intravascular space (f_{iv}), and fractional interstitial space - were estimated by fitting contrast-enhanced curves to a two-compartmental model. For a central slice through the tumor, three radial regions-of-interest depicting the core, transitional, and periphery regions were defined, and the mean values for the physiological parameters and fractional necrotic (f_N, no discernable contrast-enhancement) were determined. Results: PS-341 prevented tumor growth in all three tumors, and DCE-CT images displayed an increase in f_N, specifically within the central region of the tumors where f_N ranged from (0.0-0.19) to (0.23-0.40). This expanding core was reflected by an increased radial heterogeneity: the percent change of f_{iv} in core versus periphery ranged from (-47 to -74%) versus (-25 to 16%), and for perfusion ranged from (-36 to -78%) versus (-50 to 31%). Conclusion: The therapeutic effect of PS-341 on the intra-tumor physiology of ovarian tumors is to expand the poorly perfused necrotic core, with varying anti-angiogenic effects near the periphery.

No. 113

QUANTIFICATION OF NOREPINEPHRINE TRANSPORTER OCCUPANCY IN ATOMOXETINE TREATED MONKEYS AS MEASURED WITH [F-18]FD$_2$MENER AND POSITRON EMISSION TOMOGRAPHY

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We studied the utility of the novel norepinephrine transporter (NET) ligand (*S,S*)-[F-18]FD$_2$MeNER and positron emission tomography (PET) for assessing target occupancy with the NET reuptake inhibitor atomoxetine. Two cynomolgus monkeys were scanned four times each with a Siemens ECAT EXACT HR PET system to determine the untreated baseline NET binding potential (BP) and occupancy in various brain regions after steady-state infusion with 0.03, 0.06, or 0.12 mg/kg/h atomoxetine, modeled to mimic the exposure of low, medium and high dose atomoxetine in humans under steady-state conditions. NET BP values for cortical and subcortical regions were determined using time activity curves from 0 to 240 minutes post (*S,S*)-[F-18]FD$_2$MeNER injection in a multilinear reference tissue model with the caudate as reference region for unspecific binding. Steady-state plasma concentrations (C_{ss}) of atomoxetine and its metabolite N-desmethyl-atomoxetine were determined using LC/MS/MS. We observed an improved dose versus C_{ss} relationship with N-desmethyl-atomoxetine as compared to atomoxetine; therefore the metabolite was used as a proxy for exposure. N-desmethyl-atomoxetine Css ranged from 11 to 70 ng/mL. NET occupancy during atomoxetine treatment increased with exposure from 35% to 83%. Our results support that (*S,S*)-[F-18]FD$_2$MeNER is the first useful radiofluorinated ligand for the quantification of NET BP and occupancy *in vivo*, and that it is superior to [C-11](*S,S*)-MeNER given that a specific binding peak equilibrium is reached during the PET experiment due to the longer half-live of flourine-18.

No. 114

A NOVEL STRATEGY FOR GENERATION OF HIGH AFFINITY BINDERS TO TYROSINE KINASES

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We generated high-affinity peptide binders to the external domains of (KDR, kinase domain receptor) and c-Met (HGF receptor). We used phage-display selection and a secondary screen to identify peptide pairs binding to the kinase but not competing with one another. KDR binding peptides were secondarily screened as tetrameric biotinylated peptide-avidin conjugates for binding to KDR- and mock-transfected 293H cells. While homomultimers (1 - 4 of the same peptide per conjugate) showed enhanced binding to KDR- versus mock-tranfected cells relative to monomers, heteromultimers (two or more noncompeting peptides per conjugate) showed much stronger binding than homomultimers. Two noncompeting binding peptides were then combined into synthetic “heterodimers” using a chemical scaffold: the conjugate was ~ 5000 kDa. Peptide monomers starting from Kd > 100 nM gave heterodimers with Kd < 1 nM. Generality was verified by creating HAPs against c-Met with the same procedure. A heterodimer composed of monomers with Kd = 0.2 μM and 0.8 μM had a Kd of 0.8 nM, an improvement of 250 fold. For KDR, the heterodimers interfered with native ligand-induced autophosphorylation much more than even strongly binding monomers or homodimers. The new linker is chemically versatile as to label position and linker length within the limits tested. Our strategy seems to work as a general approach for generating strong de novo binders to the extracellular domains of receptor tyrosine kinases, and possibly other proteins.

No. 115

EVALUATION OF [F-18]PACLITAXEL (FPAC) AS A POSITRON EMISSION TOMOGRAPHY TRACER FOR PGP EXPRESSION IN CELLS AND IN HUMAN EPITHELIAL TUMOR MOUSE XENOGRAFTS

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Background: Over-expression of the membrane pump, P-glycoprotein (Pgp) is a major cause of multidrug resistance (MDR) in many tumor types. Detection of MDR prior to chemotherapy may help direct more effective treatment. Fluorine-18 labeled paclitaxel (FPAC) is a positron emission tomography (PET) tracer showing promise as an *in vivo* marker for MDR, specifically the Pgp membrane pump. Its biodistribution has been reported in rodents and non-human primate imaging has provided dosimetry estimates. Methods:We performed *in vitro* uptake studies with FPAC other known imaging markers of MDR in a human epithelial cancer cell lines: KB 8-5 cells (Pgp +), KB 3-1 cells (Pgp-). 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) was used as a control tracer. We confirmed the expected differential response to treatment with paclitaxel and performed FPAC biodistribution and microPET imaging studies in a mouse xenograft model. Results: KB 8-5 cells (drug resistant) showed reduced uptake of tetrofosmin (Tfos), Tc-99m sestamibi (MIBI), and FPAC compared with KB 3-1 cells (drug sensitive). KB 8-5 tumors showed a decreased response to paclitaxel treatment and lower uptake of FPAC in both the biodistribution and microPET studies compared with the KB 3-1 tumors. Conclusion:. Major limitations of Tfos and MIBI as MDR tracers are the inability to quantitate uptake, and the dependence of cellular retention on the presence of an electrochemical gradient. We have shown that FPAC uptake is inversely correlated with the presence of Pgp *in vitro* and *in vivo* using an established MDR epithelial cell lines, making it a promising PET tracer for the evaluation of MDR.

No. 116

OPIATE RECEPTOR POSITRON EMISSION TOMOGRAPHY OCCUPANCY IN THE DEVELOPMENT OF AN OPIATE RECEPTOR ANTAGONIST DRUG

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Recent baboon positron emission tomography (PET) imaging studies (using LY255582, study by Kumar and colleagues) documented the utility of measuring serial receptor occupancy with opiate antagonists. These antagonists have shown therapeutic benefit with caloric control in rodent models. To test the feasibility for humans we examined the PET occupancy with oral 150 mg naltrexone subjects studied with dynamic [C-11]

Diprenorphine (mu delta kappa antagonist) at two, seven and 24 hours post drug. To optimize the quantification we employed various region of interest (time activity curves) and parametric (using SRTM) reference region modeling methods (REFM) employing the occipital cortex as the reference region. Occupancy measures during the two, seven and 24 hours post drug demonstrated a peak of 90% declining to 85% occupancy with no significant difference between the REFM measures at each time point. To confirm using the more traditional approach with a radial arterial input, various one and two tissue compartment models were considered. Occupancy estimated by arterial methods compared to REFM methods was within 10% for the two methods measured at two and seven hours post drug. This demonstrates the approach of serial measures of opiate receptor occupancies of initial dosing and washout for potential future therapeutic dose selection with opiate drugs. It emphasizes the importance of verifying the appropriate mathematical models for quantification of the outcome measures depending on whether methodological accuracy (e.g. using arterial input and specific compartment models) or simpler logistics (e.g. with REFM techniques) are required for drug development decisions.

No. 117

SYNTHESIS, RADIOLABELING AND EVALUATION OF 4-METHOXY-1H-INDOLE-3-CARBOXYLIC ACID-(4-[4-(2,4-DICHLORO-PHENYL)PIPERAZIN-1-YL]BUTY))AMIDE ([11C]WLD3.001) AS DEVELOPMENT OF POTENTIAL SELECTIVE DOPAMINE D3 POSITRON EMISSION TOMOGRAPHY TRACER

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Introduction: Antagonism of the dopamine D3 receptor was suggested to be important to the therapeutic effects of antipsychotic drugs. To date the vast majority of dopamine receptor radiotracers are nonselective binding to both D2 and D3 subtypes, complicating interpretation of results. To address this issue, we synthesized [C-11]WLD3.001 and performed an initial assessment of its potential as a selective D3 receptor positron emission tomography (PET) tracer. Methods: WLD3.001 and its corresponding phenol precursor WLD3.002 were synthesized. The Log P value of WLD3.001 was assessed by an HPLC method, and *in vitro* binding assays performed. [C-11]WLD3.001 was synthesized by reacting WLD3.002 with [C-11]CH3I at 40 C for two minutes. Regional brain biodistribution and blocking studies were performed in conscious male Sprague - Dawley rats (225-300g). Results: The affinities (Ki) of WLD3.001 for dopamine D2, D3, D4 and D5 subtypes were 1,650 nM, 2.4 nM, 2,818 nM and >1000 nM, respectively. The log P value was 2.92. The regional brain biodistribution data revealed that peak uptake (%ID/g +/- SD) at 15 minutes post-injection were highest in the thalamus (0.96 +/- 0.10) > frontal cortex (0.84 +/- 0.07) > striatum (0.67 +/- 0.10) > cerebellum (0.49 +/- 0.03), with clearance of activity from all regions thereafter. Blocking studies (15 minutes) revealed ~45% saturable binding in the thalamus and frontal cortex, and ~25% specific binding in other regions. Conclusions: [C-11]WLD3.001 is a high affinity selective ligand for dopamine D3 receptors that exhibited saturable binding in the rodent brain. Further characterization of this tracer is warranted.

No. 118

DCE MAGNETIC RESONANCE IMAGING DETECTED DIFFERENTIAL RESPONSE OF METASTATIC VERSUS INDOLENT HUMAN MELANOMA TO ZD6126 TREATMENT

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It has been previously demonstrated that two melanoma cell lines obtained from a surgically excised tumor of the same patient have different phenotype: C6181cells form a metastatic tumor *in vivo* when xenografted in nude mice; microscopic necrosis or a necrotic center is common in this tumor; it even forms blood vessels through a unique mechanism called vasculogenic mimicry. On the other hand, the indolent line (A375P) is not metastatic. ZD6126, which targets the microtubular cytoskeleton of endothelial cells, is shown to induce vascular shutdown leading to tumor necrosis. We hypothesized that metastatic melanoma may have more immature vasculature, which can be blocked by ZD6126 treatment. We utilized dynamic contrast enhanced (DCE) magnetic resonance imaging (MRI) to study the acute response (within one hour after treatment). The pretreatment Ktrans values (first order rate constant for transfer of contrast agent from vasculature to tumor interstitium) of C8161 and A375P are 0.45 (±0.29, n=5) min-1 and 0.15 (±0.11, n=4) respectively. Immunostaining for CD31 shows that C8161 has higher vessel fraction than the A375P tumor (4.5% vs 2.2%, $P<0.05$). A significant reduction of Ktrans (0.16 min-1 compared to the pretreatment value of 0.45 min-1, $P<0.05$) in C tumors was observed within an hour after injection of ZD6126 (200 mg/kg), whereas Ktrans was reduced only slightly in A tumors ($P=0.1$) in response to ZD6126 treatment. Our results suggest that a radical difference in vasculature between the two melanomas could be the mechanism behind this differential response and DCE MRI could play a critical role in probing this difference.

Poster Presentations

Basic Science Poster Presentations

No. 119

EFFECT OF SINOGRAM FILTERING IN THE QUALITY OF POSITRON EMISSION TOMOGRAPHY RECONSTRUCTIONS

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Introduction and rationale: In the reconstruction of positron emission tomography (PET) studies, list mode data are usually aggregated into sinograms. This step is necessary for filtered backprojection algorithms and also for some statistical methods. Several effects, such as randomness of the positron emission, scatter, positron range and non-colinearity, degrade these sinograms. The subsequent reconstruction process propagates these errors to the final images. Since filtering in the angular direction introduces non-uniform tangential blurring, sinograms are generally filtered only in the radial direction for noise reduction. This filtering, however, also degrades resolution. Several methods have been proposed to face this problem, for instance filtering in the Wavelet or Stackgram domains. Fourier transform of a sinogram is known to show a particular shape of the spectral energy distribution. In this work, this property has been exploited to perform an adapted filtering, comparing the results with previously reported methods. Materials and methods: Data from phantoms and rodents obtained from a real PET system (rPET, SUINSA) have been used to compare different sinogram filtering techniques and to evaluate the enhancement achieved. Results and conclusions: A comparison of different methods for noise reduction in sinograms is presented. The proposed method for filtering in the Fourier domain provided the best results in terms of efficiency, noise reduction and simplicity. It achieved a SNR increase of up to 30% with no FWHM degradation. Furthermore, this correction improves the sinogram leading a visual enhancement similar to that of scatter correction methods.

No. 120

EFFECT OF MISALIGNMENTS IN SMALL ANIMAL POSITRON EMISSION TOMOGRAPHY SCANNERS BASED ON ROTATING PLANAR DETECTORS

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Introduction: Technological advances have improved the assembly process of positron emission tomography (PET) devices, resulting in quite exact geometric parameters. However, in high sensitivity and high spatial resolution systems, even minimum misalignments (submillimetric) of the

detectors may result in a noticeable degradation of the image resolution. For this reason, in such systems an exact characterization of misalignments is critical for a good reconstruction quality. While this subject is widely studied for computed tomography (CT) and single photon emission computed tomography (SPECT) systems based on cone beam geometry, it seems that this is not the case for PET scanners based on rotating planar detectors. The purpose of this work is to analyze misalignment effects in these systems and to define a protocol for geometric characterization. Materials and methods: The effects of misalignments have been simulated and the results have been validated with data from a real scanner (rPET, SUINSA), using both phantom and rodent studies. Results and conclusions: The effects of detector misalignments are presented. A testing protocol for detecting and measuring misalignments in the three axes in PET scanners based on rotating planar detectors is proposed. This protocol uses simple phantoms and is robust and easy to perform. Implementation details are given for the high-resolution animal rPET scanner. The results show the importance of detector alignment: for instance, a misalignment of 0.8 mm in one detector resulted in an increase of 14% in tangential FWHM of a point source in the center of field-of-view (FOV). The correction performed with the proposed protocol provided a significant improvement in resolution.

No. 121

ISSUES IN THE QUANTITATIVE RECONSTRUCTION OF POSITRON EMISSION TOMOGRAPHY STUDIES

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Introduction: In order to make quantitative analysis of positron emission tomography (PET) studies it is necessary to obtain an "exact" reconstructed image. This is not trivial to obtain as each step in the process from list mode data can be a source of bias or artifacts. Sinogram statistical distribution may be altered due to the acquisition process: scatter, decay, dead time, geometrical effects and crystal sensitivity. Subsequent sinogram rebinning may also change this statistical distribution. Finally, FBP may introduce DC component bias and aliasing, depending on the particular implementation used. This work analyzes the whole process to ensure that all these undesirable effects are properly compensated at every point of the reconstruction chain to guarantee a true quantitative reconstruction. Materials and methods: The study of quantitative reconstruction was applied to a real scanner (rPET, SUINSA). Different theoretical and experimental methods were tested for sinogram correction. Several methods for SSRB statistics recovery and for count recovery and aliasing elimination after FBP were tested. Results were validated on real data using a NEMA-like contrast phantom, considering attenuation and scatter. The linear behavior of detected trues versus activity in the field-of-view was verified. Results and Conclusions: A complete reconstruction algorithm for the rPET system is presented. An experimental correction of the sinogram based on an acquisition of a field flood provided best results. Counts recovering in the SSRB step and adequate slice uniformity have been achieved. Regarding FBP implementation, the Crawford method was selected for compensating DC bias and aliasing after filtering in Fourier domain.

No. 122

STATISTICAL PARAMETRIC MAPPING IN SMALL ANIMAL BRAIN ACTIVATION STUDIES

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Ultra-high resolution positron emission tomography (PET) has enabled the visualization of biochemical processes in the brain in small animals, and changes due to disease or stimuli. Conventional analysis methods using manually-drawn regions-of-interest (ROIs) to extract counts are highly subjective, and it is difficult to identify subregions of the brain. Pixel-based analysis, such as statistical parametric mapping, perform automated analysis of brain images to detect differences within or between groups. This work describes the application of pixel-based analysis to an 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) brain activation study in rats. A total of seven rats were scanned 45 minutes after injection of FDG. Each animal received two randomized scans, one with vibrissae stimulation for 15 minutes during the uptake phase of FDG, the other with no stimulation as a control. The brains were extracted from the PET images using a region-growing method, and coregistered to each other using a mutual information algorithm. Stimulated and unstimulated images were compared using pixel-by-pixel paired t-tests to determine statistically significant differences ($p<0.01$). Clusters of significant pixels were further assessed to compensate for correlated multiple comparisons (corrected $p<0.05$). Statistically significant regions were compared against conventional ROI analysis. Different methods of normalization, using either whole brain or cerebellum counts, were tested. Contralateral somatosensory cortices were activated by the vibrissae stimulation, with increased FDG uptake. Both pixel-based and ROI analyses agreed in the regions affected by the activation. Only cerebellar normalization gave the expected hypermetabolic regional differences, while whole brain normalization produced hypometabolism in regions that should be unaffected.

No. 123

SIMPLIFIED QUANTIFICATION OF SMALL ANIMAL 2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE- POSITRON EMISSION TOMOGRAPHY STUDIES USING A STANDARD ARTERIAL INPUT FUNCTION

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Arterial input function (AIF) measurement for quantification of small animal positron emission tomography (PET) studies is technically challenging and limited by the small blood volume of small laboratory animals. The present study investigates the use of a standard arterial input function (SAIF) to simplify the experimental procedure. Methods: Twelve 2-deoxy-2-[F-18]fluoro-D-glucose (FDG)-PET studies accompanied by serial arterial blood sampling were acquired in seven male Sprague-Dawley rats under isoflurane anesthesia without (every rat) and with additional (five rats) vibrissae stimulation. A leave-one-out procedure was employed to validate the use of a SAIF with individual scaling by one (1S) or two (2S) arterial blood samples. Results: Automatic slow bolus infusion of FDG resulted in highly similar AIF in all rats. The average difference of the area under curve of the measured AIF and the individually scaled SAIF was $0.11 \pm 4.26\%$ and $0.04 \pm 2.61\%$ for the 1S (six-minutes sample) and 2S (four-minutes/43-minutes samples) approach, respectively. The average difference between the cerebral metabolic rates of glucose (CMR_{glc}) calculated using the measured AIF and the scaled SAIF were $1.31 \pm 5.45\%$ and $1.30 \pm 3.84\%$ for the 1S and 2S approach, respectively. Conclusions: The use of a SAIF scaled by one or (preferably) two arterial blood samples can serve as a valid substitute for individual AIF measurements to quantify FDG-PET studies in rats. The SAIF approach minimizes the loss of blood and should be ideally suited for longitudinal quantitative small animal FDG-PET studies.

No. 124

BIOLUMINESCENCE IMAGING CONFIRMS THAT WEEKLY COMPUTED TOMOGRAPHY STUDIES DO NOT CHANGE TUMOR GROWTH IN AN ANIMAL MODEL OF BREAST CANCER METASTASIS

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The use of X-ray computed tomography (CT) to monitor tumor growth in animal models is becoming widespread. The purpose of this study was to determine whether X-ray exposure would change metastatic tumor development in an animal model. Methods. Female BALB/c nude mice

(n=28, 5 wks of age) were injected (intracardiac) with $2x10^5$ MDA-MB-435 cells, a metastatic human breast cancer line rendered firefly luciferase positive by lentiviral transduction. One group of mice (n=15) received a weekly CT scan (X-SPECT, Gamma Medica, Inc., 256 projections, X-ray tube=50 kV, 0.6 mA) for four weeks while a second group (n=13) received no CT scan. Bioluminescence imaging (BLI) was conducted weekly using the IVIS-100 system (Xenogen). A single photon emission computed tomography (SPECT)-CT study was acquired after i.v. administration of 3 mCi Technetium-99m-Methylene-diphosphonate (Tc-99m-MDP) at termination. Results. The X-ray exposure did not significantly alter the total tumor burden or number of metastatic sites; total body BLI signal increased by an average of 111.29-fold after initial injection to four weeks after injection; there was an average of 3 metastases/mouse. Region of interest analyses of SPECT-CT images showed Tc-99m-MDP uptake in the knee and temporomandibular joints was not changed by CT studies. Conclusions. Weekly CT imaging studies do not alter metastasis. Exposure to X-rays did not alter MDP uptake in normal joints. This is the first study to eliminate this source of error in experiments aimed at monitoring metastatic tumor development.

No. 125

ERRORS IN THE LOCALIZATION OF BIOLUMINESCENT SOURCES DUE TO INACCURATE KNOWLEDGE OF THE MOUSE TISSUE OPTICAL PROPERTIES - A COMPUTER SIMULATION STUDY

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A simultaneous optical- positron emission tomography (PET) imaging system is being developed to monitor the distribution kinetics of labeled tracers and cells in the mouse. We have recently shown by simulation that this system can perform tomographic imaging of bioluminescent sources if the anatomical locations of mouse tissues and their optical properties are known exactly. As the latter is not practically feasible there will always be discrepancies between the photon propagation model estimates of light fluxes reaching the detectors and the actual measurements, which could result in erroneous source localization in the reconstructed images. It will therefore be necessary to determine the minimum amount of mouse anatomical and optical property information needed to reconstruct bioluminescent sources accurately. We found that assumption of a spatially uniform tissue background of volume average optical properties was not sufficient for producing tomographic bioluminescence images. We are currently exploring the localization errors of bioluminescent sources arising from inaccurate optical properties assignments to tissue volumes defined by a segmented micro magnetic resonance imaging (MRI) mouse image. The effects of inaccurate knowledge of the blood volume, oxygen saturation levels and scattering coefficients of the gut, liver and other tissues are being examined. Source localization errors due to imperfect segmentation of the mobile gut and the presence of gas-filled bubbles within it are also being investigated. These studies are aimed at contributing to the design of a practical tomographic bioluminescence imaging protocol.

No. 126

DEVELOPMENT AND VALIDATION OF A SIMULATED HUMAN TORSO DYNAMIC FHBG- POSITRON EMISSION TOMOGRAPHY DATASET

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BACKGROUND: Positron emission tomography (PET) image analysis algorithm development depends on *in vivo* ground truth data for effective validation. Without this, one alternative approach is to test against simulated realistic data based on the physical and biological principles underlying PET image acquisition. A PET simulation protocol was recently proposed to generate realistic images using a digital brain phantom and data from published tracer kinetic studies. We extend this work to the full human torso using an annotated NURBS-based Cardiac-Torso (NCAT) phantom with organ activities labelled with clinically derived time-activity data of 9-(4-[F-18]-Fluoro-3-Hydroxymethylbutyl) Guanine (FHBG) uptake in patients lacking the reporter gene herpes simplex virus type 1 thymidine kinase (HSV1-tk). METHODS: PET SORTEO, a Monte Carlo PET simulator, was used to simulate image acquisition of the torso in seven scans by an ECAT EXACT HR+ scanner. Simulated emission scans were attenuation-corrected using transmission scans, the sinograms were combined and image reconstruction was performed using Ordered Subsets Expectation Maximisation with Gaussian smoothing. RESULTS: A simulated 4-D PET volume was generated and validated via rigid registration with real-life images at corresponding times. TACs were reconstructed using organ ROIs defined by registration to the original phantom, and showed the same shape but consistent underestimation of the original TACs due to noise and partial volume effects. CONCLUSIONS: Our results suggest that PET simulation offers a viable method of obtaining realistic PET data. We plan to extend this work to other tracer studies, and to mice using a MOBY phantom and a microPET simulator.

No. 127

SENSITIVITY ANALYSIS OF A THREE COMPARTMENT MODEL USED IN POSITRON EMISSION TOMOGRAPHY TRACER KINETIC MODELLING

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BACKGROUND: Quantitative positron emission tomography (PET) data analysis relies on fitting compartment models that describe tissue uptake of tracer to time-activity curves. The two-tissue three-compartment model is commonly used for internally metabolised tracer compounds. It requires four rate constants, which can vary by several orders of magnitude in the literature, and a blood input function in order to estimate intra-cell tracer concentration. We analyse the model and devise a validation criterion for the rate constants. METHOD: A numerical sensitivity analysis was performed on the solution to the model ODEs by calculating cell tracer concentration Ct(t) whilst varying a) pairs of rate constants across five orders of magnitude, and b) the blood input function shape and size. RESULTS: The rate constant K1 which determines transport of tracer into the cell was found to be critical in determining Ct(t), suggesting that K1 needs to be carefully measured. In contrast Ct(t) displayed little sensitivity to variation of the other parameters k2, k3 and k4. The topology of Ct(t) was invariant to changes in blood input function except when the signal exhibited high noise characteristics. We show that function sensitivity can be determined by analysis of the coefficients of the system impulse response function, and Ct(t) thresholds for which the parameters become sensitive and hence potentially unrealistic were defined using a generic physiological input function. CONCLUSIONS: A numerical test devised from sensitivity analysis of the two-tissue three-compartment model can be used to assess the validity of rate constants derived from curve fitting or de novo simulations.

No. 128

PETPARAMS: AN ONLINE DATABASE OF POSITRON EMISSION TOMOGRAPHY TRACER STUDIES

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BACKGROUND: Parameters derived from tracer kinetic modelling (TKM) studies can be used to calculate time-activity curves for use in positron emission tomography (PET) simulations, and also provide a useful point of comparison for new tracer studies, particularly in disease studies. However literature reviews of such studies are time-consuming and often non-exhaustive. A web-enabled database is established which provides easy access to a single comprehensive repository of publicly available TKM study summaries and parameters for members of the pharmacokinetic

research community. METHODS: The online database PETParams (www.petparams.com) was created using PHP/MySQL as a reference resource of PET tracer study data. The database is easily searchable and contains details of studies describing uptake of a range of tracers for healthy and disease tissue across several different species. It has been populated by a comprehensive literature review, and includes key results for each study where available, including compartment model descriptions, derived rate constants and blood/plasma input functions for tracer kinetic studies, and links to PubMed citations. Statistical reports can be generated from the database including variation in and validity of rate constants. An integrated messaging system will allow discussion of each study, and downloadable copies of the entire database will be available for local reference. The content will initially be kept up-to-date by the authors, and we hope to encourage original author involvement via provision of access to external research groups to create and maintain new entries.

No. 129

POSITRON EMISSION TOMOGRAPHY/ COMPUTED TOMOGRAPHY IMAGING OF ALPHA V BETA 3 INTEGRIN EXPRESSION IN MOUSE MODELS OF OSTEOLYTIC BONE DISEASE

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Bone metastases cause significant morbidity in many types of cancer. Current imaging methods for bone metastasis detection and monitoring require waiting several months after treatment before a response can be accurately assessed. An imaging agent targeting osteoclasts, bone degrading cells which are up-regulated in osteolytic lesions, may facilitate earlier follow-up in patients with osteolytic bone metastases. Osteoclasts express high levels of $\alpha_v\beta_3$ integrin, which binds peptides containing the Arg-Gly-Asp (RGD) sequence. Many tumor cells also express $\alpha_v\beta_3$. In order to validate an $\alpha_v\beta_3$ imaging agent for direct detection of osteoclasts, we examined two models of pharmacologically-induced osteolysis. Both TNF-α and parathyroid hormone (PTH) induce osteoclast-mediated osteolysis when either is serially injected subcutaneously at the calvarium (skullcap). The cross-bridged chelator, CB-TE2A, was conjugated to c(RGDyK) for radiolabeling with Cu-64 ($t_{1/2}$ = 12.7 h; β^+ (17.4%)). Uptake of Cu-64-CB-TE2A-c(RGDyK) by osteoclasts *ex vivo* was selectively blocked by increasing concentrations of c(RGDyK). There was no specific uptake of the tracer in β_3 negative macrophages. Biodistribution studies revealed that TNF-α treatment increased calvarial uptake of Cu-64-CB-TE2A-c(RGDyK) by 2.4-fold compared to sham-treated mice; however, TNF-α also induced elevated uptake in multiple normal tissues. Calvarial uptake of Cu-64-CB-TE2A-c(RGDyK)was increased 1.9-fold in PTH treated mice compared to vehicle-treated mice, and there was no concomitant increase in normal tissue uptake. In addition, calvarial uptake showed a linear correlation with a measure of the amount of osteoclasts on bone surface. These results suggest that Cu-64-CB-TE2A-c(RGDyK) selectively binds $\alpha_v\beta_3$ on osteoclasts and can be used to image osteolytic bone lesions.

No. 130

CELLULAR MAGNETIC RESONANCE IMAGING TO DIFFERENTIATE GLIOMA FROM RADIATION INJURY IN RAT BRAIN

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Angiogenesis in glioma are typically permeable to contrast agents, and can thus be detected by contrast-enhanced magnetic resonance imaging (MRI) or computed tomography (CT) . However, areas of radiation necrosis can also show enhancement due to active inflammatory reactions and increasing vascular permeability. Thus, distinguishing recurrent glioma from radiation necrosis becomes problematic if only changes in vascular permeability and/or volume are considered. One distinguishing characteristic, however, is that there is little active angiogenesis at the site of radiation necrosis. Moreover, there is no proof that radiation necrosis can initiate immunogenic reaction. The purpose of this study was to differentiate glioma from radiation necrosis by cellular MRI using magnetically labeled endothelial progenitors cells (EPCs) and splenocytes [from tumor bearing rats]. Ferumoxides and protamine sulfate were used to label EPCs and splenocytes. On day 7 after tumor implantation (9L) or radiation injury (75 Gy) labeled EPCs were administered intravenously and MRI was obtained on day 14. Labeled splenocytes were administered in rat on day 11 after tumor implantation and MRI was obtained on day 14. MRI showed low signal intensity areas at the periphery of the tumor in rats injected with labeled EPCs and splenocytes. However, no signal intensity change was observed in rats with radiation injury. Labeled splenocytes from control rat did not show low signal intensity area on MRI. Prussian blue staining showed iron positive cells at the periphery of the tumors but not in the area of radiation injury. Labeled EPCs and sensitized T-cells can be used to differentiate glioma from radiation necrosis by MRI.

No. 131

A FULLY 3-D RE-PROJECTION BASED RECONSTRUCTION ALGORITHM USING TIME-OF-FLIGHT POSITRON EMISSION TOMOGRAPHY FOR WHOLE BODY IMAGING

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Aim: The goal of this work is to implement an analytical re-projection based reconstruction algorithm for fully 3-D Time-Of-Flight (TOF) data, to be used (1) as a gold standard in the evaluation of different TOF systems, and (2) as an initial estimate for list-mode reconstruction. Method: The TOF projection data are mashed into a sparse set of discrete histoprojections along axial and polar angles, leading to significant reductions in the data size and the computation time. The projection data can be represented as a set of discrete points on Orlov's sphere, arranged as three rings with 18 points on each with an angular difference of 10 degrees. In the step-and-shoot acquisition the projection data/histoprojections are not complete for oblique axial angles represented by points on the two non-central rings of the sphere. Since analytical algorithms require complete projection data, the missing portions of the oblique histoprojections are obtained by re-projection, as in the non-TOF case. Results: Simulation studies have been performed to investigate the accuracy of the 3-D re-projection based reconstruction algorithm for TOF-PET. Our studies will also include results for the case of TOF list-mode iterative reconstruction starting from the image obtained by the proposed analytical reconstruction algorithm. This has the potential to dramatically speed-up convergence of the iterative reconstruction, especially for cold lesions, making it a promising tool for static and dynamic fully 3-D TOF-PET reconstruction.

No. 132

BIODISPOSITION AND METABOLISM OF [18F]FLUOROCHOLINE (FCH) IN CULTURED 9L GLIOMA CELLS AND SUBCUTANEOUS 9L TUMOR MODEL

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Fluorine-18 labeled choline analogs are currently under investigation as PET imaging tracers for cancer. The objective of this work was to study the biodisposition and metabolism of [F-18]fluorocholine (FCH) in cultured 9L glioma cells and a subcutaneous 9L glioma rat tumor model. The cultured 9L glioma cells were incubated for two hours in medium with FCH and [H-3]choline. Uptake and metabolism of tracers by cultured 9L glioma cells was compared. For subcutaneous 9L glioma rat tumor model, FCH and [C-14]choline were administered in isotonic saline by bolus injection through femoral vein. Blood was sampled from the carotid artery to compare the clearance of tracers from blood. At five and 20 minutes post-injection, the animals were euthanized, and uptake and metabolism in different organs were investigated by HPLC analysis. In this study, the uptake of both choline tracers was similar in cultured 9L glioma cells. Similar uptake was

also seen in different tissues in the rat model, including tumor. Both FCH and choline were similarly phosphorylated and oxidized in cultured 9L glioma cells. In rat, similar metabolism of tracers in tissues was also seen. Rapid blood clearance of both tracers was seen in rat with variable secretion in urine with corresponding increase of betaine forms. These results showed similar biodisposition and metabolism of FCH as compared to choline. From this study we conclude that FCH closely mimics choline in tracer studies of uptake and metabolism.

No. 133

FAST AND HIGH YIELD SYNTHESIS OF [F-18]-3'-DEOXY-3'-FLUOROTHYMIDINE USING RESIN IMMOBILIZED METHYL IMIDAZOLIUM SALTS AND AQUEOUS 18F FLUORIDE IONS

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A fast, reliable and high yield synthesis of [F-18]-3'-Deoxy-3'-Fluorothymidine ([F-18]-FLT) using novel resin immobilized methyl imidazolium (RIMI) salts, an easily synthesized nosylated precursor and aqueous $F\text{-}18^-$ ions has been developed. RIMI BF_4^- and $CF_3SO_3^-$ salts were prepared by attaching 1-methyl imidazoline to a brominated resin and exchanging the Br^- anion of the resin with the two anions shown above or by methylating a commercially available imdazoline resin to produce the imidazolium salts. These resins, suspended in acetonitrile, in the presence of Cs_2CO_3 and 5'-O-trityl-2'-deoxy-3'-nosyl-□-D-lyxofuranosylthymine were reacted with straight [18F]fluoride ions in water (up to 20% v/v). The mixture was heated at 100-120°C for 15-30 minutes to obtain the 5'-O-trityl-3'-deoxy-3'- F-18-thymidine which was deprotected with glacial acetic acid/water (4:1), to obtain 100% chemically and radiochemically pure [F-18]-FLT by HPLC in less than an hour. This method is suitable for automation utilizing a generic synthesis box in the cyclotron and is economical because the resin, after rinsing with acetonitrile, can be used again for several labelings.

No. 134

APPLICATION OF THE REFERENCE TISSUE METHOD TO A LONGITUDINAL POSITRON EMISSION TOMOGRAPHY **STUDY: A NON-INVASIVE APPROACH TO QUANTITATION**

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It is often impractical to explicitly determine the tracer input function through arterial blood sampling and metabolite analysis in laboratory positron emission tomography (PET) studies. One approach to quantifying distribution volume ratios (DVRs) that addresses this problem is the reference tissue method (RTM) proposed by Logan. In this study, we evaluated the applicability of the RTM to two radiotracers used at our institution: the dopamine D2 ligand [F-18]-4-amino-5-chloro-N-(1-((4-fluorophenyl) methyl)-4-piperidinyl)-2-methoxybenzamide (FCP) and the dopamine transporter ligand [F-18]-(1)-N-(4-fluorobenzyl)-2b-propanoyl-3b-(4-chlorophenyl) tropane (FCT). Using both the RTM and our standard graphical analysis method that requires plasma input function measurement, we analyzed PET data from FCT and FCP studies of adult male rhesus monkeys. The monkeys (n=12) were exposed over several years to various conditions of cocaine self-administration. The cerebellum, an area devoid of specific binding, was used as the reference region for both methods. For the RTM, a baseline estimate of k_2 for each tracer, $\boldsymbol{k_2}$, was derived from cerebellum k_2 values of cocaine naïve monkeys. DVRs were computed for regions throughout the brain. For the RTM, both tracers showed little dependence on the estimate of k_2. Varying k_2 as much as -50% to +75% from $\boldsymbol{k_2}$ resulted in average DVR differences of <10%. Comparing the Logan methods with and without blood sampling, using $\boldsymbol{k_2}$, average DVR differences of -1.66% ± 2.75% and 1.04% ± 3.26% were observed for FCT (n=98) and FCP (n=106) studies, respectively. We recommend the use of the RTM for DVR quantification of these ligands in PET studies of rhesus monkeys (DA 14637).

No. 135

INVESTIGATION OF BREAST TUMOR OXYGEN DYNAMICS BY NIR SPECTROSCOPY DURING RADIATION THERAPY

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The intra-tumoral oxygen dynamics of breast tumor bearing rats was studied dynamically during high dose gamma radiation therapy (30 Gy), using near infra-red spectroscopy. Tumor oxygenation was sampled in six rats bearing 13762NF mammary adenocarcinomas for 10 minutes prior to irradiation, during the irradiation period, and for 10 minutes following radiation. One cohort of rats inhaled oxygen during irradiation, and the second group of animals inhaled air. In neither group was there a discernable radiotherapy-induced alteration of intra-tumoral oxygenation status, either during the irradiation period or during the following 10-minute interval. In both groups of rats, a rapid change in vascular oxygenation was observed when the inhaled respiratory gas was switched from air to oxygen, however there was no detectable difference observed between the irradiated and untreated animals. This result implies similar physiology between the two groups, and a lack of acute vascular damage in small tumors. Previous studies that observed an acute response to irradiation were performed *ex vivo*, in normal, rat arterial endothelium. Since tumor vasculature is known to be highly aberrant and may possess incomplete or abnormal endothelium, the absence of an acute vascular response to radiation may be linked to the abnormal architecture of tumor vessels. In summary, here we demonstrate that NIRS is a novel tool for sampling the biological environment in extreme conditions that would render most other modalities useless. It's stability and high repeatability make it an ideal candidate for monitoring the tumor microenvironment over time, and in response to adjuvant intervention.

No. 136

ASSESSMENT OF APOPTOSIS WITH HUMAN AND SYNTHETIC ^{99M}TC-EC-ANNEXIN-V

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Purpose: Primary breast cancer (PBC) is often treated with induction chemotherapy (IC). The early occurrence of apoptosis is associated with higher chance to achieve pathological complete remission (pCR) that translates in improved prognosis. Therefore, early detection of these cellular events in order to predict the treatment-associated outcome is important. This study was aimed to use human and synthetic ^{99m}Tc-EC-annexin V to image tumor cells undergoing programmed cell death caused by IC. Methods: Both human and synthetic annexin was conjugated with ethylenedicysteine (EC). To quantitate cellular uptake changes during apoptotic process using ^{99m}Tc-EC-annexin V, a human lung tumor cell line (A549) were treated with either adeno virus (Adv-Bax) (at 4,000 particles/cell) and paclitaxel (12.5-150 ng/well) or a human breast tumor cell line. To non-transfected cells, transfected cells, pre-and post-paclitaxel or taxol treated cells ^{99m}Tc-EC-annexin (2 μCi/well, 1.5μg/well) were added and incubated for 0.5, Two and four hours. The findings indicated that ^{99m}Tc-EC-annexin V is useful to measure apoptotic process before and after virus transfection and chemo agent treatment. Results: The data indicated that an increased uptake (Ca. two times) of human ^{99m}Tc-EC-annexin was observed after adding virus to the cells. Similar findings were observed with synthetic ^{99m}Tc-EC-annexin-V in cells treated with paclitaxel or taxol. No detectable of human or synthetic annexin-V antibody formation was observed. Conclusions: The results indicate that apoptosis can be quantified using human or synthetic ^{99m}Tc-EC-annexin-V and that it is feasible to use both ^{99m}Tc-EC-annexin-V to image tumor apoptosis.

No. 137

LOCAL MOTION COMPENSATION FOR LIST-MODE POSITRON EMISSION TOMOGRAPHY

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Motion artifacts from respiratory and patient motion degrade image quality and hamper accurate quantification in many clinical applications of positron emission tomography (PET). In this work we have developed and tested a new motion compensation algorithm based on PET list-mode data. The algorithm calculates motion estimates for a user-specified volume of interest (VOI) with high temporal resolution. The corresponding lines of response (LORs) are shifted according to the estimates and a motion-free image of the VOI is reconstructed from the corrected list-mode data. The algorithm does not require any prior information or application-specific parameter settings. It is fast, robust and reliably corrects rigid motion of any nature. The method has been tested with several simulated datasets from geometrical and anatomical (NCAT) digital phantoms. The images of the motion-corrected data were compared with images from data, where the motion has been turned off in the simulation. The comparison shows no visible differences as long as the object in the VOI is free of elastic deformation. This is the case for e.g. the brain, small to medium sized tumors, etc. The NCAT phantom was used to study the performance of the algorithm in improving quantification of lung lesions. The standardized uptake value analysis of the motion-corrected image is in perfect conformance with the theoretically expected values.

No. 138

F-18-LABELED BOMBESIN ANALOGS FOR TARGETING GRP RECEPTOR-EXPRESSING PROSTATE CANCER

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Objective: The gastrin-releasing peptide receptor (GRPR) is over-expressed in a variety of human tumors. The aim of this study was to develop F-18-labeled bombesin analogs for positron emission tomography (PET) imaging of GRPR expression in prostate cancer xenograft models. Methods: [Lys^3]bombesin ([Lys^3]BBN) and aminocaproic acid-bombesin(7-14) (Aca-BBN(7-14)) were labeled with F-18by coupling the Lys^3 amino group and Aca amino group respectively with *N*-succinimidyl-4-[F-18] fluorobenzoate ([F-18]SFB). Tumor targeting efficacy and *in vivo* kinetics of both radiotracers were examined in male athymic nude mice bearing subcutaneous human prostate cancer PC-3 tumors by means of biodistribution and dynamic microPET imaging studies. [F-18]FB-[Lys^3]BBN was also tested for orthotopic PC-3 tumor delineation. Results: The typical decay-corrected radiochemical yield was about 30-40 % for both tracers with a total reaction time of 150 ± 20 minutes starting from [F-18]F^-. Both radiotracers exhibited rapid blood clearance. [F-18]FB-[Lys^3]BBN had predominant renal excretion. [F-18]FB-Aca-BBN(7-14) exhibited both hepatobiliary and renal clearance. Dynamic microPET imaging studies revealed that the PC-3 tumor uptake of [F-18]FB-[Lys^3]BBN in PC-3 tumor was much higher than that of [F-18]FB-Aca-BBN(7-14) at all time points examined ($P < 0.01$). The receptor specificity of [F-18]FB-[Lys^3]BBN *in vivo* was demonstrated by effective blocking of tumor uptake in the presence of [Tyr^4]BBN. No obvious blockade was found in PC-3 tumor when [F-18]FB-Aca-BBN(7-14) were used as radiotracer under the same condition. MicroPET and microCT fusion demonstrated that [F-18]FB-[Lys^3]BBN were also able to demarcate orthotopic prostate tumor. Conclusion: This study suggests that [F-18]FB-[Lys^3]BBN and PET imaging is suitable for detecting GRPR-positive prostate cancer *in vivo*.

No. 139

RGD PEPTIDE-LABELED NIR QUANTUM DOTS FOR IMAGING TUMOR VASCULATURE IN LIVING OBJECTS

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Objective: Near-infrared (NIR) quantum dots (QDs) are suitable for *in vivo* optical imaging and multiplexing. Integrin $\alpha_v\beta_3$ is significantly upregulated on invasive tumor cells and tumor vasculature. In this study we developed a peptide-labeled QD for *in vivo* optical imaging of tumor vasculature. Methods: We have labeled QD705 (λ_{em}= 705 nm) with c(RGDyK) (potent integrin $\alpha_v\beta_3$ antagonist) and the resulting QD705-RGD conjugate was tested for *in vitro* cell staining, *ex vivo* tissue staining, and *in vivo* tumor vasculature targeting. Results: After successful demonstration of the integrin $\alpha_v\beta_3$ specific targeting of QD705-RGD through both *in vitro* and *ex vivo* experiments, the probe QD705-RGD was tested *in vivo*. Athymic nude mice bearing U87MG glioblastoma tumor were administered QD705 or QD705-RGD *via* tail vein injection and the mice were imaged using a spectral imaging system. The tumor fluorescence intensity in mice injected with QD705-RGD reached maximum at six hours post-injection with good tumor-to-background contrast while no significant tumor signal was observed in mice injected with QD705. Both *ex vivo* tumor imaging and microscopic images of cryosectioned tumor slices confirmed the presence of QD in the tumor tissue of QD705-RGD but not in QD705 injected mice. Conclusion: For the first time we demonstrated that RGD peptide-labeled quantum dot QD705-RGD can specifically target integrin $\alpha_v\beta_3$ both *in vitro, ex vivo* and *in vivo*. The results reported here opens up new perspectives for integrin-targeted NIR optical imaging and will have great potential in cancer diagnosis and imaging as well as imaging-guided surgery and therapy.

No. 140

COMPUTED TOMOGRAPHY-DEFINED OBJECT BOUNDARY FOR RECONSTRUCTING MULTIPLE-PINHOLE SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY IMAGES

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Multiple-pinhole collimators have been used for either conventional rotating flat detectors or novel stationary ring detectors in single photon emission computed tomography (SPECT) to improve the sensitivity without sacrificing the resolution. However, images reconstructed from overlapped multiple-pinhole projections may have artifacts due to the loss of boundary information of the object. This simulation study demonstrates the feasibility to use the object boundary defined from co-registered computed tomography (CT) images to improve the reconstruction in the ring-detector-based multiple-pinhole SPECT. A small animal positron emission tomography (PET) scanner (MOSAIC, Philips) was converted for SPECT imaging using a collimator insert (ring radius=30mm) that had 42 helically spaced pinholes (single-knife edge, diameter=1mm, acceptance angle=90 degree) that allowed projections to overlap. A cold-rod phantom and a mouse phantom were simulated to obtain overlapped SPECT data. Images were reconstructed using a 3-D iterative maximum-likelihood expectation-maximization (MLEM) algorithm, which could be implemented either in a boundary-defined local volume (BD-MLEM) or in a non-boundary-defined global volume (NBD-MLEM). The BD-MLEM algorithm reconstructed faithful images from the severely overlapped data of both cold-rod and mouse phantoms, whereas NBD-MLEM failed. For better co-registration of CT and SPECT images, fiducial markers can be placed on the surface of the object. The fiducial markers were reconstructed accurately using NBD-MLEM. In summary, the BD-MLEM algorithm enables to use many pinholes and/or large opening angles to achieve a high sensitivity (~0.4% in this simulation study) and relatively large field-of-view (transaxial ~3cm and axial ~6cm) without substantial loss of resolution.

No. 141

NOVEL CONFIGURATIONS OF MULTIPLE PINHOLES FOR A STATIONARY RING-DETECTOR-BASED PINHOLE SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY

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Single photon emission computed tomography (SPECT) based on a full ring detector and a ring pinhole collimator is able to achieve ultra-high resolution and high sensitivity for a small field of view. The annular sampling enables stationary acquisition without rotating the detector or animal, therefore reduces acquisition time and makes dynamic studies possible. A prototype implementation of this system was recently developed on the ring detector of a small animal positron emission tomography (PET) (MOSAIC, Philips). For further improvement of the detector efficiency and angular sampling, as well as being specific for applications, various novel configurations of multiple pinholes are being studied using simulation methods. Key parameters in the configuration include the ring diameter of the collimator, the diameter and acceptance angle of pinholes, and the number and layout of pinholes. High numbers of pinholes (up to 50) are tested in novel configurations that allow the projections to overlap each other on the detector. The distances among adjacent pinholes are carefully determined to avoid any repeated overlapping pattern. A dedicated MLEM reconstruction algorithm was developed and incorporated the collimator orientation, which can be obtained from a single point source calibration before each study. As an example, a configuration with helically spaced 42 pinholes (single-knife edge, diameter=0.5mm, acceptance angle=60 degree) was designed for the mouse brain study, providing a sufficient field of view (axial=20mm, transaxial=20mm), a spatial resolution of 0.85mm and a geometric sensitivity of 0.3%. Simulation results showed faithful reconstruction with minimal artifact caused by overlapped projections. Further optimization and implementation are being developed.

No. 142

SMALL ANIMAL DUAL MODALITY IMAGING USING A MULTI-PINHOLE SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY COLLIMATOR INSERT ON A POSITRON EMISSION TOMOGRAPHY CAMERA

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To effectively study small animal models of disease *in vivo* a wide range of positron emission tomography (PET) and single photon emission computed tomography (SPECT) imaging agents need to be employed. In an effort to achieve high resolution and high sensitivity small animal SPECT imaging we propose the notion of using a multiple pinhole insert in a PET scanner. Adapting PET scanners to enable SPECT imaging is an attractive solution for many reasons. Most PET scanners are full ring detectors which offer the potential for pinhole configurations that provide full angular sampling of an object without the need for complex detector rotation. Dual modality studies can be performed without moving the subject providing perfect spatial alignment. Furthermore, enabling SPECT imaging in a PET scanner is economical as it reduces the initial investment and maintenance costs. In this study we demonstrate the feasibility of using a multi-pinhole collimator to enable SPECT imaging in the Mosaic (Philips), a dedicated small animal PET scanner. Detector performance measurements show that low energy gammas can be efficiently detected and adequately positioned by raising the photomultiplier tube (PMT) high voltage to compensate for the lower light output. A multi-pinhole mouse collimator was developed, for the specific geometry of the Mosaic, that offers high spatial resolution (1.7mm) and high sensitivity (0.25%). To demonstrate the dual modality imaging capabilities *in vivo* mouse studies were performed to study cardiac function using both 99mTc-MIBI SPECT and 2-deoxy-2-[F-18]fluoro-D-glucose-PET.

No. 143

***IN VIVO* QUANTITATION OF INTRATUMORAL RADIOISOTOPE UPTAKE USING MICRO- SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY / COMPUTED TOMOGRAPHY AND THE SODIUM IODIDE SYMPORTER (NIS) GENE**

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Purpose: To determine the ability of micro- single photon emission computed tomography (SPECT)/ computed tomography (CT) to accurately and reliably quantitate intratumoral radioisotope uptake *in vivo*, and to compare these measurements with planar imaging or micro-SPECT imaging alone. Methods: Human pancreatic cancer flank xenografts were established in ten mice. Following injection of a NIS expressing attenuated measles virus vector and I-123, planar and micro-SPECT/CT images were obtained. Tumor activity was determined by dose calibrator measurements and region of interest image analysis. Imaging of an I-123 standard was performed to determine the conversion of accumulated counts from ROI analysis into activity. Micro-SPECT/CT recovery and attenuation coefficients were also determined. Agreement and reproducibility of tumor activity measurements were assessed using Bland and Altman plots, Lin's Concordance Correlation Coefficient (CCC), and paired t-tests. Results: Intratumoral NIS expression and radioisotope uptake was detected in all mice on planar and micro-SPECT/CT images. Scatter plots demonstrate strong agreement (CCC=0.93) between micro-SPECT/CT and dose calibrator tumor activity measurements. Application of recovery and attenuation coefficient correction factors slightly improved agreement of the micro-SPECT/CT measurements with dose calibrator measurements (CCC=0.94). These plots and the limits of agreement show that the difference between dose calibrator activity measurements and those obtained with micro-SPECT only and planar imaging are more variable (CCC=0.84 and 0.78, respectively). In some cases, they are two times greater than the true measured tumor activity. Conclusion: Micro-SPECT/CT can be used to accurately and reliably quantify and monitor intratumoral NIS gene expression and radioisotope uptake *in vivo*, and is superior to quantitation using planar or micro-SPECT imaging alone.

No. 144

TRANSMISSION IMAGING ON A MICROPET SCANNER USING A CO-57 SOURCE

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The standard acquisition using a Co-57 source for transmission measurements on Micro positron emission tomography (PET) systems employs a 120-125 keV energy window. Given that the energy resolution of LSO at these energies is ~30%, much of the transmitted signal will be excluded based on energy discrimination, thereby reducing the counting statistics. As the resulting image is routinely segmented for attenuation correction, this approach is nevertheless sufficient. However, a further application of the Co-57 source is to take advantage of the improved contrast achievable at the Co-57 energies to obtain useful anatomical images, perhaps employing CT contrast agents which would not be possible at 511 keV. We use a wider 95-125 keV window to obtain an approximate four-fold increase in the count rate for the case of a 5 cm diameter Utah phantom with a PVC type I plastic insert and air cavity. Since the scatter at these energies is fairly isotropic a simple subtraction based on a fit to the observed scatter tail is sufficient. Improved contrast is observed between the plastic insert and water with the image variance reduced by a factor of ~2. The generation of accurate photon linear attenuation images at the acquired Co-57 energies may then be used for: (1) more accurate image-based scaling to 511 keV for attenuation correction of the PET emission data, (2) identification of anatomical structure, and (3) improved estimates of areas of contrast-enhanced blood pool for input function estimates.

No. 145

A PSAPD-BASED SYSTEM FOR SIMULTANEOUS MULTI-SLICE POSITRON EMISSION TOMOGRAPHY AND MAGNETIC RESONANCE IMAGING

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Positron emission tomography (PET) and magnetic resonance imaging (MRI) are two widely utilized imaging modalities that are largely complementary in the information they provide. The ability to simultaneously image with PET and MRI may benefit a range of existing molecular imaging studies that utilize both anatomic and functional imaging, as well as open up new opportunities for time-correlated PET and MRI studies *in vivo*. We report on a multi-slice PET scanner insert for a preclinical small animal 7T MRI system using magnetic field-insensitive position sensitive avalanche photodiode (PSAPD) detectors coupled, via short lengths of optical fibers, to arrays of scintillator crystals. The fibers are used to minimize interference between the radiofrequency and gradient coils, and the PET detector system. The PET system consists of sixteen modules with eighty electronic channels processed using standard NIM electronics. A multiplexer board reduces the number of channels used to obtain the position information from 64 to 8. Each PET module consists of an array of 8x8 LSO crystals each measuring 1.43x1.43x6 mm^3, coupled through a 6x6 array of 2x2 mm^2 double-clad optical fibers to a 14x14 mm^2 PSAPD. The five outputs of the PSAPDs were read out using commercially available charge-sensitive preamplifiers mounted on custom-built printed circuit boards populated with non-magnetic components. Results demonstrate that the LSO crystals can be clearly identified, and that the PSAPDs operate within a 7T magnet. MRI phantom studies show no significant deterioration in the MR image quality using spin echo sequences. We expect to present first *in vivo* studies at the conference.

No. 146

EMPLOYING □-GALACTOSIDASE AS A REPORTER FOR OPTICAL IMAGING OF STABILIZED HIF LEVELS IN TUMOURS

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Tumor hypoxia is associated with tumor aggressiveness, metastasis, angiogenesis, and resistance to therapy. Several groups have recently published the use of reporter genes as a tool for imaging tumor hypoxia non-invasively focusing their efforts upon the one key protein driving the hypoxic response, hypoxia-inducible factor (HIF). This protein is stabilized under low oxygen conditions and translocated to the nucleus where it binds to specific DNA binding domains called hypoxia-response elements (HRE). □-galactosidase is a reporter protein whose expression and activity is not dependent upon oxygen, unlike other common genetic reporters such as luciferase and green fluorescent protein (GFP). This property suggests that lacZ may be useful in the development of pre-clinical models to illuminate tumor hypoxia. Traditionally the reporter gene lacZ has been detected histologically. However, recently 7-hydroxy-9*H*-(1,3-dichloro-9,9-dimethylacridin-2-one) galactoside (DDAOG) has been shown to shift its fluorescent excitation and emission spectra after cleavage by lacZ. To test the feasibility of using lacZ as a reporter gene in hypoxic conditions, a 5XHRE-lacZ construct was used to transiently transfect human pancreatic tumor SU86 cells. These cells were cultured overnight in a 2% oxygen sealed chamber then incubated with DDAOG for two hours before fluorescence was analyzed on a Xenogen IVIS200 optical imaging system. Indeed, the cleaved fluorescent product DDAO with a far-red wavelength shift is apparent when 5XHRE-lacZ SU86 cells are incubated in low oxygen environments. Our results indicate that it is feasible to develop preclinical tumor models of tumor hypoxia using □-galactosidase as the reporter protein.

No. 147

POSITRON EMISSION TOMOGRAPHY IMAGING OF VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR EXPRESSION

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Objective: This study aimed to develop suitably radiolabeled vascular endothelial growth factor (VEGF) for positron emission tomography (PET) imaging of tumor angiogenesis and VEGF receptor 2 (Flk-1/KDR) expression. Methods: A Cys-tagged VEGF (C-scVEGF$_{121}$) was conjugated with macrocyclic chelator DOTA through the lysine side chain. DOTA-VEGF was labeled either with I-124 through direct iodination or with Cu-64 through DOTA complexation. Results: No appreciable effect on the receptor binding affinity and functional activity of DOTA-VEGF was observed as compared to C-scVEGF121. Two subcutaneous tumor models, SVR murine angiosarcoma and human breast cancer MDA-MB-435, in athymic nude mice were tested. Non-invasive microPET imaging revealed the rapid, specific, prominent uptake of the tracers in highly vascularized SVR tumor but significantly lower and sporadic uptake in the MDA-MB-435 tumor. *Ex vivo* tissue staining corroborated with the *in vivo* imaging result as the vessel density and VEGFR-2 expression was much higher in SVR over the MDA-MB-435 tissue slices. Substantial uptake in the kidneys was also observed due to both high VEGFR-1 expression in this organ and the renal clearance route of the tracer. Conclusion: This study demonstrated that radiolabeled C-scVEGF$_{121}$ with minimum disturbance on the receptor affinity, specificity and functional activity of the resulting tumor marker has great potential in clinical applications. Modification of this fusion protein at the Cys tag with even less disturbance on receptor affinity and functional activity is currently in progress.

No. 148

QUANTITATIVE POSITRON EMISSION TOMOGRAPHY **IMAGING OF TUMOR INTEGRIN □$_v$□$_3$ EXPRESSION WITH [F-18]FRGD2**

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Objective: The development of non-invasive methods to visualize and quantify integrin □$_v$□$_3$ expression *in vivo* is crucial for the success of anti-angiogenic therapy based upon integrin antagonism as well as the development of new drugs with favorable tumor targeting efficacy and *in vivo* kinetics. In this study, we labeled the dimeric RGD peptide E[c(RGDyK)]$_2$ with F-18 and evaluated its tumor targeting efficacy and pharmacokinetics of [F-18]FB-E[c(RGDyK)]$_2$ ([F-18]FRGD2). Methods: E[c(RGDyK)]$_2$ was labeled with F-18using N-succinimidyl-4-[F-18]fluorobenzoate ([F-18]SFB). The *in vivo* metabolic stability of [F-18]FRGD2 was determined. The diagnostic value after injection of [F-18]FRGD2 was evaluated in various xenograft models by dynamic micro positron emission tomography (PET) imaging followed by *ex vivo* quantification of tumor integrin level. Results: The total reaction time for [F-18]FRGD2 including final HPLC purification is about 200 ± 20 minutes. Typical decay-corrected radiochemical yield is 23 ± 2 % (n = 20). [F-18]FRGD2 is metabolically stable. The binding potential (BP) extrapolated from graphical analysis of PET data and Logan plot correlates well with the receptor density measured by SDS-PAGE and autoradiography in various xenograft models. Tumor/background ratio at one hour post-injection of [F-18]FRGD2 also gives a good linear relationship with tumor tissue integrin level. Conclusion: The dimeric RGD peptide tracer [F-18]FRGD2 with high integrin specificity and favorable excretion profile may be translated into clinic for imaging integrin □$_v$□$_3$ expression. The binding potential calculated from simplified tracer kinetic modeling such as Logan plot can be an excellent indicator of tumor integrin density.

No. 149

A THIOL-REACTIVE F-18-LABELING AGENT *N*-[2-(AMINOETHYL)MALEIMIDE]-4-[F-18]FLUOROBENZAMIDE ([F-18]AMFB) AND THE SYNTHESIS OF RGD PEPTIDE-BASED TRACER FOR POSITRON EMISSION TOMOGRAPHY IMAGING OF $\alpha_v\beta_3$ INTEGRIN EXPRESSION

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Objective: The objective of the present work was to develop a novel thiol-reactive F-18 labeling reagent for the prosthetic labeling of peptides and proteins via selective conjugation with a sulfhydryl group. Methods: *N*-[2-(aminoethyl)maleimide]-4-[F-18]fluorobenzamide ([F-18]AMFB, total reaction time 150 ± 20 minutes with non-decay-corrected radiochemical yield of 5 ± 2 %, specific activity 150~200 TBq/mmol) was thus incorporated with thiolated monomeric and dimeric Arginine-Glycine-Aspartic acid (RGD) peptides (c(RGDyK and E[c(RGDyK)]$_2$) via efficient alkylation of the free thiol group. Results: The advantage of labeling the sulfhydryl group using [F-18]AFMB over labeling the primary amino group with [F-18]SFB was confirmed. Conjugation of monomeric and dimeric sulfhydryl-RGD peptides with [F-18]AMFB was achieved in high yields (85 ± 5 % non-decay-corrected based on [F-18]AMFB). Non-invasive microPET imaging and direct tissue sampling experiments demonstrated that both tracers had integrin specific tumor uptake in subcutaneous U87MG glioma and orthotopic MDA-MB-435 breast cancer xenografts. For the monomeric tracer, U87MG and MDA-MB-435 tumor uptake were 1.27 ± 0.50 and 1.04 ± 0.28 at 60 minutes post-injection while for the dimeric tracer, the uptakes in the U87MG and MDA-MB-435 tumor were significantly higher at 2.14 ± 0.33, 2.11 ± 0.48, respectively. Conclusion: The relatively good metabolic stability and favorable pharmacokinetics of the dimeric RGD peptide-based tracer warrant further investigation in both preclinical and clinical settings for documenting tumor integrin expression. [F-18]AFMB also provides a general method of labeling thiol-containing peptides, proteins, antibodies, as well as 5'-thio-functionalized oligonucleotides in high radiochemical yield and high specific activity for successful positron emission tomography applications.

No. 150

CY5.5-MEDI-222 FOR *IN VIVO* OPTICAL IMAGING OF TUMOR XENOGRAFTS

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Objective: Here we developed fluorescent dye Cy5.5-labeled MEDI-222 for optical imaging of tumor integrin $\alpha_v\beta_3$ expression. The effect of different Cy5.5/MEDI-222 ratios was investigated to optimize the tracer performance. Methods: In this study we conjugated Cy5.5 to MEDI-222, a humanized monoclonal antibody against human integrin $\alpha_v\beta_3$, at three different reaction ratios (3:1, 10:1, and 50:1) and the resulting Cy5.5-MEDI-222 conjugates were tested for *in vivo* optical imaging of MDA-MB-435 breast cancer xenografts. Results: From the three Cy5.5/MEDI-222 reaction ratios used for conjugation, different numbers of Cy5.5 per MEDI-222 were obtained (0.83, 1.65, and 2.50 respectively). Near-infrared fluorescence imaging revealed high and specific tumor uptake for the Cy5.5-MEDI-222 conjugate with less than 1 dye/mAb. Significant quenching was observed if more than one dye molecules were attached to the antibody. The conjugate with an average of 0.83 Cy5.5 per MEDI-222 (3:1 ratio for the conjugation reaction) gave the best optical imaging result. The tumor-to-background ratios were 4.48, 6.51 and 8.72 at 50, 100 and 175 hour post injection respectively. Conclusions: The successful tumor targeting of this probe may aid in the preclinical investigation of MEDI-222 and/or MEDI-222-based therapeutics. This study indicates that extra attention needs to be paid when developing antibody-based optical imaging probes, as high dye to antibody ratio would lead to quenching because of close vicinity of the dye molecules, while very low dye to antibody ratio is also sub-optimal because of low specific activity of the probe for imaging applications.

No. 151

MOLECULAR IMAGING PERFORMANCE AND APPLICATIONS OF THE BIC SMALL ANIMAL SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY / COMPUTED TOMOGRAPHY UNIT

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Studying pre-clinical models of human diseases with combined physiological and anatomical imaging holds great potential in drug discovery. Our dual-head single photon emission computed tomography (SPECT) unit is composed of NaI crystals coupled to a 3 x 3 square array of PSPMT. The computed tomography (CT) unit consists of a micro-focus X-ray source and a CCD camera with 32 micron pixels. Small animal SPECT spatial resolution and quantification properties were assess with calibrated phantoms. A micro-volume hollow sphere and a micro-ECT phantom were employed. Metastatic disease mouse models were injected with In-111-DOTA-8-AOC-BBN(7-14)NH_2 and Tc-99m agents to assess extent of SPECT lesion detectability. Micro-sphere internal phantom diameters ranged from 3.95 to 7.86 mm with volumes ranging from 31 to 250 μL, respectively. Tc-99m activity levels ranging from 124 to 968 μCi were placed inside the micro-spheres. The micro-ECT phantom was filled with 2.1 mCi of Tc-99m. The phantoms were scanned for 360-degree, 60 projection views at 45 cm radius of rotation for 30 minutes. Projection data was reconstructed with an OSEM algorithm. Tomographic spatial resolution of 1.2 mm was achieved with the ECT phantom. Quantification phantom results suggest that below 1 mCi proportionality exists between registered counts and activity concentration in the field of view. MicroSPECT/CT was successful in visualizing In-111-DOTA-8-AOC-BBN(7-14)NH_2 and HDP uptake in tumor models. MicroSPECT was successful is visualizing receptor mediated uptake of In-111-DOTA-8-AOC-BBN(7-14)NH_2 in prostate bone metastases of PC-3 origin. Tibial lesions as small as 1.5 mm were detected with microSPECT. MicroCT co-registration confirmed the location and lesion extent of the metastatic model analyzed.

No. 152

REPRODUCIBILITY OF 2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE-MICROPET STUDIES IN XENOGRAFTED MICE

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Objective: 2-Deoxy-2-[F-18]fluoro-D-glucose (FDG) has been used to image mouse xenograft models with micro positron emission tomography (PET) for therapy response. However, the reproducibility of serial scans has not been estimated. The purpose of this study was to determine the reproducibility of FDG microPET studies. Methods: Mouse tumor xenografts were formed with murine melanoma cells, B16F10 (N=5) and human pancreatic carcinoma cells, MiaPaCa (N=3). A 10 minute microPET R4 (Concorde) scan was performed one hour after a 100 μCi FDG injection via the tail vein. A second microPET scan was performed six hours later after re-injection of FDG. Twenty-four sets of studies were performed. Mean injected dose per gram (%ID/g) values were calculated from tumor regions of interest. The coefficients of variation (COV) and differences in the mean %ID/g from studies performed on the same day were calculated to determine the reproducibility. Activity from the second scans performed after six hours were adjusted by subtracting the estimated residual activity from the first FDG injection. Results: The COV for the mean %ID/g between FDG microPET scans performed on the same day 6 hours apart was 14.5±11.0%. The difference between mean %ID/g of the two scans was 0.2±1.3%. The tumor size, body temperature, and body weight did not appear to contribute to the variability of the scans. Conclusions: FDG microPET xenograft mice studies were reproducible with moderately low variability. This level of variability is sufficient for reasonable assessments of serial changes. These results can be applied to follow tumor therapy response or for pre-clinical drug evaluation.

No. 153

SELECTIVE SPECTRAL IMAGING OF DUAL FLUORESCENT/BIOLUMINESCENT REPORTER GENES *IN VITRO* AND *IN VIVO*

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Preclinical tumor studies require repetitive non-invasive assessment of tumor development *in vivo*. Optical imaging represents a powerful tool for longitudinal visualization of tumors in living subjects. We engineered and evaluated several dual reporter gene constructs for *in vitro* and *in vivo* studies. GFP or RFP genes linked to click beetle green/red (CBG/CBR), firefly (FLuc) or Renilla (RLuc) luciferases fusion reporter genes were produced in U87 glioma cells and *in vitro* fluorescent and bioluminescent characteristics were assessed. GFP/CBG, GFP/CBR and GFP/FLuc had maximal fluorescent signal at 510 nm, RFP/RLuc - at 580 nm. *In vitro* bioluminescence was measured using IVIS (Xenogen) (table). Cells bearing reporter construct were implanted subcutaneously in mice. GFP/CBG tumors had peak signal at 515-650 nm, GFP/CBR cells - at 575-875 nm, GFP/FLuc - at 515-875 nm and could be distinguished from RFP/RLuc based on lusiferase product substrate. Interestingly, substantial shift of peak detection toward red spectrum was detected for FLuc and CBG luciferases *in vivo*. Providing high sensitivity and quantitation, dual reporter genes allow for imaging of different cell populations in the same animal using different spectral channels and substrates. The shift of light spectrum of some luciferases should be taken into consideration for studies *in vivo*.

Bioluminescence *in vitro* (photons/sec/cm2/sr x109)

emission, nm	**wt**	**GFP/Fluc**	**GFP/CBG**	**GFP/CBR**	**RFP/Fluc**
515-575	0.07	5.74	3.64	0.54	2.12
575-650	0.11	10.11	1.76	8.94	4.68
695-770	0.02	0.63	0.04	1.13	0.31
810-875	0.02	0.04	0.02	0.05	0.03

No. 154

RESOLUTION IMPROVEMENT OF SMALL ANIMAL POSITRON EMISSION TOMOGRAPHY IMAGES USING A STEP AND SHOOT ROTATING SCANNER

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High cost of positron emission tomography (PET) scanners led to designs with reduced number of detectors, at the expense of sensitivity loss. Complete angular sampling is achieved rotating the detectors. The current generalization of iterative statistical methods of reconstruction, together with the fact that iterative methods are more tolerant to incomplete angular sampling allows us to explore different rotation schemes (i.e. continuous vs. step and shoot) of the detectors to obtain the best image resolution within the minimum reconstruction time. PET data are often arranged in sinograms, subsequently employed for analytical reconstruction methods, or in LOR-histograms, where the number of counts in every line of response is considered. These latter arrangement of data is better suited for iterative reconstructions, because the physical characteristics of the scanner are related to the nature and placement of the detectors that define every LOR, rather than by their corresponding position inside the sinogram. In general, the best way to reconstruct using iterative methods is LOR histograming, which allows for optimal evaluation of the response matrix of the system. Using Monte Carlo methods, we obtained simulated PET rotating scanner data which were reconstructed by 3-D-OSEM, and compare the resolution achieved and reconstruction time when employing sinograms, LOR histograms and LIST mode acquisitions. Different rotation strategies, such step-and-shoot with different overlaps or continuous mode rotation were compared. Our results show that resolution can be improves by up to 30 % just by modifying the configuration of the rotation motion and the histogramming method.

No. 155

QUANTIFICATION SOFTWARE FOR QUANTITATIVE MOLECULAR IMAGING

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We have previously developed COmpartment Model Kinetic Analysis Tool (COMKAT), a toolkit for analyzing pharmacokinetic data. We have extended COMKAT in several ways to create a more powerful tool for analyzing dynamic positron emission tomography (PET) images. First, we created a compiled MEX file model solver and linked it against CVODES, a robust differential equation solver written in the C language. This has significantly enhanced the speed of parameter estimation and output simulation. Second, we implemented support for customized kinetic rules that include, for example, enzyme-substrate kinetics and user-defined kinetic rules. Third, we implemented a graphical user interface for loading data, selecting a model and performing simulation and data fitting. Combined with the COMKAT IMAGETOOL, a component we developed for image viewing and processing, users may draw 2-D or 3-D regions and generate time activity curves (TACs). Users may simultaneously fit several TACs using one of the many preprogrammed models or a model of their own design. Finally, we established an integrated environment to support COMKAT developers. It includes a web site with upload and download functions. It includes a bug-tracking system for reporting, confirming and resolution tracking. It also includes Concurrent Version System (CVS) repositories to facilitate version tracking and concurrent development by multiple developers. The integrated environment enables developers from other groups to participate in developing and testing COMKAT. In summary, the improvements make COMKAT a more user-friendly and powerful software for quantitative analysis of PET images. COMKAT is made available without cost to not-for-profit researchers through the website: http://comkat.uhrad.com.

No. 156

COMPARISON ON TWO-DIMENSIONAL AND THREE-DIMENSIONAL IMAGING CHARACTERISTICS AND QUALITY OF A WHOLE BODY PET-CT SCAN

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The aim of the study was to compare the clinical utility and imaging quality of a whole body positron emission tomography (PET)/ computed tomography (CT) scan in three-dimensional (3-D) and two-dimensional (2-D) modes. The study group consisted of 60 patients (56 +/-15 yrs) with a suspected diagnosis of primary or recurrent malignancy consecutively scheduled for PET. Three sequential PET-CT scans (Discovery ST) were performed 50 minutes after 260-530 MBq 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) i.v. of each patient. Every subject was studied in three standard modes: 2-D (3 or 4 minutes/ bed depending on patient weight over or under 70 Kg), long 3-D (3 or 4 min/bed) and short 3-D acquisition (1.5 or 2 min/bed). In order to avoid the influence of chronology in sequential studies, patients were included consecutively in six groups, including all the order possibilities. PET were reconstructed using iterative algorithm and one single attenuation correction CT was applied for the three studies in each patient. Two blinded observers analyzed the images and quality assessment was based on 4 items quantification (1 to 5 with 5 best): Image free of artifacts (IFA), Qualitative signal to noise (QSN), Lesion detectability (LD) and Overall image quality OIQ). Overall t-Student test for appeared samples was applied in order to detect significant differences

between different acquisition modes. Our results showed that in the 2-D acquisition method improved significantly ($p<0.01$) the image quality in comparison on 3-D in IFA and OIQ. No differences were observed in QSN (mean differences not significant between 2-D and long 3-D) and LD (2-D versus long 3-D).

No. 157

DISCOVERY OF SMALL MOLECULE PROBES THAT TARGET THE OLIGOMERIC FORM BUT NOT THE FIBRILLAR FORM OF BETA AMYLOID

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Soluble oligomeric forms of □-amyloid (a□) have been shown to be neurotoxic and their formation is proposed to precede plaque formation in the amyloid cascade hypothesis. Early detection of a□ oligomeric species prior to symptoms of cognitive decline could enable therapeutic intervention to prevent or slow disease progression. While there are several small molecule probes that bind the fibrillar form of a□, currently none are known to specifically target the oligomeric forms. We constructed a library of benzofuran-based small molecule probes for screening against oligomeric a□. Initial hits were identified as low affinity by molecular modeling and in a scintillation proximity assay. Although low affinity, one of these probes bound to oligomeric a□ with a five-fold increase over fibrils by SPA, suggesting selectivity. Additionally, a select number of these hits were validated in an "*ex vivo*" binding assay, in which either synthetic oligomers or fibrils were incubated on naive Sprague-Dawley rat brain sections, as well as human AD and human control brain tissues. In this assay our probes co-localized with oligomers, but did not bind fibrils. Lastly, we found that *ex vivo* application of probe solutions on PDAPP mouse brain sections followed by immunohistochemical detection of a□, as well as thioflavine S staining, indicated that our probes bind to ab deposits formed *in vivo*. Our data support the hypothesis that a□ oligomers have three-dimensional structures that are distinct from fibrils and which can be probed by small molecules.

No. 158

NON-INVASIVE MAGNETIC RESONANCE IMAGING OF SUPERPARAMAGNETIC IRON OXIDE-LABELED HRPE CELL TRANSPLANTS FOR *IN VIVO* FOLLOW-UP IN MONKEYS AND RATS

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Cell labeling methods using superparamagnetic iron oxide (SPIO) nanoparticles have been recently implemented to allow the *in vivo* follow-up of transplanted cells through magnetic resonance imaging (MRI). We describe here our preliminary studies regarding the safety and efficacy of SPIO labeling of transplanted human Retinal Pigment Epithelial (hRPE) cells for future *in vivo* MRI. Using the SPIO nanoparticle suspension Feridex I.V. in combination with the transfection agent protamine sulfate (Pro), hRPE cells were SPIO labeled (SPIO-hRPE) and assessed for short-term viability and proliferative activity compared to standard non-labeled hRPE (NL-hRPE) cells in culture. *In vitro* samples of various concentrations of SPIO-hRPE cells were imaged on a Bruker 7T MR scanner to assess labeling efficacy. Finally, parkinsonian rats and one monkey were transplanted with both SPIO-hRPE and NL-hRPE cells into opposite striatums and imaged up to three months post transplant. *In vivo* hRPE cell survival was confirmed post mortem by hRPE-specific immunohistochemistry and prussian blue histochemistry. Our results showed no differences in proliferative activity or cell viability up to 10 days post-seeding between SPIO-hRPE and NL-hRPE cells, and differences in MR signal intensity was demonstrated using varying Pro concentrations and SPIO-hRPE cell concentrations. *In vivo*, MRI demonstrated hypointense regions in SPIO-hRPE cell transplanted striatum compared to the NL-hRPE transplanted side up to three months post transplant, which suggests the presence of labeled cells. These preliminary results demonstrate the potential use of SPIO labeling techniques for the long term *in vivo* follow-up of clinical cell transplantation procedures.

No. 159

ANIMAL SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY WITH TC-99M-DMSA IS A HIGHLY ACCURATE TOOL TO QUANTIFY RENAL DAMAGE IN RATS AFTER TARGETED RADIONUCLIDE THERAPY

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Aim: To validate *in vivo* quantification of Tc-99m-DMSA (Dimercaptosuccinic acid) uptake in rat kidneys using single photon emission computed tomography (SPECT) compared to *ex vivo* quantification in a gamma-counter. Tc-99m-DMSA was chosen as a marker for renal tubular damage after high-dose [Lu-177-DOTA0,Tyr3]octreotate, which is used routinely in patients with neuroendocrine tumors. In repetitive treatment the kidney is the dose-limiting organ. It is therefore mandatory to have a reliable, simple tool to monitor renal function after treatment. Methods: Fifteen male Lewis-rats were injected with different activities of [Lu-177-DOTA0,Tyr3]octreotate, resulting in different degrees of renal damage after >100 days. 105-146 days after [Lu-177-DOTA0,Tyr3]octreotate-injection the rats received 50MBq Tc-99m-DMSA and four to six hours p.i., SPECT of the kidneys was acquired with the new four-head multipinhole-collimator camera NanoSPECT (Bioscan Europe Ltd., France). Immediately after imaging the rats were sacrificed and the kidneys were counted to determine the absorbed activity. The SPECT data were reconstructed iteratively and regions of interest (ROIs) were drawn manually around the kidneys. The activity in the ROIs was determined using HiSPECT and INTERVIEW software. Results: Uptake values of 0.71 to 21.87 %IA (% injected activity)were measured, dependent on the severity of renal damage. An excellent linear correlation between the determined activity *in vivo* and *ex vivo* was found with $r2=0.947$ and a slope $m=1.058$. Conclusion: Quantification of renal Tc-99m-DMSA uptake *in vivo* by multipinhole SPECT is highly accurate compared to *ex vivo* over a wide range of values. Using a diagnostic tracer like Tc-99m-DMSA, allows accurate quantification of physiological functions over time in the same animal.

No. 160

DESIGN OF AN ADAPTIVE SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY IMAGER

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We are developing an adaptive single photon emission computed tomography (SPECT) imaging system for small-animal applications. The current design is a single-pinhole, single-detector SPECT system with adjustable pinhole size, object-to-pinhole distance, and pinhole-to-detector distance. The pinhole size is selected from a set of five pinholes with different diameters via a sliding linear stage, while the object-pinhole and pinhole-detector distances are both continuously controlled by two independent linear stages. All of the adjustable parameters can be controlled through a single serial port. The ultimate objective is to have the system perform a set of initial scans to determine basic object properties. Using this information, the system will adjust the acquisition parameters based on task-based measures to acquire data optimal to the specified imaging task and object being imaged. For initial testing purposes, the system will be operated by optimizing the utilization of the available detector area. We will present an overview of the system design, initial results, and a discussion of adaptive imaging strategies. This work was

supported under NIH/NIBIB Grants R01-EB002146, P41-EB002035, and R37-EB000803.

No. 161

A NEW COLLIMATED MICRO COMPUTED TOMOGRAPHY DEVICE FOR COMBINED IMAGING AND RADIOTHERAPY OF LIVING MOUSE MODELS

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Treatment of small animals with radiation is limited to planar fields shaped using lead blocks, rendering precise localization of radiation and treatment of deep-seated tumors impossible. It is important to develop conformal radiotherapy techniques for the laboratory in order to ensure the relevance of experimental models of cancer therapy to the clinic, as well as to further explore the utility of radiation-activatable genes in biological research. We have engineered an experimental conformal radiotherapy system based on a commercially available micro computed tomography (CT) scanner (eXplore RS150, GE Medical Systems, Milwaukee, WI). A dose rate of 2 Gy/minute can be achieved using 120 kVp, 50 mA X-rays, permitting the delivery of therapeutic doses of radiation in reasonable (1-30 minute) exposure times. A variable aperture collimator has been constructed to facilitate dose shaping, based on a stereotactic radiosurgery technique. The collimator consists of a planar array of six trapezoidal 1 cm thick brass blocks mounted on a rigid frame. By driving motion of the blocks along the frame, the size of the central hexagonal aperture can be adjusted. The complete collimator assembly consists of two concentric collimator plates, offset by 30 degrees to produce a dodecagonal beam profile. Block motion is achieved via a rotational motor controlling two driving plates in parallel using a sprocket-and-chain mechanism. Power and serial communication capability to the collimator is provided by interfaces on the scanner gantry. Using this system, we can image and treat small animals in a single examination, bringing clinical radiotherapy capabilities to the laboratory.

No. 162

OPTIMISATION OF THE MICRO POSITRON EMISSION TOMOGRAPHY COMPONENT OF THE AMISSA IMAGING SYSTEM

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The Subatomic Research Institute (IReS, France) is developing A Multi-modality Imaging System for Small Animal, called AMISSA. Within this framework, a new micro positron emission tomography (PET) design is under study. The key point of this project is that the crystals are oriented along the axis of the scanner, and regrouped into several rings. A ring of detector is made of several modules arranged around the animal. Each module consists in a 12x12 matrix of 1.5x1.5x20 mm3 LYSO crystals. At both ends of the module, the matrix is read by a multianode PMT. With such a design, the transverse position of the interaction is given by the crystal address, while the axial position is given by the light sharing between both ends. This architecture makes the depth of interaction information directly accessible. Using additional concentric rings of modules, the detection efficiency can reach the detection solid angle. The geometry of the scanner is optimized using a Monte Carlo simulation (openGATE). Three rings of, respectively, 10, 10 and 20 modules are required to reach a detection efficiency greater than 10 %, with a spatial resolution close to 1 mm3.

No. 163

CENTRALIZED POSITRON EMISSION TOMOGRAPHY AND POSITRON EMISSION TOMOGRAPHY/ COMPUTED TOMOGRAPHY IMAGE ANALYSIS; ORGANIZATION, CHALLENGES AND SOLUTIONS

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The rapid growth in utilization of positron emission tomography (PET) and PET/ computed tomography (CT) is leading its use as a marker of treatment response in clinical drug development and cooperative therapeutic trials. This utilization requires a high level of standardization beyond purely diagnostic studies. Large scale cooperative therapeutic trials require multi-center, multi-vendor participation with centralized, standardized qualitative and quantitative image assessment to insure objective, unbiased and reproducible data assessment. Secondary to the rapid technological advances in PET and PET/CT, state of the art commercial systems from a variety of vendors are being installed. This variety generates many challenges with data acquisition, data handling, data analysis and co-registration for centralized therapeutic response assessment. This scientific presentation highlights the infrastructure of our centralized image analysis laboratory as it pertains to processing of PET and PET/CT images for clinical trials. Included are examples of standardized criteria for PET and PET/CT imaging including procedures dealing with data acquisition, data transmission, data storage, quality assurance, post processing and analysis of the images. The centralized image processing laboratory also provides the technical infrastructure to enable rapid transfer and storage of studies as well as communication between participating sites via state of the art communication capabilities.
Laboratory
Establishing centralized image processing centers will lead to establishing guidelines and procedures for use of PET and PET/CT in clinical trials in multi-center and multi-vendor setting. The end goal is to help pioneer the future of PET and PET/CT image analysis by standardization of image acquisition and processing protocols independent of institution and instrumentation vendor.

No. 164

IMAGING ALPHAVBETA6 EXPRESSION *IN VIVO* USING MICROPET

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Integrins are a family of cell-surface heterodimeric transmembrane receptors that mediate cell/cell and cell/extracellular matrix interactions. The integrin alphaVbeta6 is expressed exclusively on epithelial cells but at low or undetectable levels in normal adult tissues. However, alphaVbeta6-expression is increased dramatically following injury or inflammation and in many different epithelial cancers. Thus, we have developed imaging agents that selectively target alphaVbeta6 for the early detection of carcinoma.
We have generated several positron emission tomography (PET) and optical imaging candidates based on small peptides. Different prosthetic groups were employed to determine their effect on peptide activity. Inhibition of ligand binding to recombinant alphaVbeta6 was assessed by ELISA achieving an IC50 of 2nM (parent peptide) and 2-3nM (4-fluorobenzoyl-peptide, DOTA-peptide). F-18 radiolabeling was performed on solid phase and all compounds were purified using RP-HPLC. *In vivo* imaging was performed in nude nu/nu mice bearing DX3puro and DX3purobeta6 tumors on opposite flanks. Following bolus injection intravenous (iv), intraperitoneal (ip), or subcutaneous (sc) imaging was performed over a six hours (F-18) or 24 hours (Cu-64) period. Best results we obtained with the 4-[F-18]fluorobenzoyl-peptide. Tumor uptake was fast (<30min, iv, sc) and showed high selective retention in the alphaVbeta6-expressing tumors at three hours post injection. This is the first time alphaVbeta6 has been successfully imaged *in vivo*. We believe these data will herald improved therapy for carcinomas that express high levels of alphaVbeta6 such as oral squamous cell carcinoma.

No. 165

ITERATIVE VS ANALYTIC RECONSTRUCTION METHODS FOR POSITRON EMISSION TOMOGRAPHY'S: COMBINING THE BEST OF BOTH APPROACHES

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Dedicated small animal positron emission tomography (PET) scanners have become one of the main tools in biomedical research. New technologies and new reconstruction methods have been developed to reach the high spatial resolution and sensitivity that these studies require. Among them, statistical reconstruction algorithms like OSEM, have shown superior image quality than conventional analytic reconstruction techniques, like Filtered Back-Projection (FBP). One of their key advantages is the ability to incorporate an accurate model of the PET acquisition process through the use of a modeled system response matrix (SRM). These two families of emission tomography reconstruction methods have been developed independently of each other, and this has created some difficulties in both approaches. For example, there is a lack of knowledge about how to find the optimal filter for the FBP reconstruction, or how to get rid of the increasing noise in the image as the iteration number progress in OSEM. Frequency analysis of PET data, commonly applied in analytical methods, can provide useful information for statistical reconstruction. On the other hand, the main parameters of the SRM can be used to deduce analytically how to create a filter for FBP. A link between system response matrix parameters and the filters employed in FBP reconstructions is established in this work. Based on such a relationship, we propose a new method that combines data processing in the frequency domain, based on the SRM properties with the advantages of iterative reconstruction. The improvement in the quality of the images reconstructed with this new method is quantified.

No. 166

SMALL ANIMAL POSITRON EMISSION TOMOGRAPHY SCANNERS DESIGN OPTIMIZED FOR STATISTICAL RECONSTRUCTION METHODS

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Positron emission tomography (PET) Scanners are commonly designed bearing in mind analytical reconstruction methods. High sampling density is one of the main design goals. On the other hand, iterative methods are less sensitive to the sampling density, their performance being more related to the properties of the System Response Matrix. In small animal PET scanners, iterative techniques have proved to yield superior image quality. Specific design strategies can be followed in order to obtain optimal results with iterative techniques. For example, reducing the size of the crystals beyond certain point will not further improve the resolution of iterative methods because the average number of counts in each Line of Response will decrease and the relative importance of noise will be larger. We discuss the main issues (number of LOR's, size of the crystals, noise level) to be consider during the design of high resolution and high sensitivity PET scanners, in order to optimize the images obtained with iterative reconstructions, and comment on the improvement achievable in the image quality of a typical clinical study, and on the quantitative estimate of design parameters.

No. 167

REPRODUCIBILITY OF BIOLUMINESCENCE AND MICRO POSITRON EMISSION TOMOGRAPHY IMAGING MEASUREMENTS IN TUMOR BEARING MICE

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Quantitative micro positron emission tomography (PET) with 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) and bioluminescence imaging are increasingly used to monitor the effects of therapy in murine tumor models. The reproducibility of these measurements is largely unknown. Methods: SCID-mice bearing U251 glioblastoma xenografts transfected with Renilla luciferase were imaged twice within two to four days by FDG-PET (microPET Focus, 60 minutes after i.p. injection of 200 uCi FDG under isoflurane anesthesia) and bioluminescence imaging (Xenogen, IVIS system, 15 minutes after injection of i.p injection of 100ul of 0.2 ug/ul coelenterazine in PBS). For the FDG-PET studies, mice were fasted and warmed by a heating pad (30°C). Tumor FDG-uptake was expressed by tumor/liver ratios (T:L). The optical signal was quantified by maximum photons/second/cm^2/steridian. The inter-tumor variability (for all animals scanned on one day) and the intra-tumor variability (for two serial measurements in individual mice) were compared by coefficients of variation (CV). Results: The average T:L ratio was (2.10±0.62, 16 mice, 2 scans). The CV for intra- and inter-tumor analysis of T:L was 20% and 28%, respectively. The inter- and intra-tumor CV for optical imaging was 59% and 49%. The average tumor diameter in the baseline and the follow-up scan was 7.5 mm and 8.1 mm, respectively. Conclusion: microPET imaging of tumor FDG-uptake provides more reproducible quantitative parameters than bioluminescence imaging with Renilla luciferase. However, even under carefully controlled conditions, tumor FDG uptake in mice demonstrates a significant inter- and intra-tumor variability that needs to be considered when designing studies assessing treatment effects by microPET.

No. 168

SMALL-ANIMAL SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY / COMPUTED TOMOGRAPHY PRE-CLINICAL IMAGING SYSTEM

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We have built prototypes of a new micro single photon emission computed tomography (SPECT)/ computed tomography (CT) system designed for small-animal preclinical imaging applications, including quantitative biodistribution of radiolabeled molecules, dynamic uptake/washout kinetics, and multiple isotope imaging. These prototypes will be evaluated at several preclinical research sites in early 2006. The microSPECT system consists of a fixed ring of CZT gamma-ray detectors. A cylindrical multi-pinhole collimator was designed for small field-of-view applications (eg, heart, brain). In addition, a unique cylindrical multi-slit collimator was designed for whole-body and dynamic studies, one size with a 2.5 cm transaxial field of view for mice and another 8 cm for rats. As the collimator rotates continuously or in stop-and-shoot mode, projections are acquired in list mode for iterative reconstruction. Septa provide axial slice definition. The axial field of view is 8 cm, the in-plane spatial resolution is 0.5 mm for mice and 2.5 mm for rats, and the system sensitivity is 0.015% for mice and 0.030% for rats. A higher sensitivity (0.055%), lower resolution (1.5 mm) collimator for mice was also designed. This scanner will perform in 10-15 minutes whole-body studies that now take an hour or more. Dynamic studies with 10-second timing resolution are enabled by this design. A microCT imager is located adjacent to the microSPECT on the same gantry axis and a horizontal bed moves the animal by servo-motion control. The CT images are used for attenuation and scatter correction of the SPECT images, as well as anatomical reference in fused SPECT/CT images.

No. 169

MONITORING OF DISEASE PROGRESSION BY *IN VIVO* BIOLUMINESCENCE IMAGING AND MAGNETIC RESONANCE IMAGING IN AN ANIMAL MODEL OF LEUKEMIA

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Purpose: Imaging technologies are increasingly used for animal experiments of tumor models. We examined the feasibility and reliability of a multimodality approach using *in vivo* bioluminescence imaging (BLI) and magnetic resonance imaging (MRI) for the monitoring of disease progression in an animal model of leukemia. Methods: Murine pro-B cell line Ba/F3 was transduced with firefly luciferase and p190 BCR-ABL, and mice were inoculated with the cells intravenously. Imaging studies, including *in vivo* BLI and MRI of living mice and *ex vivo* BLI of excised organs, were performed one, two, three, and four weeks after inoculation. Disease progression in a given mouse was observed longitudinally by *in vivo* BLI and MRI. Results: The *in vivo* BLI demonstrated extensive light emission throughout the body, and the whole-body signal on *in vivo* BLI increased with time after inoculation. The *ex vivo* BLI showed predominant light emission in the liver, spleen, and bone marrow, and the signal for each organ correlated with the whole-body signal. MRI enabled accurate volume measurement of the liver and spleen, visualized hepatic nodules, and aided in localizing sources of light emission on *in vivo* BLI. The volumes of the liver and spleen measured by MRI correlated with the signals of the respective organs measured by *ex vivo* BLI. Longitudinal imaging studies allowed the assessment of disease progression for each mouse. Conclusions: BLI and MRI allow repetitive, whole-body, quantitative evaluation of extensive disease induced by the intravenous inoculation of leukemia model cells.

No. 170

***IN VITRO* VALIDATION OF BIOLUMINESCENT MONITORING OF CELL PROLIFERATION AND THERAPEUTIC RESPONSE IN LEUKEMIA MODEL ANIMALS**

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Purpose: The application of *in vivo* bioluminescence imaging to noninvasive, quantitative monitoring of tumor models relies on the positive correlation between the intensity of bioluminescence and tumor burden. We performed cell culture studies to investigate the relationship between bioluminescent signal and viable cell numbers in luciferase-expressing cells. Methods: Interleukin-3-dependent murine pro-B cell line Ba/F3 was transduced with firefly luciferase to generate cells expressing luciferase stably under the control of a retroviral long terminal repeat. The luciferase-expressing cells were transduced with p190 BCR-ABL to give factor-independent proliferation. The cells were cultured under various conditions, and bioluminescent signal intensity was compared with viable cell numbers and the cell cycle stage. Results: The Ba/F3 cells showed stable luciferase expression and autonomous growth after transduction with both luciferase and p190 BCR-ABL. The cells implanted into mice subcutaneously or intravenously were visualized by *in vivo* bioluminescence imaging. The bioluminescent intensities approximately reflected cell proliferation and responses to imatinib in cell culture studies. However, the luminescence per viable cell was influenced by the concentration of interleukin-3 in factor-dependent cells and by the stage of proliferation and imatinib concentration in factor-independent cells, implying the impairment of the proportionality between viable cell number and bioluminescent signal intensity. Luminescence per cell tended to vary in association with the fraction of proliferating cells. Conclusions: The signal intensity obtained by *in vivo* bioluminescence imaging may depend not only on viable cell number but also on proliferative activity.

No. 171

A LARGE FIELD-OF-VIEW HIGH RESOLUTION PANEL DETECTOR POSITRON EMISSION TOMOGRAPHY SCANNER FOR IMAGING ANIMALS

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A large field-of-view LSO positron emission tomography (PET) scanner (Siemens Molecular Imaging) has been developed for imaging low levels of radioactivity. Five large area panels of dimension 52 cm (axial) x 37 cm (transaxial) incorporates a total of 10,080, 4 mm x 4 mm x 20 mm LSO crystals resulting in a spatial resolution of 4.5 mm and a sensitivity of 2.3%. Emission and transmission data are acquired simultaneously while the panels rotate at 30 rpm. In addition to clinical patient studies, this design is particularly suited for imaging larger animals such as dogs that exceed the maximum size for imaging with a microPET scanner. The large 70 cm diameter port facilitates the imaging of anesthetized dogs. The whole-body bio-distribution 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) can be acquired dynamically for a field-of-view of 53 cm, allowing multi-organ kinetics to be followed. In veterinary medicine, where PET scanning is still largely unexplored, the availability of the scanner allows dogs with natural occurring tumors to be imaged and to be followed response to therapy. Data can be acquired in dynamic or static mode. Following acquisition, the emission data are and histogrammed from a list mode file into a fully 3-D sinogram set and reconstructed using 3-D OSEM. This paper will present results following multi-organ kinetics in dogs with naturally occurring cancers, to determine response to radiation and chemotherapy.

No. 172

MONITORING OF 2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE UPTAKE IN TUMOR-BEARING MICE BY USING HIGH-SENSITIVITY PROJECTION IMAGING: COMPARED WITH POSITRON EMISSION TOMOGRAPHY IMAGING

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Purpose: The efficacy of using high-sensitivity projection imaging to measure metabolic activity in tumor-bearing mice was evaluated and compared with that of using positron emission tomography (PET). Methods: A PPIS-4800 (Hamamatsu) and a microPET R4 (Concorde) were used for projection and tomographic imaging, respectively. Different thickness of six disks filled with F-18 solution and five Balb/C mice were scanned by microPET and PPIS. The mice were scanned 7, 10, 14, 17 days after clone-carcinoma cell implanted in the right legs. The scan time of microPET was 3.3 times longer than that of PPIS. Results: The activity ratios of disk obtained from PPIS were 1: 1.3: 2: 2.53: 2.81: 3.59, with corresponding solution-thickness ratios of 1: 1.5: 2: 2.5: 3: 3.5. The radioactive concentrations of disks obtained from microPET were 1: 1.35: 1.4: 1.34: 1.44: 1.39, which should be actually identical. The errors in measured activities with microPET were among 34%-44%, while those with PPIS were 0% - 13%. Monitoring tumor-to-muscle (T/M) ratios in mice were 1.25, 2.47, 4.83, 4.01 by PPIS, and 1.59, 2.44, 3.2, 2.71 by microPET. The maximum T/M thickness ratio was 1.65 in the day 17. The accumulations of 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) in tumor monitored by PPIS has similar trend to that of microPET, where it should be noted that the uptake values measured with PPIS include the increase of the tumor mass. Conclusion: Using PPIS for massive scans of monitoring FDG accumulated in tumor-bearing mice could be achieved. Further study in thickness dependent recovery of activity estimation is on working.

No. 173

USE OF A PIXILATED LYSO DETECTOR FOR BOTH POSITRON AND SINGLE PHOTON IMAGING APPLICATIONS

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Purpose: The purpose of this research is to evaluate possibilities of using LYSO-based detectors to apply to both single photon and positron imaging. Methods: The imaging detector unit was developed and fabricated in our lab. It was exposed to Ge-68(511keV) and Co-57(122keV) flood sources to evaluate its performance in different gamma energy bands. To assay influences of ^{176}Lu nature background, especially in low count rate condition, two-hour background measurements were made. Then tests of 1mm pinhole collimator with 2mCi Tc-99m source were processed. Results: The results showed that whole crystal array could be successfully recognized and distinguished. Performance analysis results were in attached table. In long-term background measurements, a specific count rate of about 122cps/cm^3 was obtained. When an energy window (122keV±25%) was set, the background count rate became 5cps/cm^3. After collimation, signal count rates of more than 800cps were measured. It was 10 times higher than background counts (69cps). Conclusion: This imaging detector unit shows good imaging ability in both high and low gamma energy band. Even under influence of ^{176}Lu nature background, it shows a meaningful count rate in collimated conditions. It is concluded that this detector unit is potential for both single photon and positron imaging applications

Detector performance analysis

Gamma energy (keV)	511	122
Intrinsic resolution(mm)	0.64±0.19	0.78±0.22
Peak-to-valley ratio	4.64±2.1	5.05±2.8
Energy resolution	18%±5%	22%±5.1%
Uniformity	0.59±0.1	0.56±0.09

No. 174

OPTICAL IMAGING AND REAL-TIME PCR ANALYSIS OF ADENOVIRUS BIODISTRIBUTION

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Success of adenovirus gene therapy relies on the ability to deliver the genetic material into target cells. This study was undertaken to assess the biodistribution of the adenovirus via various routes of injection. We administered 1x10^8 infectious units of adenovirus consisting of the constitutive CMV promoter expressing firefly luciferase gene in SCID mice. Gene expression was monitored by optical imaging at 2, 4, 7 and 14 days post intravenous, intraprostatic, and intraperitoneal viral injections. The lungs, liver, kidneys, spleen, and prostate were isolated and imaged for luciferase activity on day 14. To quantify the level of adenovirus in the tissue, 100ng of genomic DNA was used for real-time PCR. Primers were designed to amplify 150 bases of adenoviral E2 region. Intravenous and intraperitoneal injection resulted in highest expression and viral DNA in the liver, while intraprostatic injection resulted in prostate dominant distribution. Propensity of adenovirus to be transported into lymph nodes was examined by administering the virus into the forepaw. Robust vector mediated expression and DNA was observed in the ipsilateral brachial and axillary lymph nodes. A time-course of vector-mediated intraprostatic expression in immune-competent BALB/C host showed that the expression was below the threshold of detection at three weeks, but it persisted beyond five weeks in immune-deficient mice. These quantitative and longitudinal assessments on the *in vivo* distribution and expression of viral vector is valuable towards designing the most appropriate route of administration to achieve efficient gene delivery to the targeted site.

No. 175

DEVELOPMENT OF A POSITRON EMISSION TOMOGRAPHY SYSTEM FOR SIMULTANEOUS SMALL ANIMAL POSITRON EMISSION TOMOGRAPHY AND MAGNETIC RESONANCE IMAGING

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Clinical and preclinical multimodality imaging systems like combined positron emission tomography (PET) and X-ray computed tomography (CT) have shown the great benefit of fused functional and morphological image data. In contrast to CT, magnetic resonance imaging (MRI) has much better soft tissue contrast and does not result in additional radiation dose, which is especially important for preclinical imaging studies with small animals. Furthermore, a combined PET-MRI system could allow simultaneous imaging. We developed a PET detector based on avalanche photodiodes (APDs) fitting in the limited radial space between gradient- and RF-coil of a dedicated 7 Tesla animal MRI system (Bruker Biospin, Germany). Each detector consists of a 12x12 LSO crystal block (Siemens, USA) coupled to a 3x3 APD array (Hamamatsu, Japan). The APDs are read out by integrated charge sensitive preamplifiers. Both are located on a dedicated circuit board, using only non magnetic parts, meeting the space limitation. The detectors underwent extensive tests outside and inside the MR scanner also during MR imaging. The studies revealed that neither the performance of the PET detector nor the MR images were influenced by each other. Coincidence measurements, outside the MR, with 2 detectors (6 ns timing resolution) have been conducted and a first PET phantom image was acquired. These encouraging results achieved with the MRI compatible PET detector proved the feasibility of such a combined system for simultaneous PET-MR imaging. Thus, we are currently concentrating on building a complete detector ring fitting inside the 7 Tesla MR bore for multimodality mouse imaging.

No. 176

PHARMACOKINETIC STUDIES FOR DOPAMINERGIC SYSTEM EVALUATION WITH I-124 LABELED RADIOTRACERS AND SMALL ANIMAL POSITRON EMISSION TOMOGRAPHY: PILOT STUDY

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I-123 has been widely used in labeling radiotracer for investigating dopaminergic neurotransmission with single photon emission computed tomography (SPECT). Despite of usefulness of I-123 in labeling radiotracer for dopamine SPECT study and recent achievement of SPECT for small animal imaging, there is limitation to evaluate pharmacokinetics in animal study using SPECT and I-123 labeled radiotracer. In this study, we examined the applicability of I-124 labeled IPT as well as □-CIT, which are well-known dopamine radiotracer, in small animal study. Rat (240 ± 10g) under anesthesia was placed on bed of microPET-R4 scanner. The I-124 □-CIT and IPT (37MBq respectively) were injected to rat, respectively. Immediately after the injection, PET acquisition was started for totally one day for □-CIT and three hours for IPT, respectively. The acquired PET data was sorted to temporally framed sinogram and reconstructed to dynamic images using FORE rebining and 2-D-OSEM. By placing regions of interest on dynamic images, time-activity curves (TAC) of striatum (STR) and occipital lobe (OCC) were obtained respectively and used in kinetic analysis. On the image of both radiotracers, the regions of STR and OCC were clearly differentiated and the TACs of both regions showed similar patterns with those labeled with I-123 in human, but with shorter time course than that of human. The quantitative values of binding potential

were discriminated between □-CIT and IPT. This preliminary results shows that PET imaging with I-124 labeled dopamine radiotracer could be useful in pre-clinical study for new drug development or disease model evaluation, prior to I-123 SPECT study.

No. 177

2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE KINETIC MODELLING BY MICROPET: USING THE LIVER AS SURROGATE FOR THE BLOOD SAMPLING DERIVED INPUT FUNCTION IN MICE

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Aim: Modeling of 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) kinetics in mice is desirable for studying tumor models and transgenic animals. Blood sampling for the determination of the input function poses problems due to surgical catheter insertion, small blood volume and early fast temporal changes. In this study we examined if data derived from a liver volume of interest (VOI) could be used as surrogate for the *ex vivo* derived input function. Methods: In 10 mice with a femoral artery catheter, blood samples were drawn during the 60-90 minute list mode data acquisition using a micro positron emission tomography (PET) Focus 220 (n=8-15, size 5-15 □l), were weighed, well counted and converted into PET equivalent counts and corrected for plasma FDG content. The obtained values were compared with the liver data derived from images. To determine differences the brain glucose uptake rate (Ki) calculated by graphical analysis (minute 5 - 60) and applying either the liver or the blood sampling data. Results: Blood and liver absolute FDG content correlated (r^2=0.45, p=0.03) at 60 minutes; the correlation between the activities integrated over 60 minutes even displayed a highly significant correlation (r^2=0.86, p<0.001). When applying the blood sampling and liver data in graphical analysis of brain FDG uptake, Ki(liver) differed by 15.0±8.8% from Ki(blood). Ki(liver) and Ki(blood) correlated significantly (y=1.027x + 0.0002, r^2=0.91).Conclusion: Liver time activity curves might be used as a surrogate for the blood sampling derived input function in graphical analysis of FDG studies in mice.

No. 178

MULTIPLE POSITRON EMISSION TOMOGRAPHY PROBES USING A SINGLE CPCU CONFIGURATION AND COMMON SYNTHESIS STEPS

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The Center for Molecular and Genomic Imaging (CMGI) at the University of California, Davis, provides radiolabeling and imaging services to support preclinical research of novel probes and imaging modalities. Since opening in November 2004, the project of the core radiochemistry facility has been to provide a menu of positron emission tomography (PET) reagents. Using a GE Tracerlab FxFN with simple modifications, we have validated methods for automated production of 9-[(4-[F-18]fluoro)-3-hydroxymethylbutyl]guanine (FHBG), 1-(3'-deoxy-3'-[F-18]fluoro-□-D-pentofuranosyl)thymidine (FLT), and 1-[F-18]fluoro-3-(2-nitro-imidazol-1-yl)-propan-2-ol (FMISO) with decay corrected (DC) product yields of 20, 37, and 27%, respectively. Materials and Methods. ABX precursors N^2-(p-anisyldiphenylmethyl)-9-[(4-(p-toluolsulfonyloxy))-3-p-anisyldiphenylmethoxy-methylbutyl]guanine (Tosyl-FHBG; 4 mg), 3-N-Boc-1-[5-O-(4,4'-dimethoxytrityl)-3-O-nitrophenylsulfonyl-2-deoxy-□-D-lyxofuranosyl]thymidine (Boc-FLT; 5 mg), and 1-(2'-nitro-1'imidazolyl)-2-O-tetrahydropyranyl-3-O-toluenesulfonyl-propanediol (NITTP; 20 mg) were used to produce FHBG, FLT, and FMISO, respectively. A post-reaction vessel 3 way valve was added to the GE Tracerlab FxFN to allow C18 Sep-Pak purification of radiolabeled, unhydrolyzed FHBG intermediate; intermediate purification was not necessary for FLT or FMISO. The F-18 processing step common to all synthesis programs included 2 azeotropic distillations. All products were purified by C18 HPLC and eluted in PBS:ethanol 92.5:7.5, suitable for injection without additional formulation. Results. FHBG, FLT, and FMISO were produced with DC product yields of 20 (±2.5), 37 (±12), and 27 (±6)%, and synthesis times of 85, 55, and 55 minutes, respectively. Discussion. Three core F-18 reagents for PET studies have been produced using a single CPCU configuration and common F-18 processing and purification programs, and delivered in an injection-ready medium. This approach simplifies the process of producing multiple PET probes to support diverse studies.

No. 179

THE X-RAY COMPUTED TOMOGRAPHY COMPONENT OF THE NANOSPECT/ COMPUTED TOMOGRAPHY SMALL-ANIMAL IMAGING SYSTEM

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In previous works we have presented our multiplexing multi-pinhole single photon emission computed tomography (SPECT) imaging technique and its extensive applications in small-animal molecular imaging. SPECT combined with X-ray computed tomography (CT) introduces anatomical information and improves acquisition (helps define axial region of interest), reconstruction (attenuation correction) and data analysis (aids segmentation). In this work we present a description of an X-ray CT upgrade to a dedicated small-animal SPECT system (the NanoSPECT). The NanoSPECT houses up to four gamma cameras outfitted with multi-pinhole apertures providing submillimeter SPECT resolution. The X-ray source and detector are mounted on the back of the high-precision gantry and thus share the same axis of rotation as the SPECT system. Helical scanning is employed by both modalities and is performed by translating of the animal through the SPECT and CT fields of views. The system is capable of acquiring partial- or full-body mouse and rat images ranging from 40 to 270mm. This variable axial-length feature is also present in the SPECT modality. The X-ray source is a 90kVp microfocus (18□m) tube. The X-ray detector is made up of a 1024x2048 array of 48□m pixels (49.2x98.6mm2) and reads out at a rate of 2.7fps. The geometric magnification of the system is 1.3 providing a reconstructed CT resolution below 150□m (below 1.0 mm for SPECT). Reconstructions are performed using a ray-tracing based filtered backprojection and the system is setup for image acquisitions ranging from quick low-dose to high-resolution studies. We will present a wide range of dual-modality phantom and animal studies.

No. 180

OBJECTIVE FUNCTIONS FOR IDENTIFYING ARTIFACTS IN POSITRON EMISSION TOMOGRAPHY/ COMPUTED TOMOGRAPHYATTENUATION IMAGES

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In single-voltage- computed tomography (CT)-based attenuation correction (AC) for positron emission tomography (PET), the CT-image is converted to attenuation values appropriate for 511-keV imaging. This transformation is not unique, and depends strongly on effective atomic number. Consequently, transformation algorithms contain built-in expectations about the makeup of the scanned material. Under some conditions, including scans performed with CT-contrast media, algorithms fail, leading to inaccuracies in the transformed 511-keV attenuation image that propagate to the final attenuation-corrected emission image. However information about 511-keV attenuation is also present in the emission data. Transformed 511-keV attenuation images containing inaccuracies are inconsistent with the emission data. We are developing methods which evaluate these inconsistencies so as to correct errors in CT-based 511-keV attenuation images. These artifact-correction algorithms consist of an objective function that accurately discriminates artifactual AC from artifact-free AC, combined with an estimation procedure. In this simulation study, we examine the performance of two objective-function classes:

likelihood-based and sinogram-consistency-condition-based (SCC). Several phantom anthropomorphic activity and attenuation distributions were generated and "scanned" producing 128 noise-variates at each of 7 emission-sinogram count levels from $1000\text{-}10^7$. Artifactual attenuation images were produced by making various modifications to the true attenuation. Accuracy in identifying the correct AC compared to artifactual AC was computed for all cases. Likelihood-based functions were found to be highly accurate even at high noise levels or with small-sized artifacts. SCC functions are less accurate, but also computationally less expensive, and may be optimal for particular types of AC inaccuracies.

No. 181

A COMPARISON OF THE GE EXPLORE OPTIX PRECLINICAL OPTICAL IMAGING SYSTEM TO A CONTINUOUS WAVE IMAGING SYSTEM

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In this study GE Healthcare's preclinical optical imaging system, the eXplore Optix, was compared to a continuous wave (CW) imaging system. The goal was to perform a quantitative comparison the eXplore Optix to other fluorescence imaging systems. The systems were compared on sensitivity and contrast-to-noise (CNR). Eight solid polyester resin phantoms were created to cover a range of biologically relevant scatter and absorption values. The phantoms contained fluorescent targets ranging from 10 nM to 100 nM concentrations of Cy5.5. Full two-dimensional topographic scans were performed using these tissue mimicking phantoms on the eXplore Optix system and a continuous wave system. A laser power of 0.350 mW was used with a two second integration time per point and a 1.5 mm scan step size. The same phantoms were imaged with a CW system with a one-minute acquisition time.For each phantom the CNR for all 2 mm deep targets were plotted versus the known target concentration. A linear regression determined the relationship between the CNR and the concentration. Detectable targets were observed to have a CNR greater than 0.7. The concentration expected to produce a CNR of 0.7 was identified as the minimum detectable concentration. In conclusion, the eXplore Optix imaging system is 5.5 times more sensitive to low concentration targets than the CW imaging system. In terms of absolute amounts at a depth of 2 mm, the eXplore Optix system can detect just 800 fmoles of fluorescent dye while the CW system requires a minimum of 4400 fmoles for detection.

No. 182

AN ANTI-PROSTATE STEM CELL ANTIGEN DIABODY FOR PROSTATE CANCER TARGETING

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Prostate cancer has become an attractive yet difficult disease for antibody-based targeting. Prostate Stem Cell Antigen (PSCA) is a membrane bound glycoprotein (27 kDa) which is overexpressed in localized, androgen independent, and metastatic bone lesions of prostate cancer. We developed an antibody fragment [diabody (scFv dimer); 50 kDa], generated from a humanized 2B3 monoclonal antibody which targets PSCA. Surprisingly, the humanization process caused a four-fold reduction in affinity against PSCA. By protein modeling, six framework mutations were specifically chosen for a potential increase in binding affinity. Thus, two distinct diabodies were created, parental and affinity matured, both with an eight amino acid linker peptide $[(GGGS)_2]$. The apparent affinities were determined by Biacore (K_D = 5.41 nM and 1.89 nM). Size exclusion chromatography revealed that homogenous dimers are favored by the parental, but not the affinity matured, diabody. In addition, different linker lengths (5 vs. 8 amino acids) and storage conditions were explored in order to obtain a homogenous dimer preparation. *In vitro* PSCA binding was demonstrated by immunofluorescence using PSCA-positive prostate cancer cell lines. Diabodies were radioiodinated and retained 17% and 22% immunoreactivity for the parental and affinity matured diabodies, respectively. A micro positron emission tomography study with I-124-labeled diabody demonstrated effective localization to the PSCA-positive tumor in LAPC-9 xenografted SCID mice. Biodistribution studies with I-124-labeled diabody showed a tumor uptake of 1.22 %ID/g. Through protein engineering, traits such as binding affinity and conformation have been optimized for the 2B3 diabody. We are currently evaluating the effect affinity has on tumor targeting.

No. 183

ULTRA-HIGH SPATIAL RESOLUTION PINHOLE TOMOGRAPHY USING XSPECT

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Geometric Detector Response Calibration (GDRC) has been incorporated into the FLEX XSPECT system to achieve ultra-high spatial resolution single photon emission computed tomography (SPECT) image using a pinhole aperture. The XSPECT system is equipped with two 5-inch by 5-inch small-field-of-view (SFOV) pixellated scintillator gamma cameras. When mounted with pinhole collimators, the XSPECT system can scan small animals *in vivo* at various radii of rotation. Originally, due to the size of the pinhole aperture, as well as the misalignment between detectors and axes of rotation, the spatial resolution of the reconstructed image could only reach ~1.2 mm when a 1 mm pinhole aperture was used in the scanner. In order to compensate for these geometric misalignments, a total number of 7 parameters that characterize the SPECT system geometry were defined and studied. These parameters describe the relationship between the axes of rotation and the detector plane, and can be calibrated easily by running a tomographic scan using a simple phantom, which consists of two point sources at a fixed distance. A previously developed 3-D OSEM pinhole image reconstruction algorithm that takes into account these 7 parameters, in addition to the pinhole aperture geometry, were implemented and tested using different phantoms. A reconstructed image spatial resolution of ~0.6 mm with radius-of-rotation of 2.5 cm was observed when the 1.0 mm pinhole aperture was used.

No. 184

LABELING OF G-RICH OLIGONUCLEOTIDES WITH N-SUCCINIMIDYL 4-[F-18]FLUOROBENZOATE ($S^{18}FB$)

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Background: G-rich oligonucleotides (ONs) are emerging as a new class of non-antisense ONs whose anti-cancer therapeutic effect is related to the formation of G-quartets. Labeling G-rich ONs with F-18 might be beneficial for investigating the mechanism of the therapeutic effect and for developing a potential radiopharmaceutical for cancer diagnosis using positron emission tomography (PET). Thus, the aim of this study is to explore this possibility by labeling three single-stranded G-rich ONs with $S^{18}FB$- GRO5 (TGTTG), GRO15 (GTT-GTT-TGG-GGT-GGT), and GRO26 (GGT-TGG-GGT-GGG-TGG-GGT-GGG-TGG-GG). Methods and Results: $S^{18}FB$ was synthesized with yields of 55-65% (decay-corrected) within 45 minutes. GR05, the shortest sequence being tested, was conjugated first with $S^{18}FB$ in order to be cost-effective. Many factors were investigated to optimize the labeling yields. The yields were 42%, 70%, 84%, and 99% at 59, 118, 236, and 472 nmol/100 µL of GRO5 respectively. Reaction temperature (22 and 40 ^oC), reaction time (15 and 30 min), and borate buffer concentration (25 and 50 mM) showed minor effects on the labeling yields. Two long single-stranded G-rich ONs, GR015 and GR026, were subsequently labeled with the same procedures and the yields were 80 ±2% at 93 nmol/100 □L (n=3) and 47±3% at 98 nmol/100 □L (n=3) respectively. Conclusions: This preliminary study demonstrates that single-stranded G-rich ONs can be labeled with $S^{18}FB$ with reasonable yields at low temperatures which are highly critical for the stability of the G-quartet structure. The next step is to label the double-stranded G-rich ONs with $S^{18}FB$.

No. 185

MAGNETIC RESONANCE AND OPTICAL IMAGING OF HUMAN MELANOMA MOUSE XENOGRAFTS WITH DIFFERENT METASTATIC POTENTIAL

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Finding the biomarkers of tumor metastatic potential by imaging methods would be helpful in treatment planning and in the design of agents that modify the tumor phenotype. We report that four methods that are potentially transferable to the clinic - dynamic contrast enhanced magnetic resonance imaging (MRI) (DCE-MRI), $T_{1\rho}$-weighted MRI, ^{31}P-MRS, and low temperature fluorescence imaging (that could be performed on biopsy specimens) - can distinguish between indolent (A375P) and aggressive (C8161) human melanoma xenografts in athymic nude mice. DCE data analyzed by the BOLus Enhanced Relaxation Overview (BOLERO) method in conjunction with concurrent measurements of the arterial input function yielded a blood transfer rate constant, which measures blood perfusion and vessel permeability, that was significantly higher in the core of the indolent tumor than in the core of the aggressive tumor. Histological staining indicated that aggressive tumors have more blood vessels but fewer functional blood vessels than indolent tumors. Indolent tumors exhibit $T_{1\rho}$ values that are significantly higher than those of aggressive tumors at spin-locking frequencies greater than 500Hz. ^{31}P-MRS showed that the ratio of phosphomonoester versus NTP was higher in aggressive tumors than in indolent ones. The mitochondrial redox ratio, Fp/(Fp+NADH), where Fp and NADH are the fluorescence of oxidized flavoproteins and reduced pyridine nucleotides, respectively, of aggressive tumors is much higher (more oxidized) than that of indolent tumors and often shows a bimodal distribution with an oxidized core and a reduced rim. Further study with mouse xenografts of various levels of metastatic potential is in progress. Funding support: NIH P01-CA56690-09A2.

No. 186

DETERMINATION OF BLOOD OXYGEN SATURATION BY PCT SMALL ANIMAL SCANNER

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Purpose: The purpose is to determine the oxygen saturation value (SaO2) of blood from the photoacoustic absorption spectra as measured by the photoacoustic computed tomography (PCT) small animal scanner. Materials and Methods: A circulation system, consisting of a tonometer, an oxygen electrode, and a pump, was devised to produce blood samples with different pO2 values. The tonometer controlled the amount of dissolved oxygen within the blood by continuously bubbling air and nitrogen into the sample, and the oxygen electrode was used to monitor blood pO2 values. Six samples ranging from 0 to 250 mmHg were drawn and scanned in an optical photo-spectrometer, a PCT scanner, and a clinical blood gas analyzer (pO2, pH, hemotocrit). The SaO2 values were determined from the photoacoustic absorption spectrum (710-920nm) based on a linear combination model of oxy-hemoglobin and deoxy-hemoglobin curves. Similarly, the ratio of optical absorption values within the visible spectrum (500-600nm) was used to determine the SaO2 after removing the effects of scattering. Results: The dissociation curves (pO2 versus SaO2) as determined by optical absorption and photoacoustic spectra were compared to the theoretical dissociation curve for hemoglobin. The range of the SaO2 residuals from the optical absorption data is from 1% to 7% and that of photoacoustic data is from 0.1% to 6%. Conclusions: We have shown that NIR PCT-spectroscopy can be used to measure the SaO2 of blood samples. These studies will provide the baseline data for future studies imaging tumor hypoxia in small animals.

No. 187

SYNTHESIS OF F-18-LABELED RAPAMYCIN DERIVATIVES

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The immunosuppressant rapamycin is a highly specific inhibitor of a mammalian protein kinase called target of rapamycin (mTOR), which has a central regulating role in cell growth and proliferation. The mTOR pathway is up regulated in many human cancers and its inhibition (e.g., with rapamycin analogs) results in cytostatic effects. Three different rapamycin derivatives are now in clinical trials for treating various cancers in humans. The high affinity of rapamycin for mTOR complexed with a cellular protein called FKBP12 (K_d= 2 nM) makes it also an attractive molecule for specific tumor imaging with positron emission tomography (PET). To this end, we have synthesized several fluorinated rapamycin derivatives by 2- and 4-fluorobenzoylation at 42-*O*-position or 2- and 4-fluorobenzylation at 7-position of rapamycin, respectively. All these fluororapamycin analogs were extensively characterized by 600 MHz ^{1}H, ^{13}C, ^{19}F, HMBC and HMQC 2-D NMR experiments and high resolution mass spectroscopy. The IC_{50} values of these fluorinated analogs for inhibition of mTOR function were also determined in U87 cell line. As a model analog, we have synthesized F-18-labeled 42-*O*-(4-fluorobenzoyl)rapamycin by a two-step procedure, first preparing no-carrier-added 4-[F-18]fluorobenzoic acid then condensing it with rapamycin. The pure desired product was isolated by semi-preparative HPLC in 4±2.3% (n = 10) radiochemical yield. *In vitro* assays with F-18-labeled rapamycin analog showed that the probe was taken up by U87 glioblastoma cells as well as U87 cells overexpressing EGFRvIII and restored with PTEN. MicroPET imaging with ([F-18]fluorobenzoyl)rapamycin is currently being performed on SCID mice bearing the same human tumor xenografts.

No. 188

CHARACTERIZATION OF DOXORUBICIN-INDUCED CARDIOTOXICITY IN RAT HEARTS BY ^{99M}TC-SESTAMIBI IMAGING

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Doxorubicin (DOX) may induce cardiotoxicity in cancer patients. Early damage from DOX occurs in mitochondria, a critical area for production of myocyte energy and for accumulation of ^{99m}Tc-sestamibi (MIBI). The purpose of this study was to characterize MIBI images in rat hearts with DOX-induced cardiotoxicity and to test the capability of a new small-animal single photon emission computed tomography (SPECT) imager, FastSPECT-II, in determining radiopharmaceutical kinetics. Methods: Eleven rats were treated with DOX (5 mg/kg) injected intraperitoneally every third day for a total of four injections. Six rats were imaged by FastSPECT-II (Group I) and five were imaged by FastSPECT (Group II), an earlier SPECT system. Another five animals received carrier-vehicle (DMSO/water) injection and FastSPECT-II imaging (Control group) using the procedure as in Group I. Immediately after injection of MIBI through a jugular-vein catheter, dynamic cardiac images were acquired for two hours. Cardiotoxicity was determined by histological analysis. Results: MIBI images in DOX-treated hearts had different characteristics from those in the control hearts; findings were confirmed by histology and autoradiography. Qualitatively, images acquired by FastSPECT-II were similar to those acquired by FastSPECT in the rats with cardiotoxicity. FastSPECT-II demonstrated better temporal resolution than FastSPECT in time-activity analysis. DOX-treated hearts had lower late retention of MIBI compared to control hearts. Conclusions: MIBI uptake and distribution is altered in rat hearts with DOX injury. MIBI imaging may be useful for early noninvasive detection of DOX cardiotoxicity before cardiac function deteriorates. FastSPECT-II provides a practical means to investigate cardiovascular radiopharmaceutical kinetics.

No. 189

SOLID PHASE RADIOLABELING OF PEPTIDES WITH 2-[F-18]FLUOROPROPIONIC ACID

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The use of biomolecules such as peptides, proteins or nucleic acids radiolabeled with F-18 as agents for positron emission tomography imaging is rapidly growing field. Incorporation of F-18into biomolecules including peptides is exclusively carried out by employing a variety of prosthetic groups. The 4-[F-18]fluorobenzoic acid (4-[F-18]FBA) is the most common prosthetic group used for radiolabeling, it can be relatively easily prepared, activated as succinimidyl fluorobenzoate ([F-18]SFB) and conjugated to the purified peptide in aqueous media. However, the in-solution conjugation of [F-18]SFB to the peptide is limited to the amino acid sequences bearing only one acylation-prone nucleophilic group otherwise a complex mixture of non-selectively radiolabeled products is obtained. To overcome this, we previously developed a solid phase approach for incorporation of the 4-[F-18]FBA into protected peptides attached to the solid support. 2-[F-18]fluoropropionic acid (2-[F-18]FPA) offers an interesting alternative to the widely used 4-[F-18]FBA. In this report, we present the method for labeling peptides on solid phase with (2-[F-18]FPA). The racemic 2-[F-18]FPA was prepared according to the published procedure using 9-methylanthranyl 2-bromopropionate as a precursor. The 4-[F-18]FPA was coupled either to the N-terminus of the peptide or to the $N^{\square}$ amino group of lysine using HATU/DIEA activation followed by triflouroacetic acid mediated cleavage of the product.

No. 190

IMAGING IN PRE-CLINICAL MODELS AND EXPERIMENTAL THERAPEUTICS

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Funded by the National Cancer Institute since late in 1999, the NCI-Mouse Models of Human Cancers Consortium (NCI-MMHCC) has rapidly advanced the science of *in vivo* and *in vitro* cancer modeling by altering germlines of laboratory mice, developing a variety of organotypic co-culture systems, and grafting human tissues into immuno-compromised mice. The 25 groups who comprise the Consortium - 300 members at 75 institutions in the US and abroad - are expert in many aspects of basic, translational, clinical, and epidemiological cancer research, and in the design and use of pre-clinical model systems. The 300-member NCI-MMHCC cooperates with the NCI to evolve a systems biology approach to human cancer research. They collaborate with the NCI Center for Bioinformatics (NCI CB) to provide the databases and bioinformatics infrastructure to integrate descriptive models information with comparable human disease data. The Consortium recently launched a Pre-Clinical Testing Demonstration Project, designed to define when, where, and how the various types of cancer models, including genetically engineered mice, contribute to the discovery-development-delivery continuum for derivation of novel cancer interventions and their use in early clinical trials. The project includes development of a laboratory informatics infrastructure that will fully integrate with clinical and prevention trials informatics efforts of the NCI CB and permit cross-species comparisons. The incorporation of state-of-the-art imaging modalities significantly enhances the ability of pre-clinical modelers to observe the natural history and clinical course of cancer *in vivo* and to verify observations and test hypotheses derived from imaging *in vitro* model systems.

No. 191

BUILDING AN OPTICAL IMAGING TOOLKIT FOR APPLIED *IN VIVO* RESEARCH

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Optical-based *in vivo* imaging reagents, combined with specialized instrumentation and animal disease models, can provide valuable biological information ranging from the molecular to the morphological, from the pre-clinical setting to the basic research lab. We are developing optical imaging reagents and kits designed to function in the near infared (NIR) wavelength region of the spectrum where living tissue is relatively transparent. Access to well-characterized optical tools (labeled proteins, contrast agents, and imaging standards) should make this imaging modality more valuable for *in vivo* applications as image-based monitoring of physiology continues to gain acceptance. Our presentation will include data on applications of protein labeling kits, NIR dyes and dye conjugates, light emitting colloidal materials and fluorescent semiconductor nanocrystals in biological investigations using living animals.

No. 192

CORRELATION OF POSITRON EMISSION TOMOGRAPHY STANDARDIZED UPTAKE VALUE MEASUREMENTS BETWEEN DEDICATED WORKSTATIONS AND A PACS-INTEGRATED WORKSTATION SYSTEM

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The purpose of this study was to evaluate the clinical utility of a positron emission tomography (PET) analysis module of a Picture Archiving Communication System (PACS) workstation in comparison to a dedicated PET interpretation workstation. Images of 32 patients were reviewed at dedicated PET and at PACS-integrated workstations. Mean standardized uptake values (SUVs) were calculated for the liver and the lung. Maximum SUVs were recorded for the bladder and an index lesion. The time spent for SUV estimations was recorded. Pearson coefficients between the workstations ranged from 0.96 to 0.99 for bladder and lesion maximum SUVs. For liver and lung average SUVs the coefficients varied from 0.53 to 0.98. The mean time spent to perform all measurements was 122.6 s for the dedicated workstations and 134.6 s for the PACS-integrated system. The correlation of SUV estimations between dedicated PET and PACS-integrated workstations is very good, especially for maximum SUVs. For routine reading a PACS workstation with a PET analysis module offers an excellent alternative to the use of a dedicated PET workstation.

No. 193

SIMULTANEOUS DUAL-ISOTOPE POSITRON EMISSION TOMOGRAPHY IMAGING

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Single photon emission computed tomography (SPECT) imaging allows performing dual-isotope studies based on the different energy spectra of the isotopes used. On the contrary, positron emission tomography (PET) imaging does not allow this procedure since positrons annihilation yields two 511 KeV gamma rays regardless of the original isotope/s used. However, when two different positron emitter isotopes are administered simultaneously, it is possible to statistically discriminate their activity concentration as a function of their different decay periods. To enable this approach dynamic acquisition of emission data must be performed with no decay correction. Then, the time-data in every pixel is fitted to a weighted sum of the expected decay functions for every isotope by a non-linear least squares method, so that these weights represent an estimation of the activity concentration of every isotope. Limitations to this technique include the assumption of a constant activity concentration and the decrease in the signal to noise ratio of the single-isotope images. In this work, we have implemented this methodology and evaluated the impact of different practical factors such as the number of temporal bins or the benefits of a smoothing strategy for improving the signal to noise ratio in the images. We have validated the accuracy and precision of this methodology with phantoms containing different concentrations of 2-

deoxy-2-[F-18]fluoro-D-glucose and 13N-NH3, as well as performing several experiments with living rodents. This approach could be extended to the clinical practice providing reductions in the scan time and therefore higher patient throughput, for example in myocardial viability studies.

No. 194

IMAGING CANCER USING GENETICALLY ENGINEERED LIGHT-EMITTING BACTERIA

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Cancer research has long sought a magic bullet that would selectively target malignant cells. In this study, we exploited that E. coli injected into tumor-bearing mice selectively target and proliferate in solid tumors by employing optical imaging technique. pUC19 plasmid encloning *Lux* or *GFP* was transformed into wild type (MG1655) or mutant E.coli strains. For stably expressing *lux*, *lux* was cloned with asd (aspartate □-semialdehyde dehydrogenase) gene and transformed into asd defective E. coli (MG1655asd$^-$/asd$^+$*lux*). These bacteria were i.v. or i.p. injected into tumor mice (CT26, C6, MCF-7, B16F10, ARO). The imaging signal from MG1655*lux* was detected initially at liver (20min), thereafter at tumors for at least one week in both nude and Balb/c mice. MG1655asd$^-$/asd$^+$*lux* produced stronger and longer (for two weeks) signal from tumor than does MG1655*lux*. Directly injected MG1655asd$^-$/asd$^+$*lux* was transiently observed at central necrosis followed by spreading to the peripheral proliferative area. Flagella defective (FlhD$^-$) and aerotaxis defective (Aer$^-$) mutant completely failed to reach tumor loci. Regulatory mutants defective in sensing environmental stress (ppGpp$^-$, rpoS$^-$) could not reach and proliferate in tumor. Directly injected FlhD$^-$ strains into tumor necrotic portion just stayed there and didn't proliferate to adjacent area in tumor. E. coli strongly targeted solid tumor regardless of host immune status. Our results support that the targeting of tumor by E.coli is an active process with flagella function and the mechanism would be possibly related to aerotaxis. Live attenuated E. coli would be applied as a delivery vehicle of varying imaging markers or therapeutic molecules.

No. 195

IMAGING OF SOLID TUMOR USING NEAR-INFRARED EMITTING PURPLE BACTERIA

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The near-infrared region is a fascinating part of *in vivo* small animal imaging. It has been known for many years that purple bacteria such as *Rhodobacter sphaeroides* use a special chlorophyll for photosynthesis that absorbs specifically in near-infrared region. If biological material absorb specifically in the near infrared, it is likely that they use some of the quantum energy for structural and other changes of the absorbing molecules and, thus, re-emit light of lesser quantum energy. In this study, we explored tumor targeting capacity of *R. sphaeroides* in tumor bearing mice. *R. sphaeroides* 2.4.1 strains were cultured in sistron's minimal medium A (SIS) at 32°C. Xenograft tumor model has been established by subcutaneous injection of CT26 mouse colon cancer cell line. 1X10^8 *R. sphaeroides* cells suspended in 100ul of PBS were injected via tail vein with 1-cc insulin syringe into tumor bearing mouse. *In vivo* fluorescence imaging has been done after 20 minutes to 36 days of purple bacteria using indocyanine (ICG) emittion filter (E, m=810~835nm). Under the spectroflurimeter, the excitation and emission peak were measured at 780 and 1050 nm, respectively. Near-infrared imaging signal from *R. sphaeroides* was initially detected at liver for three days but at the necrotic region of tumor mass. The imaging signal continuously increased until 36 days after injection. We successfully imaged cancer with near-infrared fluorescence bacteria. Our result indicate that near-infrared fluorescence purple bacteria are able to be used to monitor bacterial trafficking in living tumor models.

No. 196

***IN VIVO* MONITORING EMBRYONIC STEM CELL IMPLANTATION INTO RAT CORPUS CAVERNOSUM BY OPTICAL IMAGING SYSTEM**

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The conventional method for the analysis of stem cell transplantation depends on postmortem histology. Here, we have sought to demonstrate the feasibility of a longitudinal monitoring of implanted cell survival in living animals by optical imaging techniques. Mouse embryonic stem (ES) cells were obtained from ATCC (ES-E14TG2a). ES cells were cultured in the DMEM supplemented with 3.7 g/L sodium bicarbonate, 1% penicillin and streptomycin, 1.7 mM L-glutamine, 0.1mM □-mercaptoethanol, 5 ng/mL mouse leukemia inhibitory factor (LIF), and 15% fetal bovine serum (FBS) with or without a feeder layer and cultured for five days in standard medium plus LIF. ES cells were then transfected (MOI=100) with Ad-CMV-Fluc. Our experimental Sprague-Dawley rats (n=7) were given with different numbers of ES cells (10^5, 10^6, $5x10^6$) expressing Fluc into corpus cavernosum. Cell survival was assessed histologically and/or by optical bioluminescence imaging which was conducted using a cooled CCD camera (Xenogen), beginning on the day after the transplantation. In cell cultures, firefly luciferase activity correlated linearly with cell numbers from 10^5 to $5x10^6$ (r^2=0.95). In living animal imaging, imaging signal activity correlated linearly with cell numbers injected from 10^5 to $5x10^6$ at each time point (r^2=0.62 ~0.98). In all three groups of rats, imaging signal was detected in rat genital area from the second day to the forty-seventh day after cellular injection. Adenovirus mediated transient expression of firefly luciferase reporter gene in ES cells was feasible to monitor cell survival over a month after transplantation and were noninvasively monitored with a bioluminescence optical imaging system.

No. 197

INTRAVITAL MOLECULAR IMAGING OF EARLY EVENTS OF TUMORIGENESIS AND MALIGNANT PROGRESSION IN GFP-MET TRANSGENIC MICE

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Met and its ligand HGF/SF play important roles in normal cellular processes, as well as in tumorigenesis and metastasis. Aberrant Met-HGF/SF signaling is often associated with poor clinical outcome. We generated transgenic mice expressing functionally active green fluorescent protein (GFP)-Met chimeric receptor for following early transforming events, *in vivo*. In parallel, we developed high resolution optical molecular imaging methods for intravital molecular imaging (IMI) at subcellular resolution. Within several months, male mice spontaneously developed sebaceous gland tumors that are metastatic to the lung, kidney, and liver. IMI analysis of GFP-Met mice show enhanced fluorescence in cells of the sebaceous glands. Using IMI, we detected proliferating single cells in sebaceous glands preceding tumor formation. These cells show high GFP-Met levels relative to adjacent normal tissue. We also observed single cells, spreading from the tumor mass that express high GFP-Met levels and appear to be single-cell precursors of metastases. In addition, IMI allowed us to monitor Met internalization following HGF/SF treatment *in vivo.* Thus, we have developed an IMI method combined with a novel GFP-MET animal model system that allows real time quantitative evaluation, of Met receptor activation in tumorigenic and malignant lesions at sub-cellular levels. This technology can serve as a tool for pre-clinical evaluation of

therapeutic strategies targeting the Met receptor. This work was supported in part by the NIH research grant (P50CA93990).

No. 198

XENOGRAFT MURINE MODEL OF HUMAN RHABDOMYOSARCOMA EVALUATED BY SMALL ANIMAL POSITRON EMISSION TOMOGRAPHY

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This study was approved by the Ethical Cometee of the University of Bologna. Aim of this study was to develop a murine xenograft model of human rhabdomiosarcoma that can be evaluated by 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) Small Animal positron emission tomography (PET). We studied 24 nude mice, administrated with rhabdomyosarcoma cells from RH-30 cell line. Mice were divided into five groups and administrated with different number of cells in the subcutaneous tissues (SCT) of the abdomen, dorsal SCT or peritoneum(IP). Each animal underwent four FDG-PET scans (GE,eXploreVistaDR) under gas anaesthesia. We administrated 20MBq of FDG iv. Uptake time was 60', acquisition time 20'. Images were reconstructed with OSEM2D and TBR calculated. PET scan was carried out at different time points. All the animals were sacrificed and histology was available to verify PET results.

	Gr1	Gr2	Gr3	Gr4	Gr5
Nr of animals	4	8	4	4	4
Nr of cells	1*E6	7,5*E6	8,6*E6	2,9*E6	6,9*E6
Inoculum	SCT abdomen	IP	dorsal SCT	dorsal SCT	dorsal SCT
PET after inoculum	1,2,3,4 wks	1,2,3,4 wks	1,2,3,4 wks	2d, 5d, 12d, 20d	2d, 5d, 12d, 20d
First positive PET	2 at 2 wks	4at 2wks, 3 at 3 wks	4 at 1wk	3 at 2d	3 at 2d, 1 at 12d
Animals which developed the tumor	2	7	4	3	4

The incidence of the tumor does not depend on the total number of cells administrated. IP administration guarantees a high incidence of the tumor but calculation of TBR and early identification of the cancer is difficult. SCT administration gives the possibility to quantify tumor metabolism and to predict tumor growth after just 2-D from inoculum. Dorsal masses are easier to identify compared to abdominal SCT masses. Gr5 is the strongest model.

No. 199

IMMOBILIZATION OF LUNG CANCER CELLS ONTO MICROCARRIERS FOR THE EVALUATION OF RADIOPHARMACEUTICAL KINETICS

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Objective: A 3-D culture system using the microcarrier and spinner flask technologies will be useful for assisting the development and qualification of radiopharmaceutics, but the first step is to develop methodologies for cell immobilization onto microcarriers. Methods: HTB177 cells, a non-small cell lung tumor cell line, were immobilized onto Cytodex-3 microcarriers in DMEM (Dulbecco's Modified Eagle Medium) culture medium with gentle stirring inside a spinner flask placed in a CO_2 incubator. Four different stirring times (five or 10 minutes for every 30 or 45 min) and two different durations (four and six hours) were studied. For each spinner flask, 2x10^5 cells/mL were inoculated on 10 mg/mL microcarriers in 30 mL of DMEM at an initial stirring speed of 30 rpm. Spin speed and DMEM volume were then adjusted to 60 rpm and 100 mL after four and six hours and remained the same until day 4. Viable cell numbers were determined by counting 5 mL of a microcarrier sample.
Results:

Average viable cell numbers for each group on Day 4 are summarized below:

Group	Spin Sequence	x10^5 viable cells/5 mL
1 (n=4)	5 min/30 min, 4 hr	4.83+/-2.76
2 (n=2)	5 min/45 min, 4 hr	6.83+/-2.58
3 (n=4)	10 min/30min, 4 hr	4.1+/-1.7
4 (n=2)	10 min/45 min, 4 hr	6.83+/-0.39
5 (n=4)	5 min/30 min, 6 hr	3.43+/-1.09
6 (n=2)	5 min/45 min, 6 hr	3.45+/-1.2
7 (n=4)	10 min/30 min, 6 hr	3.28+/-0.77
8 (n=2)	10 min/45 min, 6 hr	5.1+/-2.12

There was no statistical significance between any two groups differed with only one parameter. Conclusions: Spin sequences have minimal effect on immobilizing lung cancer cells onto Cytodex-3 microcarriers which can be used in a 3-D culture system to evaluate radiopharmaceutical kinetics.

No. 200

EFFECT OF GLUCOSE CONCENTRATION AND INSULIN ON 2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE UPTAKE IN LUNG CANCER CELLS

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Objectives: Although a fasting protocol is used in positron emission tomography (PET) imaging with 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) for lung cancer diagnosis, blood glucose and insulin still vary somewhat in these patients. Thus the effect of glucose concentration and insulin on FDG uptake was investigated in HTB177 and HTB183, which are two human non-small lung cancer cells derived from two separate metastatic sites. Methods: Both cells were grown in RPMI-1640. Six glucose concentrations (0, 2, 5, 11, 20, and 30 mM) were studied in four groups: I (177, no insulin, n=18), II (177, insulin, n=18), III (183, no insulin, n=15), and IV (183, insulin, n=15). 10 μCi (0.37MBq) of FDG was added to 1 mL of medium containing 1 to $3x10^6$ cells for all groups. 0.54 IU of insulin was used to maximize the effect. All samples were immediately incubated for 60 min and were then counted in an automated □counter after being washed with cold buffer twice. % FDG update versus glucose concentration was further fitted by a tri-exponential function, with the third exponent fixed at -0.01. Results: FDG competed non-linearly with glucose for uptake into cells with 0 mM of glucose being the highest and 30 mM being the lowest. Compared to HTB177, HTB183 cells took up more FDG at all glucose concentrations. Conclusions: The preliminary data indicates that there is no significant effect of insulin on %FDG uptake in these two cell lines. The non-linear effect of glucose concentration on %FDG uptake can be adequately described by a tri-exponential function.

No. 201

AN IMPROVED PRECURSOR FOR THE SYNTHESIS OF FLT

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3'-Deoxy-3'-[F-18]fluorothymidine (FLT) is increasingly being used as a cellular proliferation biomarker in a variety of oncology imaging applications including the identification of brain tumors (gliomas), breast, pancreatic, rectal, and lung cancers. Recently the synthesis of this labeled

compound has gone through several cycles of improvement, with each newly introduced precursor and accompanying method improving the yield and simplifying the synthetic process. Initial efforts used a precursor with *O*- and *N*-protecting groups and a nosylate leaving group; the synthesis of FLT from this precursor was difficult to automate and gave low yields. Next was a precursor with an internal (2,3'-anhydro) leaving/protecting group; this precursor allowed the use of automated systems but still gave modest yields. More recently, the idea of using *O*- and *N*-protecting groups and a 3'-leaving group was revived by groups in Germany and Korea with an improvement in yield and simplification of synthetic process. With the goal of taking this iterative development process to its logical conclusion, we have developed a precursor which keeps all of the previous improvements and also offers a cleaner HPLC purification process. We have prepared 3-*N*-Boc-5'-*O*-Boc-3'-*O*-nosylthymidine and successfully used it to produce FLT. Using this new precursor, we have achieved radiochemical yields in the range of 50-60% (decay corrected; n = 40) routinely and reliably with radiochemical purities exceeding 99 %.

No. 202

***EXPLORA RN*: A GENERAL PURPOSE AND FULLY AUTOMATED NUCLEOPHILIC [F-18]FLUORINATION SYSTEM**

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A general purpose automated synthesis system for the production of fluorine-18 labeled biomarkers has been developed. This new module uses the proven valve-and-tubing design approach, and features all of the components needed to produce a wide variety of F-18-labeled compounds using [F-18]fluoride ion. The system consists of a nucleophilic fluorination module, associated hardware such as an integrated HPLC, and a computer running the SynChrom control software. The module may be operated manually using the Graphical User Interface, or by using step-wise programmed synthesis methods that are easily written and modified by the user. The software records eight different parameters in-process and allows GMP compliant production complete with all necessary documentation. The module features a closed reaction vessel which can be heated to 250°C, and an integrated cooling capability to reduce the overall synthesis time. The built-in semi-preparative HPLC has both UV and radioactivity detection capabilities. Three shielded radiation detectors allow in-process feedback. The chemically inert solenoid valves hold pressures up to 4 bar. A vacuum pump allows for drying of [F-18]fluoride ion and solvent evaporation. Solid-phase extraction capability allows the use of various solvents for the fluorination reaction and the subsequent purification of the product. The automated system has been used to produce a variety of F-18-labeled compounds such as FLT, FHBG, and a number of proprietary biomarkers in good yield and high purity.

No. 203

PEPTIDE LINKERS ARE NOT NECESSARILY NEEDED FOR EFFICIENT NON-INVASIVE FLUORESCENCE-BIOLUMINESCENCE REPORTER BI-FUSION FOR IMAGING IN LIVING MICE

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Background: Reporting efficiency from a bifusion construct is modulated in part by the length and nature of the linker. Our purpose was to maximize reporting efficiency in intact cells and tumor xenografts by optimizing the linker length. Methods: Vectors (CMV promoter-tluc-linker-egfp) constitutively expressing thermostable luciferase (Tluc) and enhanced green fluorescent protein (eGFP) bifusion containing eight different combinations of linkers ranging from 0 to 21 amino acids (a.a.) in length. A 'linker' containing a single base pair served as an additional control. The system was studied in transiently transfected cells *in vitro* and cell-implants in living mice by both luminescence and fluorescence imaging. Results: The Tluc activity derived from the fusion construct containing 0 a.a. (no linker) or 19 a.a. was 65±5% of the activity of the cells expressing from Tluc alone. The cells expressing fusion protein with linkers 10, 13, 18, and 21 a.a. are 25±5% of the single constructs. The others: single bp, 5 a.a. and 8 a.a. linkers make for weaker reporters with 5%, 15% and 11%, respectively, of the levels seen with Tluc alone. Fluorescence analysis of the eGFP component by luminometry, microscopy and FACS analysis demonstrated the same pattern with the linkers mentioned above when compared to eGFP alone. Transiently transfected 293T cells implanted in living mice after 24 hours (N=3), also showed similar results. Conclusion: Fusion reporter constructs are useful for multimodality imaging applications. This study showed that fusion constructs containing 'no linker' should be considered when optimizing reporting efficiency from the fusion reporter.

No. 204

NON-INVASIVE DUAL MODALITY OPTICAL IMAGING OF BREAST CANCER THERAPY

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Introduction. The mTRA-8 agonist antibody (Ab) against human death receptor 5 (DR5) induces apoptosis in most TRAIL-sensitive tumor cells. Recent evidence suggests that Fc□RII mediates mTRA-8-induced DR5 aggregation, a required component of death signaling. Improved imaging methods are needed to monitor this process. The present research developed imaging methods to achieve this goal in live mice bearing breast cancer xenografts. Hypothesis. The fluorescence lifetime of Cy5-labeled DR5 agonist antibody can monitor receptor aggregation in breast cancer xenografts. Methods. *In vitro*. A luciferase-positive clone of MDA MB231 (2LMP) was grown in 96-well plate ($5x10^3$/well). mTRA-8 and hTRA-8 (humanized) were added (0-10□g/ml, ±cross-linking (CL) antibody), and bioluminescence images were collected. *In vivo*. 2LMP-Luc cells were implanted subcutaneously in flank of 3 groups of athymic nude mice: wild type expressing normal repertoire of Fc□Rs (n=8 and n=3), Fc□RII knockout ($2x10^6$/mouse, n=4), and Fc□RI knockout ($4x10^6$/mouse, n=2). Bioluminescence images were acquired twice weekly. Cy5-mTRA-8 (50 ug) was injected intravenously in all mice and time domain fluorescence images were collected daily. Results. *In vitro* bioluminescence imaging showed effective, concentration-dependent killing of 2LMP-Luc cells with anti-DR5 Ab. CL-antibody was not required for hTRA-8. Bioluminescence imaging showed similar growth of tumors in all strains. Fluorescence lifetime of Cy5-mTRA-8 showed the following trend: Fc□RII knockout mice > wild type mice > Fc□RI knockouts. Conclusions. Increased fluorescence lifetime in Fc□RII knockouts indicated less DR5 aggregation, signifying a requirement for Fc□RII. Decreased lifetime in Fc□RI knockouts (more aggregation) may be evidence of competitive inhibition of Fc□RII by Fc□RI.

No. 205

KINETIC MODELING OF CELL PROLIFERATION RATE WITH 3'-DEOXY-3'-F-18-FLUOROTHYMIDINE IN MOUSE POSITRON EMISSION TOMOGRAPHY STUDY: COMPARISON BETWEEN IMAGE-DERIVED INPUT FUNCTION AND TAIL-VESSEL BLOOD SAMPLING DATA

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We have demonstrated before that image-based kinetic modeling using K 3'-Deoxy-3'F-18-fluorothymidine (FLT)-value is a robust indicator for alternations in thymidine kinase activity after irradiation. Until now, quantitative evaluation using dynamic study has not been optimized mainly due to lack of accurate and less meticulous method to obtain input function. In this study, we compare the input function data and K FLT values measured by image-based method and blood sampling data in the mammary tumor bearing mice (n=7). Dynamic microPET were acquired for 60 minutes after injection followed by microCT acquisition. Regions of interests (ROIs) for tissue uptake were defined at 60min. ROIs for blood input function was defined based on the left ventricle image at 15 seconds. Partial volume and attenuation were corrected. The rate constant (K1, k2,

k3, k4) were estimated using Kinetic Imaging System developed at our laboratory. The K FLT uptake constant was defined as K1*k3/ (k2 +k3). During dynamic scanning, serial micro-blood samples (8-10µl, 12x1min, 5x10min) were collected with capillary tubes after pricking tail vessels and then assessed with gamma well counter. Input function from image-based analysis correlated well with that from blood-sampling method. (r-square =0.95). K FLT uptake values from images alone and image-blood sampling hybrid method were 0.046±0.005 and 0.050±0.007, respectively (p>0.5). Image-derived analysis is ideal for extraction of input function. However, when blood sampling is mandatory in mouse studies, tail vessel blood collection with its easy access and comparable accuracy may substitute for the arterial sampling that can only be accomplished with microsurgery.

No. 206

EVALUATION OF CURVE FITTING METHODS USED IN COMPARTMENTAL MODELING

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Objectives: To assess the confidence in fitted parameters resulting from compartmental modeling of positron emission tomography (PET), single photon emission computed tomography (SPECT), Dynamic Contrast Enhanced- magnetic resonance imaging (MRI)
or computed tomography (CT) studies, performance of two curve-fitting methods: Levenberg-Marquardt (LM) and MINPACK-1 (MP) was investigated. The speed, global convergence failure rate and accuracy of the fitted parameters were evaluated and compared. Methods: Based on a two-compartment model, each method was applied to two data sets: i) time-activity curves (TAC) were simulated using combinations of kinetic parameters covering a range of physiological values. Different levels of Gaussian noise were added and repeated 100 times for each TAC. ii) A dynamic 2-deoxy-2-[F-18]fluoro-D-glucose (FDG)- positron emission tomography (PET) brain dataset was simulated using PET-SORTEO with typical rate constants. Voxelwise fitting of TACs from gray and white matter regions simulated parametric mapping under realistic PET image noise. Starting points for the algorithm search were generated randomly in parameter space and non-negativity constraints for each kinetic parameter were applied. Success of global convergence was defined by comparing to chi-square values obtained from known parameters. Results: MP was four times faster than LM. For the first data set, the averaged failure rate at different noise levels ranged 2-10.6% (MP) and 30.6-41.6% (LM) for parameters close to zero, and 0.3-0.4% (MP) and 5.2-6.3% (LM) in other cases. For the second data set, the failure rate was 0.04% (MP) and 3% (LM). The fitted parameters showed bias and varying deviation indicating a poor fit to Gaussian distribution. Conclusions: MP significantly outperformed the LM method. Using multiple search starting points reduced the failure rate.

No. 207

ONE CLICK ALIGNMENT AFTER SMALL ANIMAL ILL-POSITIONED ACQUISITION

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Introduction: Small animal studies are not always acquired in perfectly controlled conditions. This leads to reconstructed images that sometimes are ill-positioned: the animal is rotated or translated from the expected position. The study must be reoriented using a manual reformatting tool until the result has the desired orientation or position. To avoid this manual step, we propose a totally automatic procedure making use of the image principal axes and centroid, combined with an auto-registration process. The result is a correctly positioned study. Materials and Methods: For every study, the centroid is translated to the image center, locating the image in the middle of the Field of View. Due the left-right symmetry in the transaxial plane, only the principal axis with bigger eigenvalue is used to align the image. Thus this axis becomes aligned with the image Z direction. Finally, to correctly solve the orientation in the transaxial plane, the transformed study is co-registered with a mirrored version. The result has the left-right symmetry plane in the saggital plane, and consequently the transaxial plane becomes correctly aligned. Results: The method has been tested with 15 PET rat brain images, and the result has been validated by an expert user. All images were correctly aligned with no user intervention. This algorithm corrects ill-positioned acquisitions and aligns the studies to a common orientation very quickly and with no user intervention.

No. 208

AUTOMATIC PRE-ALIGNMENT OF MULTIMODALITY RAT BRAIN IMAGES USING PRINCIPAL AXES TRANSFORMATION

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Introduction: The use of small animal studies of different modalities is nowadays widespread, since functional studies like positron emission tomography (PET)
or single photon emission computed tomography (SPECT) are better analyzed when mapped to the underlying anatomical structure coming from magnetic resonance imaging (MRI) or computed tomography (CT). The different positioning of the animals in the scanners results in the need for automated image registration methods. One important drawback of the existing algorithms is their limited capture range: if case of large initial misregistration (large translations and rotations) the optimization may not converge to the right solution, and the images do not become correctly co-registered. To avoid this problem we propose the use of a pre-alignment step based on the principal axes transformation. Materials and Methods: To test this approach we acquired three rat brain image pairs from different modalities: CT with 2-deoxy-2-[F-18]fluoro-D-glucose (FDG)-PET, MR with FDG-PET and MRI with alpha-methyltyrosine PET. These studies were co-registered using external markers, being the resulting transformation our gold standard. 100 random transformations were applied to every co-registered pair to provide different values of know initial misregistration. The images were realigned by Normalized Mutual Information with Powell optimization using or not the pre-alignment step. Results: Automatic registration method alone provided good results (>85% success) when initial misalignment did not exceed 10 mm in translation and 15º in rotation, but not more than 30% of success was achieved for larger initial misalignments (up to 20 mm and up to 45º). On the contrary, when using the pre-alignment step the proportion of successful registrations increased noticeably (> 70%).

No. 209

MULTIMODALITY WORKSTATION FOR SMALL ANIMAL IMAGE VISUALIZATION AND ANALYSIS

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Small animal studies of different modalities require certain image processing tools to be properly visualized and analyzed. We present a multimodality workstation that performs these tasks for different image modalities: positron emission tomography (PET) and computed tomography (CT). Different import formats are accepted (RAW, Interfile, Analyze, DICOM) and also exported. The animal studies can be displayed in different ways, providing always a tri-planar viewer that shows transaxial, coronal and sagital views synchronized. Several basic tools are always available, like window/level and color table setting, image reformatting (affine transformation), distance and angle measures or line profiles. Analysis module allows segmenting three dimensional Regions of Interest (3-D ROIs) using not only manual but also semi-automatic methods like thresholding and region growing. The system calculates several parameters for the resulting ROIs like volume, total and mean activity or temporal curves in case of dynamic studies. Registration module focus on image fusion: Manual registration methods depending on 3-D

landmarks and automated registration algorithms (Normalized Mutual Information) are offered and resulting images can be displayed together using several fusion modes. Arithmetic operations can also be performed on two studies. Finally, several 3-D rendering tools are available for single and multiple studies.

No. 210

COMPARISON OF DIFFERENT TECHNIQUES TO SELECT THE BACKGROUND REGION FO TBR EVALUATION OF XENOGRAFT TUMORS IN MICE USING 2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE

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Xenograft tumors have a high and fast growing rate in mice and the tumor uptake can easily be studied using 2-deoxy-2-[F-18]fluoro-D-glucose (FDG). In particular TBR (tumor to background ratio) calculation is a simple tool to evaluate tumor uptake and its changes in time. Due to the small dimensions of the animals and to the positioning of the lesions it is very easy to draw the tumor region of interest (ROI) but it is quite difficult to draw the background region. In this work we compared TBR values obtained using respectively peri-tumoral, heart cavity, muscles and lung regions of interest as background. We acquired 20 nude mice affected by xenograft rabdomiosarcoma using a GE Explore Vista small animal PET one hour after the injection of about 18.5-37 MBq of FDG. TBRs were calculated dividing the max count value in the tumor ROI by the mean count value in the background ROI. ROIs have been placed by two different expert operators and the relative percentage differences between the two operators have been evaluated. The best results in terms of reproducibility of ROI positioning, according with the visual analysis and semplicity of ROI drawing were found using lung as background region. In this case the difference between the two operators was less than 5% compared with the 20% obtained with peri-tumoral regions that gave the worst results.

No. 211

LABELING OF LYMPHOCYTES FOR *IN VIVO* CELL TRAFFICKING

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In vitro labeling of cells for subsequent non invasive tracking of the labeled cells *in vivo* is a powerful tool in basic research. In general, cell labeling can be performed by small iron particles for visualization by magnetic resonance imaging, by labeling the cells with a fluorochrome for optical imaging, or by radio labeling the cells to detect them by nuclear imaging (e.g. positron emission tomography (PET)). We compared in this work all three different labeling methods by using specific Th1 cells and assessed viability, functionality (interferon-gamma (IFN-□) expression by ELISA) and double strand brakes (phosphorilated histone H2AX-□) of the cells at different time points after labeling. For each experiment 106 Th1 cells were incubated for three hours with [Cu-64]PTSM (20 - 60 □Ci) , six hours with iron particles, and five minutes with a Cy5 dye cell labeling solution. Labeling of the Th1 cells with iron particles showed no significant influence of the viability or functionality of the cells. However, an internalization of the iron into the cells could not be reached. Labeling of 106 Th1 cells with 20 □Ci [Cu-64]PTSM reduced cell viability from 95% to about 89% three hours and to about 50% 24 hours after labeling. IFN-□ was reduced by a factor of two and a significant increase (factor of 10 compared to unlabeled cells) of the histone H2AX-□was measured for the radioactive labeled cells. First *in vivo* studies with Cu labeled cells in an inflammation mouse model revealed cell trafficking into single lymph nodes.

No. 212

LOCALIZATION OF RADIOCONTRAST REAGENTS IN MICE USING THE KODAK *IN VIVO* FX IMAGING SYSTEM

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The following study evaluates the ability of standard radiographic contrast medium to enhance anatomical detail in mice imaged using the *in vivo* FX X-ray system (Kodak Molecular Imaging Systems, New Haven, CT). The utility of Optical Molecular Imaging for non-invasive localization of fluorescent, luminescent or isotopic labels is expanding a rapid pace. A major challenge for this application has been the generating detailed representations of the underlying anatomy to allow for precise determination of signal origin. Tomographic approaches such as computed tomography (CT) scan can provide such detail but instrumentation has not allowed for integration for optical data in a cost effective manner. Here we described the use of a combination of digital X-ray capture instrumentation and iodinated contrast medium to generate high-resolution images in a format amenable to co-registration with optical data from the same animal. SKH1 mice were surgically prepared with an indwelling catheter inserted in the right internal jugular vein as described previously (Yale University IACUC# 2003-10701). For imaging mice were anesthetized (ketamine/xylazine) and positioned on the instrument platen above the energy transducing phosphor screen. X-ray images were acquired using a 60 seconds exposure to 35 kV potential energy beam. Baseline images were acquired prior to (t0) and immediately after injection (200µl) of nonionic, water-soluble Omipaque (GE Medical Systems, Princeton, NJ). Subsequent images were acquired at 10 minutes intervals. Image contrast was enhanced by subtracting t0 images from subsequent time points. Localization initiates in heart, followed by excretion through the kidneys with final pooling occurring in the bladder. Support: NIH-U24DK59635

No. 213

A COMPACT SMALL-ANIMAL IMAGING SYSTEM INCORPORATING PARALLEL-HOLE SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY AND MULTIPINHOLE STANDARD/HELICAL SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY

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We have incorporated multipinhole helical (and ordinary) single photon emission computed tomography (SPECT) in our parallel-hole SPECT imaging system. Previous efforts have demonstrated the utility of parallel-hole SPECT with this system based on two detectors incorporating Hamamatsu R3292 PSPMTs. Recent expansion with a "mouse-sized" detector incorporating a pair of 2"x2" Hamamatsu H8500 modules enhanced the system further. To address limitations in spatial resolution of our parallel-hole detectors and the sensitivity of our detectors with single-pinhole collimators we have tested a combination of multipinhole and helical SPECT with enlarged field of view and enhanced resolution and sensitivity. The multipinhole SPECT system is based on the 110mm circular detector equipped with pixellated (1x1x5mm^3/pixel) NaI(Tl) scintillator on a rotating gantry. A helical orbit was effected by adding a rack providing movement of the animal along the axis of rotation of the gantry. The system is capable of organ-specific multipinhole SPECT or whole-body multipinhole helical SPECT. Features are compared among the various modes based on phantom studies.

No. 214

REDUCED RADIOACTIVITY IN URINARY BLADDER FOLLOWING DIURETIC ADMINISTRATION IN MICE

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Many radiotracers utilized in molecular imaging studies are excreted in the urine; consequently, the urinary bladder begins to show high radioisotope activity within a short time after tracer administration, which persists as a 'hot spot' in positron emission tomography (PET) or single photon emission computed tomography (SPECT) images for several hours. Identification and quantification of activity in prostate tumors or other structures close to the bladder can therefore be difficult. We hypothesized that administration of the diuretic, furosemide (Lasix), would significantly reduce radioactivity in the bladder. Male CD-1 mice (n=6) were randomly injected with either furosemide (10 mg/kg, ip) or with a comparable volume of vehicle (saline, ip) under isoflurane anesthesia, followed by 2-deoxy-2-[F-18]fluoro-D-glucose (FDG; 0.2 mCi; tail vein). After two hours (awake; food and water ad libidum), they were anesthetized and imaged (one hour) in a small animal PET scanner (microPET II). After one to two weeks, the procedure was repeated, except administration of furosemide or saline was reversed. Images were reconstructed (MAP), and regions of interest (ROIs) were drawn around the bladder to determine the total bladder activity, expressed as percent of injected dose. Data were compared by Student's t-test for paired data, and differences were considered significant if $P < 0.05$. Percent activity in the bladder was significantly less ($P=0.0025$) in furosemide-treated (4.0 +/- 0.3%) compared to saline-treated (11.1 +/- 1.0%) animals (mean +/- S.E.M.). Treatment of mice with a diuretic prior to performing a nuclear imaging study can significantly reduce radioactivity in the urinary bladder, which may allow for better discrimination of structures that lie close to the bladder.

No. 215

A MODEL THAT COMPLEMENTS AN IMAGING ASSAY FOR THEORETICAL AND EXPERIMENTAL INVESTIGATION OF *IN VIVO* PROTEIN-PROTEIN INTERACTIONS

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Affiliations: Departments of Radiology and Bioengineering, Molecular Imaging Program at Stanford (MIPS) and Bio-X Program, Stanford University Purpose: This work provides a formal qualitative and quantitative model of the *in vivo* interactions between hsp90, p23, and bioluminescent versions of the two proteins which are part of a reporter protein complementation assay. The assay consists of using bioluminescent imaging for indirect monitoring of *in vivo* protein-protein interactions. Ansamycin antibiotics which target the hsp90-p23 interaction are being evaluated using the assay. Methods: The main activities in the development of the model are object and rule selection, structural and functional analysis, hypothesis generation, and iterative improvement. Concepts from set theory, graph theory, and calculus are employed. The tools and methods are general and can be used for modeling other protein-protein interactions. The model defines an endogenous and experimental system that includes two and four monomer concentrations, respectively, and six rate constants. Results: Four protein complexes and twelve protein-protein interactions are expected in the endogenous system; 25 complexes and 98 interactions for the experimental system. The tetramer configurations predominate as the monomer concentrations increase. Less p23 than hsp90 is present in the complexes at steady state. The steady state concentration of the endogenous complexes decreases in a nearly linear fashion as the concentration of the experimental monomers increases. Conclusions: Behaviors of the experimental system are expected to parallel those of the endogenous system. The model facilitates the theoretical and image-based investigation of hsp90-p23 interaction and the evaluation of therapeutic agents that target the interaction.

No. 216

COMPARISON OF IMAGE RECONSTRUCTION METHODS FOR MICROPET MEASUREMENT OF SEROTONIN TRANSPORTER OCCUPANCY

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INTRODUCTION: 3-D maximum a-posteriori reconstruction (MAP) has been shown to improve spatial resolution and reduce statistical noise in micro positron emission tomography (PET) images compared to filtered back-projection (FBP). However, MAP has also been shown to give inaccurate quantitative results in some phantom studies. To investigate the quantitative accuracy of MAP under *in vivo* imaging conditions, serotonin transporter (SERT) binding parameters measured with MAP were compared with those measured with FBP. METHODS: ^{11}C-DASB microPET studies performed in anesthetized rats at baseline (N=5) and after Paroxetine administration (10upk-2mpk, N=16) were reconstructed with MAP and FBP. Studies conformed to IACUC regulations. Each reconstruction used the same voxel size, and neither included attenuation nor scatter correction. MicroPET images were co-registered with a segmented rat brain atlas for calculation of regional radioactivity concentrations. For both MAP and FBP, C-11-DASB specific binding in the thalamus was calculated using the area under the curve from 30-60 minutes using the cerebellum under full DASB blockade as the reference region. Occupancy by Paroxetine was calculated against the average specific binding at baseline. RESULTS: MAP images appeared sharper and less noisy than FBP images. Measurements of C-11-DASB specific binding and occupancy with the two reconstruction methods were well correlated (r^2~1.0). C-11-DASB specific binding measures were higher with MAP than FBP by ~7% (linear regression slope = 0.93), while SERT occupancy measurements were very similar (linear regression slope = 1.0). CONCLUSIONS: MAP reconstruction of C-11-DASB rat microPET studies resulted in improved image quality relative to FBP. Furthermore, MAP reconstructed data permitted reliable SERT quantification.

No. 217

ROBUST POSITRON EMISSION TOMOGRAPHY EXPERIMENT DESIGN FOR ESTIMATING RECEPTOR CONCENTRATION

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Receptor concentration quantification using positron emission tomography (PET) is an effective tool for evaluating brain and heart physiology and pathophysiology because it allows probing the molecular interactions *in vivo*. A difficulty in this task is the determination of an experimental protocol that leads to precise estimates of the receptor concentration. The problem is even more challenging in heterogeneous populations with varied physiological states. The standard approach for optimizing experimental protocols achieves precise estimates for only a subpopulation of individual with similar physiological states. We present a methodology to design a robust two-injection protocol that provides precise estimates of myocardial □-adrenergic receptor (□-AR) concentration in normal and pathologic states. Methods A two-injection protocol of the high affinity □-AR antagonist [F-18]-(S)-fluorocarazolol was designed based on a synthetic population of individuals representing a wide range of □-AR concentrations. The design figure of merit was calculated for each individual and timing and dosage of ligand injection were optimized to make the worst-case precision least bad. The resultant protocol was applied to pigs before and after chemical sympathectomy which induces □-AR upregulation. Results *In vitro* assays on hearts harvested from euthanized pigs demonstrate sympathectomy increases the receptor concentration. Consistent with this, the PET-estimated □-AR concentrations revealed the increases. Importantly, the precision of the PET estimates of receptor concentration demonstrated high precision in normal and pathologic states. The methodology presented here could be applied to other applications such as dopamine receptor concentration estimation in the brain which can differ from one region to another.

No. 218

A NEW TECHNIQUE FOR RECONSTRUCTING POSITRON EMISSION TOMOGRAPHY DATASETS FROM ROTATING SCANNERS

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Introduction: The positron emission tomography (PET) image reconstruction can be formulated as an equation system where each element of a system response matrix (SRM) represents the probability of detecting in every line of response (LOR) an annihilation event emitted from every voxel of the image volume. In the case of scanners based on pairs of opposite detectors fixed in a rotating gantry this SRM can be decomposed into a projection and rotating components. In this work we propose a new strategy to invert the SRM. Material and Methods: The reconstruction strategy is based on separating the process into two steps: 1) the projection part is solved using the pseudo-inverse of the projection component and then 2) this estimated image is rotated and added to the final image. This strategy reduces the computation requirements to invert the complete SRM, maintaining 3-D information. This new reconstruction was tested with real data from a hot Derenzo-like phantom and data from a mouse study (20-40 g), acquired in list mode during continuous rotation of the gantry. The data were organized as a histogram of LORs' with an angular binning of 1º, covering 180º. Results: Our reconstruction achieved results visually indistinguishable from those obtained by 3-D-OSEM, while reducing by a factor of two the computation time. Moreover, the pseudo-inverse strategy permits a finer control of the noise level in the final reconstructed image. Conclusion: Our method represents an intermediate solution between the speed of analytical algorithms and the quality of standard iterative reconstruction applied to rotating PET scanners.

No. 219

TEMPORAL ASPECTS OF KINETIC PARAMETER ESTIMATION USING LIST-MODE AND HISTOGRAM DATA

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Background: This work is concerned with dynamic positron emission tomography (PET) reconstruction along the lines proposed by Nichols (TMI,2002) and Kamasak (TMI,2005). In the former case, spatial time activity curves are characterised by B-spline coefficients estimated directly from list-mode data. In the latter, histogram (hist-mode) data are used to reconstruct compartment model rate constants. Methods: We propose an alternative approach in which rate constants are directly reconstructed from either list-mode or hist-mode data, based on the observation that emissions originating from each contributing exponential-mode in the compartment model are independent and identically distributed samples drawn from an inhomogeneous Poisson distribution, as suggested by Snyder (TMI,1984). Specifically, we concentrate on the temporal aspects of the problem by making the simplifying assumption that photon pairs are detected directly and study the bias and covariance properties of the resulting estimator. We have developed an approximation formula for the covariance of the fitted parameters for 1- and 2-compartment models under the assumption of an unbiased estimator for both hist- and list-mode and compared the results to multiple realizations of simulated data. Results: Experimental data were simulated using count levels ranging from 100 to 2,200 counts, and rate constants typical of metastatic colorectal cancer and brain glioma 2-deoxy-2-[F-18]fluoro-D-glucose human PET uptake. Comparison of covariance between hist- and list-mode implementations indicates a small loss of information through temporal binning, whilst variance and count level were seen to be correlated. Conclusions: Estimation of kinetic parameters from list-mode data is theoretically achievable and covariance in parameter estimates can be usefully predicted.

No. 220

COMPARISON OF BOOTSTRAP METHODS APPLIED TO POSITRON EMISSION TOMOGRAPHY DATA FOR IMAGE NOISE ESTIMATION

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Overview: Assessing the reliability of data measurement is an important challenge in quantitative data analysis. A number of bootstrap re-sampling methods have been proposed for estimating regional noise in reconstructed images from a single measurement. This study compares four methods to assess the accuracy with which they predict noise properties of the data. Theory: A natural approach to applying the bootstrap method to positron emission tomography (PET) data consists of forming multiple realizations by drawing random events from a list-mode data-set (method-A). This is theoretically grounded but often inconvenient. An alternative approach consists of temporally dividing the sinogram into sub-sinograms and drawing rows from this population (method-B). We have extended it to the cases where entire sub-sinograms and where single pixels are drawn (methods-C, D). Methods: A uniform cylinder filled with Germanium[68] was imaged for five minutes on a microPET-R4. 500 list-mode replicates were generated (method-A), then histogrammed using 3-D-binning. The original list-mode data were binned and re-sampled into 500 sinograms, according to each of methods B, C and D. All four methods were compared in sinogram space according to various figures of merit including comparison of covariance, and chi^2 tests to assess goodness of fit to different distributions. Results: All methods produced Poisson distributed bins. Sinogram-based methods were found to yield significantly lower mean values than the list-mode approach. Moreover, method B introduced artificial correlations in the sinogram. These discrepancies in the estimation of noise properties indicate that sinogram-based methods should be used with caution, and list-mode methods used when practical.

No. 221

A NOVEL PLASMID VECTOR FOR POSITRON EMISSION TOMOGRAPHY IMAGING OF THE BALANCED REPORTER-THEREPEUTIC LINKED *EX VIVO* CARIDAC TRAANSGENE EXPRESSION IN LARGE ANIMAL HEARTS

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Previously we have shown the feasibility of noninvasively monitoring *ex vivo* delivered therapeutic cardiac transgene expression in the whole heart of large animals by positron emission tomography (PET) imaging a co-transfected reporter gene that were contained in two separate plasmids. To achieve more balanced reporter and therapeutic transgene expression, recently, we constructed two new plasmid vectors. Plasmid I (PI) contains a herpes simplex virus type 1 mutant thymidine kinase (sr39tk) as the reporter gene and a recombinant human interleukin-10 (hIL-10), an immunosuppressive cytokine as the therapeutic gene driven by two identical CMV promoters. Plasmid II (PII) contains a CMV promoter-driven reporter gene and a SV40 promoter-driven therapeutic gene. Twenty-three rabbits underwent heterotopic functional cervical heart transplantation. Plasmids were complexed with liposome, then *ex vivo* intracoronarally delivered into the donor hearts before implantation. In donor hearts treated with PI, the gene transfer efficiency was similar for reporter and therapeutic genes (15±2% vs. 15±2%). However, the efficiency for reporter gene was significantly higher than therapeutic gene (15±2% vs. 12±2%, $p<0.05$) In PII group. A significant correlation was observed between the expression of sr39tk gene and the total myocardial [F-18]-FHBG accumulation quantified in percent of intravenously injected [F-18]-FHBG dose in both groups ($p<0.001$). In PI group, the correlations between hIL-10 and sr39tk gene expression, and hIL-10 expression and activity of [F-18]-FHBG were significantly higher than that in PII group. We concluded that two identical promoters driven reporter and therapeutic

gene is an optimal vector design for quantitative PET imaging the therapeutic transgene expression

No. 222

FEASIBILITY STUDY OF SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY IMAGING WITH MICROPET SCANNER

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We conducted a preliminary study to use micro positron emission tomography (PET) for single photon emission computed tomography (SPECT) imaging. This new imaging capability can have significant technical and economic impacts to the preclinical imaging research, and allows combined SPECT/PET imaging for potential new applications. A parallel-hole collimator insert (5(tangential)x10(axial)x2.5(radial) cm^3 size, 1 mm hole diameter) was placed inside a microPET Focus120 scanner (~ 1.5 mm PET image resolution). The 2-D projection images of a Tc-99m line source (0.4 mm diameter) were acquired by operating the scanner in single photon detection mode. The measured spatial resolution (FWHM) was 2.7 mm with the source-collimator distance at ~1 mm. The resolution degraded to 3.0, 3.4 and 3.7 mm when the source-collimator distance was increased to 10, 20 and 30 mm accordingly. To study the scatter effects, a 2 cm thick water phantom was placed between the collimator and the line source. The resolution degradation was minimal. These initial experiments demonstrated the feasibility to acquire SPECT images using a microPET, and indicated that in order to achieve good SPECT image quality, it is critical to optimize the collimator design and scanner data acquisition to overcome some fundamental performance limitations posed by a PET system which was designed for detecting coincident 511 KeV photons instead of single photons using collimator. In this study, investigations on some key technical issues will be reported, particularly the scatter effects due to the fact of low energy resolution in detecting low energy single photons in SPECT mode, and collimator design for optimal balanced SPECT imaging performance.

No. 223

REGISTERING SPECT AND MAGNETIC RESONANCE IMAGING FOR NON-INVASIVE LOCALIZATION OF STEM CELLS GRAFTED IN THE INFARCTED RAT MYOCARDIUM

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This abstract demonstrates the application of mutual information (MI) based registration of radionuclide and magnetic resonance imaging (MRI) in an effort to use multimodality imaging for non-invasive localization of stem cells grafted in the infarcted myocardium in rats. Radionuclide imaging such as single photon emission computed tomography (SPECT) inherently has high sensitivity and is suitable for tracking of labeled stem cells, while high resolution MRI is able to provide detailed anatomical and functional information of myocardium. Thus, co-registration of SPECT images with MRI will map the location and distribution of stem cells on detailed myocardium structures. For validation, SPECT data were simulated using a Monte Carlo based projector that modeled the pinhole-imaging physics assuming non-zero diameter and photon penetration at the edge. Translational and rotational errors of the registration were examined with respect to various SPECT activities, and they are averagely about 0.50mm and 0.82 degree, respectively. Only the rotational error is dependent on activity of SPECT data. Stem cells were labeled with ^{111}Indium oxyquinoline and grafted in the ischemic myocardium of a rat model. Dual tracer small animal SPECT images were acquired, which allowed simultaneous detection of ^{111}In-labeled stem cells and of [^{99m}Tc]sestamibi to assess myocardial perfusion deficit. The same animals were subjected to cardiac MRI. A MI-based registration method was then applied to SPECT and MRI images. By co-registration, the ^{111}In signal from labeled cells was mapped into the akinetic region identified on cine MRI; the regional perfusion deficit on the SPECT images also coincided with the akinetic region on the MRI.

No. 224

EXPANDING THE CYS-DIABODY FORMAT TO NEW ANTIGEN SYSTEMS; ANTI-CD20 AND ANTI-HER2 ANTIBODY FRAGMENTS FOR TARGETED *IN VIVO* IMAGING

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Engineered antibody fragments derived from therapeutic monoclonal antibodies offer increased versatility and enhanced pharmacokinetics for tumor imaging and targeted therapy. Previous work in our lab has resulted in the creation of a cys-diabody specific for carcinoembryonic antigen (CEA). Cys-diabodies are dimers of fragments containing the variable light (V_L) and variable heavy (V_H) domain of an antibody joined by a peptide linker of 5-8 amino acids, and an added C-terminal cysteine residue. The cysteine allows for site-specific conjugation of labeling agents away from the antigen-recognition sites. Disulfide bonds between the cysteines of two V_L-V_H monomers lead to stable, covalently-bound dimers of 55kDa. Radiolabeled anti-CEA cys-diabodies have been successfully employed in microPET studies to image tumors in nude mice bearing subcutaneous, CEA-positive xenografts. In order to evaluate whether the cys-diabody format can be expanded to targets other than CEA, an anti-CD20 cys-diabody and an anti-HER2 cys-diabody were assembled. These antibody fragments are derived from the therapeutic monoclonal antibodies rituximab and trastuzumab, used to treat non-Hodgkin's lymphoma and breast cancer, respectively. Anti-CD20 cys-diabodies have been successfully expressed as covalent dimers in mammalian cell culture, purified by cation and anion exchange chromatography, and characterized by size exclusion chromatography. SDS-PAGE analysis and Western blotting demonstrated proper size and monomeric form in reducing conditions. Fragments were labeled with ^{124}I (95.2% labeling efficiency) and retained high immunoreactivity (38%). Preliminary microPET experiments have been performed using nude mice bearing subcutaneous CD20-positive xenografts to visualize targeting. Anti-HER2 cys-diabodies have been expressed in mammalian cell culture and await further study.

No. 225

INCREASED RADIOIODINE UPTAKE OF ANAPLASTIC THYROID CARCINOMA CELLS BY ADENOVIRUS-MEDIATED SODIUM IODIDE SYMPORTER GENE TRANSFER

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We analyzed increased radioiodine uptake in anaplastic thyroid carcinoma cells (ARO cells) due to adenovirus-mediated human sodium iodide symporter (hNIS) gene transfer, by manufacturing recombinant adenovirus (rAd) encoding the hNIS gene and transfecting it into ARO cells. For *in vivo* analysis, 1.5 □ 10^8 pfu of rAd was injected intratumorally into ARO cell xenografts in the right thighs of nude mice (n=12); normal saline containing ARO cells was injected into left thigh tumors as a control. Two, 3, 4 and 6 days after rAd injection, images were taken 60 minutes after injecting 5.5 MBq of ^{131}I intraperitoneally. Right/left (R/L) count ratios acquired of xenografts were calculated for each mouse (n=12). RT-PCR and immunohistochemical staining were performed to determine hNIS mRNA expression in tumors. The iodide uptake of rAd transfected ARO cells increased up to 233 fold at 120 minutes after ^{131}I injection versus the controls. Mean R/L count ratios on 2, 3, 4 and 6 days after rAd injection were 2.85, 2.54, 2.31, and 2.18, respectively. By RT-PCR and immunohistochemical staining, hNIS expression was highest two days after injecting rAd and then gradually decreased. Radioiodine uptake was increased in ARO cells by rAd-mediated hNIS gene transfer both *in vitro* and *in vivo*. These results suggest the possibility of radioiodide therapy for non-iodide concentrating tumors by hNIS gene transfer.

No. 226

DIAGNOSIS OF PROSTATE CANCER (PC) WITH PROSTASCINT^R FUSION COMPARED TO NEEDLE CORE BIOPSY

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This study compared the diagnosis of localized prostate cancer (LPC) by ProstaScint^R with CT fusion, to the results of needle core biopsies (NCB) to determine the accuracy and validity of the ProstaScint^R method. Pathology reports for 214 patients were retrospectively compared to the results of the ProstaScint^R fusion scans. The ProstaScint^R monoclonal antibody CT fusion technique utilized the Case Western Reserve University (CWRU) protocol, with single photon emission computed tomography (SPECT) and non-contrast computed tomography (CT) scan of the pelvis. The fusion results were compared to NCB pathology information performed with sextant to 60 core biopsies at various institutions. Diagnostic results from both techniques were transposed to Microsoft Excell 2003^{TM} and analyzed using Systal Ver. 11.00.01©. For patients with LPC, ProstaScint^R with CT fusion identified 168 patients (78.5%) with PC. Similarly, pathology reports from NCB also identified 168 patients with PC, resulting in: Efficiency/Accuracy = 67.3%, Sensitivity = 0.792 and Specificity = 0.240. The positive predictive value was 79.17% and negative predictive value was 23.91%. However, agreement between the two diagnoses was inadequate: Cohen's □ = 0.031. For LPC, agreement between ProstaScint/CT fusion and NCB was a Sensitivity of 79.2%, with a Specificity of 24%, due to the poor spatial resolution of SPECT. High numbers of positive cases (for LPC) inflated Sensitivity. Agreement may also be related to the number of cores sampled with NCB. Regardless, we suggest that NCB remain the "gold standard" for diagnosis of LPC, with utilization of the ProstaScint^R /CT fusion method for adjunct diagnoses of lymph node involvement/ extraprostatic spread.

No. 227

EFFECTS OF MORPHINE SELF-ADMINISTRATION ON BRAIN GLUCOSE METABOLISM IN RATS

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Introduction: Chronic exposure to opiates has been shown to influence neural activity in brain regions related to the rewarding process. It also induces neuroadaptations which lead to addiction. We have found in previous works that morfine self-administration produces neuroadaptative changes in brain areas of Fischer-344 rats. The aim of this study is to examine the effect of chronic self-administration of morphine on cerebral glucose metabolism. Materials and Methods: Two groups of male Fischer-344 rats studied: Group-A (N=7): intravenous morphine self-administration (10-15 mg) (1 mg/kg/injection in 12 hour daily sessions for 15 days) and Group-B (N=7): saline, with the same pattern. Positron emission tomography study was performed after the last self-administration. PET scan was performed 35 min after intravenous injection of 2-deoxy-2-[F-18]fluoro-D-glucose (FDG). Images were reconstructed by 3-D-OSEM and regions of interest (ROIs) were drawn on coronal sections. Results: ROI analysis revealed a significantly lower metabolism in thalamus (p=0.048) and frontal cortex (p=0.021) in Group-A. Discussion: Endogenous opioid system might have a role in thalamic nuclei activity given the high-density of □-opioid receptors. Hypo-metabolism in the thalamus suggests an inhibition of neural activity mediated by □-opioid receptors during morphine self-administration. Hypo-metabolism in frontal cortex suggests a reduction in neuronal activity induced by the presence of morphine, which agrees with the metabolic effects of other drugs of abuse in this area, as reported for humans. Conclusions: Results demonstrate that morphine self-administration changes cerebral glucose metabolism in areas related to rewarding system, and suggest that there are brain metabolic changes induced by opiate abuse.

No. 228

EFFECT OF CANNABINOID EXPOSURE DURING ADOLESCENCE ON CEREBRAL GLUCOSE METABOLISM IN ADULT RAT

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Introduction: Despite the widespread use of cannabinoids by adolescents, literature on long-term neurobiological effects of cannabinoid-exposure during adolescence is scarce. The aim of this work is to analyze cerebral glucose metabolism in adult rats which had been pre-exposed to cannabinoids. Materials and Methods: Two groups of female Wistar rats studied: Group-A (N=5): the cannabinoid receptor agonist CP-55,940 (0.4 mg/kg) was administered daily from day 28 to 38 post-natal; Group-B (N=5): vehicle, with the same pattern. PET study was performed on day 100 post-natal. 2-Deoxy-2-[F-18]fluoro-D-glucose (FDG) was injected and scan was performed after 35 min. Images were reconstructed by 3-D-OSEM and regions of interest (ROIs) were drawn on coronal-sections. For SPM analysis, image sets were realigned, smoothed and a brain mask was applied. Resulting images were analyzed with the SPM2 software. Results: ROI analysis didn't find any statistically significant region. SPM analysis revealed significantly higher metabolism in amigdalo-entorhinal-area (p=0.05) and lower metabolism in frontal cortex (p=0.05) in Group-A. Discussion: Hypo-metabolism in frontal cortex agrees with the metabolic effects of other drugs of abuse reported for humans. Metabolic increase in amigdalo-entorhinal-area has also been reported in humans, and is probably associated with greater emotional liability induced by the adolescent cannabinoid pretreatment. Conclusions: Results demonstrate that chronic administration of CP-55,940 during adolescence resulted in significant cerebral glucose metabolism changes in the adulthood, suggesting that pre-exposure to cannabis might alter cerebral activity in the adult brain. SPM presents significant advantages over ROI-analysis when subtle differences are involved.

No. 229

THE EFFECT OF □$^+$ ENERGY ON PERFORMANCE OF A SMALL ANIMAL POSITRON EMISSION TOMOGRAPHY CAMERA

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The effective spatial resolution of a positron emission tomography (PET) scanner is determined in part by the initial energy of the positron, which is a function of the radionuclide. For F-18 (E_{max} = 0.633 MeV) the mean positron range in water is small (0.6 mm). However, many other useful positron-emitting nuclides have higher energies, for example Ga-68 (E_{max} = 1.899 MeV, mean range 2.9 mm) has one of the highest. The performance of a non-rotating, 16 module quad-HIDAC (High Density Avalanche Chamber) small animal PET scanner was measured using both F-18 and Ga-68 to represent the extremes of high and low positron energy. The sensitivity and count rate performance - scatter fraction and noise-equivalent count rate (NEC) - were measured for both isotopes. Data were also collected for a spatial resolution phantom with rectangular arrays of holes of diameter 2.0, 1.5, 1.0 and 0.5 mm with the centres separated by 4.0, 3.0, 2.0 and 1.0 mm respectively. The sensitivity of the system was found to be 8.1 cps/kBq and is not affected by positron energy. The NEC, measured for both 70 cc and 200 cc cylindrical phantoms, was approximately linear up to 30 MBq, but shows a rapid drop-off above this value. The spatial resolution phantom showed that 1 mm objects are just resolved with F-18, but none of the targets are resolved for Ga-68. In conclusion, spatial resolution is dominated by the choice of isotope down to 1 mm, with sensitivity and count-rate data being independent of positron range.

No. 230

EVALUATION OF MICRO POSITRON EMISSION TOMOGRAPHY AND MICRO COMPUTED TOMOGRAPHY IMAGE REGISTRATION

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Aim: To measure the accuracy of micro positron emission tomography (PET) and micro computed tomography (CT) image registration Introduction: The registration of PET and CT images is becoming extremely popular for human investigations especially after the introduction of combined PET/CT systems. Right now combined animal microPET and microCT are under development. However no such system is available on the market, therefore it is necessary to perform separate scans and to register the two sets of images. Methods: Cylindrical phantom PET scans (GE eXplore Vista) and CT scans (GE eXplore Locus) were acquired using a multimodality bed. Ten fiducial markers obtained using small (1,5mm) spherical zeolites beads soaked with 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) were placed on the phantom surface. Three different registration algorithms: rigid R (6 parameters), similarity S (7 parameters) and full affine FA (12 parameters) were used to co-register the images. All algorithms were available in Microview® (GE healthcare). The registration accuracy was measured by estimating the root mean square (rms) distance between coordinates of pairs of corresponding marker centroids after registration. Results: The rms was respectively equal to: 0,17 mm, 0,14 mm and 0,10 mm for R, S and FA registrations. Student's *t*-test were also performed on the square difference between coordinates of pairs for each registration mode. The *p* values were equal to p=0.012 between R and FA registrations and p=0.07 between S and FA methods. Conclusions: Results shown that FA registration perform better than R and S methods. Future work will investigate *in vivo* registration accuracy using small animals images.

No. 231

BIODISTRIBUTION OF 6-DEOXY-6-[F-18]FLUORO-D-GLUCOSE IN POSITRON EMISSION TOMOGRAPHY

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Introduction: 2-Deoxy-2-[F-18]fluoro-D-glucose (FDG) is the most common clinical positron emission tomography (PET) radiopharmaceutical. As compared to glucose, substitution of fluorine for a hydroxyl at the 2-carbon position makes FDG a poor substrate for the sodium glucose transporters (SGLTs) in the proximal tubules of the kidney. Consequently a significant amount of excreted radioactivity collects in the bladder. This leads to image artifacts and degrades tumor conspicuity in the lower abdomen and pelvic region. Herein, we report on an alternative glucose analog: 6-deoxy-6-[F-18]fluoro-D-glucose (6FDG). Prior *in vitro* data suggests 6FDG is an effective substrate for the SGLTs and therefore may mitigate problems associated with urinary excretion. Furthermore, 6FDG is not believed to be phosphorylated so it is a potential tracer for glucose transport. Methods: Rats were imaged in a micro PET scanner following intravenous injection of 6FDG (n=8) under baseline and three days later following treatment with phlorizin, a potent blocker of the SGLTs. Reference studies were done using FDG (n=8). Results: Time activity curves of 6FDG captured from the heart, brain and liver exhibited rapid uptake into the cells and rapid clearance. Images collected following 6FDG injection showed markedly less bladder activity than those collected following FDG. Pre-treatment with phlorizin increased bladder activity especially for the 6FDG scans. These results suggest that 6FDG is a viable substrate for the SGLTs and may reduce hot bladder artifacts. 6FDG may be useful for evaluating glucose transport independent of phosphorylation by hexokinase that occurs with FDG. Whether 6FDG accumulates in tumors is the subject of future investigation.

No. 232

ISOFLURANE EFFECTS ON CARDIAC FDG UPTAKE: MASKING OF SIGNIFICANT ALTERATIONS OF SUV VALUES IN A MOUSE KNOCK-OUT MODEL

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Mouse models of cardiovascular disease along with non-invasive 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) imaging of the mouse heart using small animal positron emission tomography (PET) systems have become increasingly popular as these tools and techniques have advanced. PET imaging of mice requires anesthesia to reduce movement, calling into question kinetic parameters obtained under anesthesia. We examined five wildtype mice (WT) and five cardiomyocyte specific PPARgamma knockout mice (KO) which causes increased GLUT1 transport and blocks insulin-mediated GLUT4 glucose uptake. Mice were imaged with and without isoflurane gas anesthesia for 10 minutes on two different days using tail vein injected FDG, 45-minute uptake in a Siemens microPET Focus220 and a single set of images reconstructed. For the non-anesthetized studies, we allowed 45 minutes for uptake and clearance, followed by imaging under isoflurane. For anesthetized studies, mice were knocked down prior to injection and kept under constant 2% anesthesia. Standardized uptake values were determined using the average of four small regions in the myocardial wall, normalized for body weight and injected dose. Under conscious uptake, a significant difference was measured between WT and KO mice (0.61±0.17 vs. 1.5±0.77; p=0.05), however this was not seen under anesthetized uptake (p>0.5). Isoflurane significantly increased heart uptake in WT (p=0.015) and also raised the standard deviation of the measurements, masking out the difference between the mice. For many experiments, isoflurane is a suitable and preferred anesthetic agent; however, there are certain cases when the effects on blood flow and organ uptake may mask a significant difference in metabolic function.

No. 233

MOLECULAR IMAGING TECHNIQUES FOR IMMUNOCOMPROMISED RODENTS

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Physiological factors and handling techniques can alter preclinical molecular imaging uptake characteristics of positron emission tomography (PET) probes in rodents. To reduce, control or moderate these effects, we have extended our imaging facility support to include various heating methods, simplified gas anesthesia and streamlined use of our isolated imaging chambers for barrier imaging of immunocompromised mice and rats in PET and computed tomography (CT). All rodents are anesthetized using 1-2% isoflurane; providing an unending constant level of anesthesia. The system operates on constant pressure, using orifices to meter flow, enabling simple on/off ball valves for point-of-use control over the anesthesia. One vaporizer can easily support multiple usage points; in our case, 1-2 microPET systems, microCT, 4 induction boxes and 2 lines for prep work. Preset on/off heating is provided everywhere animals are present, either through warming plates, recirculating water baths, heated induction boxes or heating under the isolation chamber bed. Proper heating to normal physiological temperatures has proven essential to prevent hypothermia and reduce 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) brown fat uptake. The anesthesia and isolation chamber systems have been used for 6000+ PET and CT imaging experiments over the past 26 months and have needed only minor repair or adjustments. Similar heating, anesthesia and isolation chambers have been designed for use with optical imaging and have been in routine use for one year. Accurate and reproducible results from PET, CT and optical systems require attention to handling and physiological conditions before, during and after experiments. Easy to use equipment and standard techniques also assist investigators and regulatory approvals.

No. 234

AQUEOUS NICKEL-NITRILOTRIACETATE MODIFIED FE3O4-NH3+ NANOPARTICLES FOR MODULAR ORIENTATION CONTROLLED AUTO-ASSEMBLY OF CELL TARGETING LIGANDS AS MR CONTRAST AGENT

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A comprehensive totally aqueous phase synthesis of NTA-Ni modified superparamagnetic Fe_3O_4 nanoparticles was prepared with the particles diameter is 6.2 ± 1.1 nm. The hemocompatibility of these nanoparticles were performed using human whole blood, which shows no detactable hemolysis activity of the nanoparticles at all dosages ranging from 0.1 nM to 10 mM particle concentrations. Furthermore, the Fe_3O_4-NTA-Ni nanoparticles are able to act as a modular designed unit rendering molecular orientation control, which was demonstrated in the receptor mediated targeting for cancer cells expressing specific integrins using RGD-4C-6-His fusion peptide as the ligand to self-assemble on the nanoparticle through surface immobilized Ni-NTA. In this report, the prepared Fe_3O_4-NTA-Ni nanoparticles were engineered as a molecular orientation controlled module to perform efficiently selective targeting of cancer cells with □↓□3 or □↓□5 integrin expression through RGD-4C-6-His, and the results show a prominent selectively targeting to cancer cells with the integrin expression. Only few non-specific stainings were detectable in the normal keratinocytes. On the other hand, nanoparticles alone failed to target HCDB1 cells and showed only background staining. These results also show promising potential for the future development of magnetic resonance based molecular imaging, targeted hyperthermia, and regional chemotherapy.

No. 235

QUANTITATIVE ASSESSMENT OF TUMOR GLUCOSE UTILIZATION FOR PREDICTION OF RESPONSE TO TREATMENT WITH EGFR KINASE INHIBITOR

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Background/Aim: Gefitinib ("Iressa") is an epidermal growth factor receptor (EGFR) kinase inhibitor that has recently been approved for treatment of advanced non-small cell lung cancer (NSCLC). However, only ~10% of unselected patients benefit from Gefitinib therapy. In this study, we investigated whether positron emission tomography with the glucose analog 2-deoxy-2-[F-18]fluoro-D-glucose (FDG)- positron emission tomography (PET) may allow early differentiation of Gefitinib sensitive and insensitive tumors. Materials and Methods: We studied a panel of NSCLC lines with a spectrum of Gefitinib sensitivity. FDG-uptake was measured at various treatment time points, and glucose transport and hexokinase activity were examined. FDG uptake in-vivo was assessed by microPET imaging of tumor bearing SCID mice. Results: In sensitive cell lines, there was a dramatic (up to ~70%) decrease in FDG-uptake as early as two hours after incubation with Gefitinib. At the same time, immunoblots demonstrated translocation of glucose transporters (GLUT3) from the plasma membrane to the cytosol. Uptake rates of the metabolically stable glucose analog, 3-O-methyldeoxyglucose, were reduced 2.6-fold. Interestingly, there was only a modest reduction of hexokinase activity. These metabolic alterations preceded changes of S-phase fraction, thymidine uptake and apoptosis. MicroPET studies demonstrated a 50% decrease of tumor FDG uptake after two doses of Gefitinib. Gefitinib resistant cells demonstrated no measurable changes in FDG-*uptake in vitro* and *in vivo*. Conclusion: Our results indicate that glucose metabolic activity closely reflects response to Gefitinib therapy. These findings are very promising for the clinical use of FDG-PET to predict response to Gefitinib therapy early in the course of treatment.

No. 236

INTRAVENOUS, INTRAPERITONEAL, AND SUBCUTANEOUS ROUTES OF ADMINISTERING CU-64-DOTA-HB22.7 DISPLAY EQUIVALENT TUMOR TARGETING ABILITY

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B lymphocyte signaling via surface molecule CD22 has been shown to increase proliferation *in vitro*. HB22.7 is a mouse monoclonal antibody that binds CD22 and blocks CD22 ligand binding. Previous studies demonstrated that HB22.7 reduces human lymphoma xenograft volume in nude mice. We have developed a Cu-64-DOTA-HB22.7 antibody, for *in vivo* imaging of lymphoma. Cu-64with its multiple emissions has applications both in imaging and in therapy. DOTA was conjugated to HB22.7 in varying ratios and all conjugated antibodies were determined to maintain immunogenicity via flow cytometry. The Cu-64-DOTA-HB22.7 antibody was injected into lymphoma xenografted mice either intravenously, intraperitoneally, or subcutaneously and biodistribution assessed using microPET. Imaging was performed at 3, 24, and 48 hours post-injection. Cu-64-DOTA-HB22.7 demonstrated highly specific tumor targeting by 48 hours. Tumor targeting was equivalent regardless of route of administration. These findings establish the potential of Cu-64-DOTA-HB22.7 as a radioimmunotherapeutic or lymphoma-specific imaging agent. Furthermore, these findings provide evidence that more accessible routes of administration can achieve equivalent results in terms of imaging, and may lead to more efficient and accurate administration of antibody-based therapeutics in mice.

No. 237

IMPACT OF SYSTEM MODELING ERROR ON THREE STRATEGIES FOR INCORPORATION OF SCATTER INFORMATION IN POSITRON EMISSION TOMOGRAPHY RECONSTRUCTION

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In statistical image reconstruction for positron emission tomography (PET), the reconstructed image quality largely depends on the system matrix as well as the scatter correction method used, especially for the case of a large attenuating medium where the measurement process is dominated by photon attenuation and scatter. Accurate system and scatter modeling can improve image quality, but whatever the method employed systematic and/or random errors will always exist in the system model to some extent, inevitably impacting final reconstructed image quality. Theoretical expressions have been derived to study the error propagation from the system matrix to the reconstructed images for the case of the expectation maximization (EM) algorithm. The effect of system and scatter modeling errors for three different scatter correction methods are considered: a) scatter subtraction, b) adding scatter as a constant term to the forward model and c) a unified model where the scatter is completely modeled within the system matrix itself. First order approximations are used to derive the theoretical expressions for the error propagation, which account

for errors in both the system matrix and the scatter estimates (when used outside the system matrix). These expressions are validated using simulated data. A close agreement is found between the measured and theoretically derived error images, with the unified system model being least sensitive to the errors. The theoretical expressions are useful to determine the required accuracy for the system matrix and scatter estimation, and indicate cases where it is suboptimal to not model photon scatter within the system matrix itself.

No. 238

A VARIABLE-SIZE VOXEL SCHEME FOR SMALLER PHANTOMS AND FASTER MONTE CARLO SIMULATIONS

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Monte Carlo simulation of the passage of radiation through matter is a powerful tool that has been used in tomography (positron emission tomography (PET)/ single photon emission computed tomography (SPECT) or computed tomography (CT)) simulations, dosimetry and radiation therapy planning. Partly due to the increase in power observed in computers and the need for greater accuracy, geometrical objects described in simulations became more complex with a greater level of details. A common way to described complex geometries is through the use of voxellized volumes, such as phantoms created after segmentation of CT or MRIs. Depending on resolution, the number of voxels can reach 10^8. This puts a high demand on memory and, depending on the software used, phantoms that large may not be accommodated by 32-bit architecture processors. Even with 64-bit processors, the installation of large amounts of memory can be expensive and impractical, especially on cpu clusters typically used by Monte Carlo. However, high resolution is often not required throughout the entire phantom. For example, in dosimetry applications, one is concerned with target volumes and sensitive organs which constitute only a fraction of the entire phantom. In imaging applications, high resolution is only needed to maintain smooth surfaces. In this paper, we report a technique to implement a variable-size voxellized phantom to reduce memory and CPU requirements. The technique has been implemented in GATE. Memory reduction of up to 85% and CPU reduction of up to 70% have been observed. For dosimetry applications, the average dose in a region was similar whether the region is compressed or not.

No. 239

NEW FLUOROGENIC SUBSTRATES FOR IMAGING □-GALACTOSIDASE ACTIVITY IN LIVING SUBJECTS

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Reporter genes, such as LacZ expressing □-galactosidase, are useful tools in biomedical research in particular for gene expression studies. Many substrates have already been developed for imaging the activity of this enzyme, either by fluorimetry, spectrophotometry or even by MRI. However, most of them are not suitable for high-throughput *in vivo* imaging studies, either because they require the sacrifice of the animal, or because the associated imaging techniques are constraining to use and expensive. Recent development of fluorescence imaging allows the detection of biological events *in vivo* in small animals. The advantages of these optical methods are their sensibility, the low cost of the instrumentation, and the absence of constraints which exist for nuclear techniques like positron emission tomography (PET) and single photon emission computed tomography (SPECT). For all these reasons, we developed new fluorescent substrates able to detect LacZ gene expression in living transgenic mice. Theses new substrates are □-galactoside derivatives that are synthesized via multi-step synthesis pathways. After their synthesis, these new fluorescent substrates have been characterized and tested *in vitro* with the enzyme in solution, and on transfected cultured cells, before being injected *in vivo* in living transgenic mice.

No. 240

INHIBITION OF DEFLUORINATION OF [F-18]FCWAY IN RAT *IN VIVO*.

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Objectives: [F-18]FCWAY is used in human subjects for positron emission tomography (PET) imaging of brain 5-HT_{1A} receptors, but suffers from significant defluorination and troublesome skull uptake of radioactivity. Our aim was to inhibit defluorination of [F-18]FCWAY in a rat model. Methods: Defluorination was examined in *i*) phosphate buffer (0.1M; pH 7.4), *ii*) rat whole blood and *iii*) rat liver microsomes with and without NADPH. Inhibitors of cytochrome P450 2E1 enzymes (cimetidine, diclofenac and miconazole) were tested for their ability to inhibit defluorination in rat liver microsomes. The effects of miconazole dose on skull radioactivity uptake, spillover and image contrast after *i.v.* [F-18]FCWAY administration to rat were studied with PET. Results: Defluorination of [F-18]FCWAY occurred in rat liver microsomes only in the presence of NADPH, and not in phosphate buffer or rat whole blood. Defluorination of [F-18]FCWAY in rat liver microsomes alone or in the presence of cimetidine, diclofenac, or miconazole, each for 30 minutes, was 13, 13, 5 and 2% respectively. After pretreatment of rats with 0, 15, 30 or 60 mg/kg miconazole nitrate, PET indicated skull uptake of radioactivity at 90 min after *i.v.* [F-18]FCWAY administration was 540, 300, 180 and 82% standardized uptake value (SUV), while brain radioactivity was 137, 75, 68 and 89% SUV, respectively. The respective *ex vivo* measures of brain radioactivity were 41, 47, 52 and 74% SUV. Conclusions: Miconazole effectively suppresses defluorination of [F-18]FCWAY in rat *in vivo*, possibly by competitive inhibition of cytochrome P450 enzymes. This reduces the impact of partial volume effect and consequently improved PET image contrast.

No. 241

EFFECTS OF ADMINISTRATION DOSE, SPECIES AND STRAIN VARIATIONS ON MICROCT IMAGING CHARACTERISTICS OF A LONG-ACTING BLOOD-POOL AGENT IN NORMAL RODENTS

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Objective: An injectable polyiodinated triglyceride lipid emulsion that remains truly intravascular for hours owing to its macromolecular-surface modification has been developed. This study was designed to evaluate effects of dose, strain and species variation on the *in vivo* imaging efficacy using microCT. Material and Methods: The contrast agent was obtained from Alerion Biomedical, Inc. (San Diego, CA). Three doses (7.5, 10 and 15 mL/kg) were given to female Sprague-Dawley rats, and two doses (10 and 15 mL/kg) were administered to female C57Bl/6 mice. Other strains (Balb/c, FVB, Nu/Nu and Scid) were injected with 15 mL/kg. Anesthetized animals (2-3 animals/group) were scanned using a GE eXplore Locus microCT scanner prior to and at indicated time intervals following administration of the agent. Relative tissue densities were determined in blood and liver at predetermined time points for comparison between each group. Results: Imaging data showed that relative contrast enhancement profiles of selected tissues were dose dependent in both rats and mice. Moreover, species variation strongly influenced the imaging characteristics of the agent. Blood clearance, as measured by a decrease in vascular contrast intensity, was significantly faster in rats than in mice. Among female mice, the FVB group displayed a slower rate of vascular clearance, while the other strains all exhibited a comparable rates that were somewhat faster than that observed for the FVB. Conclusions: *In vivo* imaging results demonstrate that the vascular contrast agent provided prolonged vascular contrast enhancement in a variety of rodent models, albeit with slight and manageable differences between strains.

No. 242

EFFECTS OF DOSE, SEX, AND SPECIES VARIATIONS ON IMAGING EFFICACY OF A HEPATOCYTE-SELECTIVE CONTRAST AGENT IN NORMAL RODENTS

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Objective: A chylomicron-like lipid emulsion containing a polyiodinated triglyceride (ITG) for selective delivery of ITG analogs directly into the hepatic parenchyma following administration has been developed. This study was designed to evaluate effects of dose, sex, and species variation on the *in vivo* imaging efficacy of the hepatoselective contrast agent in normal rodents. Material and Methods: The liver contrast agent was obtained from Alerion Biomedical, Inc. (San Diego, CA). Two (10 and 15 mL/kg) and three different doses (2.5, 5, and 10 mL/kg) were tested in FVB mice (n=3) and Sprague-Dawley rats (n=2), respectively. Anesthetized animals were scanned using a GE eXplore Locus microCT scanner (80 kVp, 450 μA and 400 steps) prior to and at predetermined time points after injection. Relative tissue densities were determined in the blood and liver. Results: Noticeable liver contrast could be achieved following injection of 5 mL/kg. Both vascular and liver enhancement profiles in mice and rats were dose dependent. Blood clearance and liver sequestration in SD rats were significantly faster than in FVB mice. No differences in tissue enhancement profiles between female and male SD rats were observed, while female FVB mice displayed a faster rate of blood clearance and liver uptake than male FVB mice. Conclusions: *In vivo* imaging results show that the hepatocyte-selective contrast agent provided excellent hepatic contrast enhancement in the rodent models tested. Species variation strongly influenced the imaging characteristics of the liver contrast agent, while effects of sex on the enhancement profiles were only observed in FVB mice.

No. 243

MICRO COMPUTED TOMOGRAPHY EVALUATIONS OF A LONG-LASTING BLOOD POOL AND HEPATOBILIARY CONTRAST AGENTS FOLLOWING MULTIPLE INTRAVENOUS INJECTIONS IN NORMAL MOUSE MODELS

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Objective: Two novel submicron oil-in-water lipid emulsion delivery systems that closely resemble chylomicron remnants and localize lipophilic iodinated contrast agents to the intravascular compartment or liver parenchyma have been developed. In this study, *in vivo* imaging characteristics of both agents were evaluated in normal mice following multiple intravenous injections. Material and Methods: Imaging agents were obtained from Alerion Biomedical Inc. (San Diego, CA) and administered to various strains of mice (C57Bl/6, Balb/c, FVB and Scid) one to two times/week for up to four weeks via tail vein injection (10-15 mL/kg). Images were acquired using a GE eXplore Locus microCT scanner (80 kVp, 450 μA and 400 steps) at predetermined time points prior and after injection. In addition, animals were monitored for signs of abnormal behaviors throughout the study. Results: Cumulative increases in liver contrast enhancement were observed with the liver agent, especially for the two times/week dosing regimen, whereas vascular intensity completely returned to background level after three days. Tissue enhancement profiles obtained throughout repeat dosing protocols were similar to those observed following a single injection. Both agents were well tolerated in all mice tested, when given as often as twice a week at a dose of 10 mL/kg. Conclusions: Acceptable injection tolerance and efficacious imaging profiles observed in mice with both contrast agents in single or multiple dosing regimens provides substantial flexibility and convenience for longitudinal studies using micro computed tomography. Moreover, the design of these agents allows for simultaneous evaluation of both anatomical and functional properties of the liver and vascular system.

No. 244

EFFECTS OF ADMINISTRATION ROUTES ON MICRO COMPUTED TOMOGRAPHY IMAGING CHARACTERISTICS OF A LONG-ACTING VASCULAR CONTRAST AGENT IN A MURINE MODEL

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Objective: An injectable polyiodinated triglyceride lipid emulsion that remains truly intravascular for hours owing to its macromolecular-surface modification has been developed. This long-acting blood pool contrast agent has been used in a number of micro computed tomography (CT) imaging procedures for both visualization and quantitative vascular characterization. This study was designed to evaluate the *in vivo* imaging efficacy of the agent as a function of administration route. Material and Methods: A pegylated chylomicron-like vehicle containing a polyiodinated triglyceride [1,3-bis[7-(3-amino-2,4,6-triiodophenyl) heptanoyl]-2-oleoyl-glycerol] packaged within its lipophilic core (approx. 52 mg I/mL) was obtained from Alerion Biomedical Inc. (San Diego, CA). Anesthetized female C57Bl/6 (3 animals/group) were scanned using a GE eXplore Locus microCT scanner (80 kVp, 450 μA and 400 steps) prior to and at predetermined time intervals following administration of a single dose (15 mL/kg bw) via three different routes (i.v., i.p. and retroorbital injection). Relative tissue densities were determined in the blood (inferior vena cava) and liver at each time point for comparison between each group. Results: Imaging data showed similar *in vivo* enhancement characteristics of the agent following intravenous, intraperitoneal, or retroorbital injection. No differences in the vascular enhancement profiles between i.v. and retroorbital venous sinus routes were observed. A delay in the time to peak vascular intensity was noted following i.p administration, but the magnitude of the peak enhancement was comparable to the other administration routes. Conclusions: The administration routes can be used interchangeably with one another. This may be advantageous for investigators without technical expertise in tail vein injection.

No. 245

INTEGRATED MICROFLUIDICS AS AN ENABLING TECHNOLOGY FOR SYNTHESES OF RADIOLABELED IMAGING PROBES

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We have demonstrated a technology platform for performing multi-step chemical syntheses in automated integrated microfluidic devices. The synthesis of a [F-18]radiolabeled molecular imaging probe, 2-deoxy-2-[F-18]fluoro-D-glucose (FDG), in an integrated microfluidic device was chosen as a proof-of-principle study. This multi-step synthesis composed of five sequential processes, i.e., [F-18]fluoride concentration, water evaporation, radiofluorination, solvent exchange, and hydrolytic deprotection, was demonstrated with high radiochemical yield and purity, and shorter synthesis time relative to conventional automated synthesis. In this case, multiple doses of FDG for positron emission tomography (PET) imaging studies in mice were prepared. By utilizing such a synthetic platform, other molecular probes, including 3'-deoxy-3'-[F-18]fluorothymidine ([F-18]FLT) and 2-(1-{6-[(2-[F-18]Fluoro-ethyl)-methyl-amino]-naphthalen-2-yl}-ethylidene)- malononitrile ([F-18]FDDNP) which share similar synthetic approach have also been successfully synthesized. These results constitute a proof of concept for performing sequential synthetic processes at the nanogram to microgram scale in an automated fashion, and also demonstrate how integrated microfluidics can generalize, accelerate, diversify and lower the cost of labeling processes for a wide range of molecular imaging probes.

No. 246

DEVELOPMENT AND EVALUATION OF A MULTI-PINHOLE SPECT METHOD FOR A SMALL ANIMAL SPECT IMAGING SYSTEM

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The purpose of this study is to develop a 3-D multi-pinhole (MPH) single photon emission computed tomography (SPECT) method for a small animal SPECT imaging system and to evaluate the effects of MPH collimator design and imaging geometry on the reconstructed image quality. In a Monte Carlo study, MPH projection data were generated from a digitized Defrise phantom and a realistic digital mouse phantom using MPH collimators with different pinhole numbers, hole patterns and imaging geometries. A Gamma Medica-Ideas X-SPECT small animal imaging system fitted with MPH collimators of different pinhole numbers and patterns were used in an experimental study. Projection data were acquired from a physical Defrise phantom, an ultra-resolution SPECT phantom and mice injected with Tc-99m MDP. All MPH projection data were reconstructed using an iterative 3-D OS-EM based MPH image reconstruction method with accurate correction for system misalignments. The 3-D MPH images from the simulation study were compared to the phantom images and were evaluated in terms of normalized mean square error and normalized standard deviation over selected regions-of-interests (ROIâ™s). Those from the experimental studies were compared to the corresponding SPH images for image artifact generation and image noise reduction. Our results indicate that MPH SPECT provides increased detection efficiency and lower image noise as compared to SPH with concomitant increased image artifacts and distortions which are also dependent on the pinhole pattern. We conclude that MPH SPECT with significant increase in detection efficiency and minimal artifacts and distortions is feasible with careful considerations of the different contributing factors.

No. 247

INITIAL RESULTS OF A POSITRON EMISSION TOMOGRAPHY/ COMPUTED TOMOGRAPHY SMALL-ANIMAL IMAGING DEVICE WITH CO-PLANAR GEOMETRY

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In this work we report initial results from a prototype of a small-animal positron emission tomography (PET)/ computed tomography (CT) system based on a common rotating gantry. The PET system consists of two detector modules based on MLS arrays and four large-area, flat-panel type PS-PMTs. The CT scanner uses a micro-focus X-ray tube and a semiconductor X-ray detector in a cone-beam geometry. Space for opposed PET detectors and the CT scanner has been allocated on the same face of the gantry disk, thus achieving a co-planar geometry that perfectly aligns the trans-axial and axial centers for both image modality systems. Shields around the detectors reduce cross modality contamination due to scatter in the sample when it is illuminated by the X-ray source. The gantry rotates 360 degrees to provide complete data sets for the CT image reconstruction program that implements a fast version of the FDK algorithm. OSEM algorithms (2-D and 3-D) as well as FBP are available for PET image reconstruction. Sequential acquisition protocols minimize the scan duration, and CT information can be used to implement PET imaging corrections. The co-planar geometry of this system provides intrinsically co-registered datasets, and eliminates the need for animal repositioning to change modality imaging. Avoiding undesired movement of the animal or attached accessories reduces the time required to perform the experiment and minimizes movement errors. The compactness and ergonomics of the system save space and enable direct visual monitoring of the animal.

No. 248

PARALLEL HOLE COLLIMATOR DESIGN FOR A DUAL PROJECTION IMAGING SYSTEM

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Several research groups have developed tomographic imaging systems for small animal research that combine anatomical images with physiological data from nuclear imaging modalities. The radiation dose associated with these imaging systems is often high, and imaging times can be quite long, ranging from several minutes to several tens of minutes for detailed applications. Many investigators make use of 3-D data from these tomographic imaging systems to compute single projections (sagittal or coronal) or a maximum intensity projection image (MIP) to demonstrate the distribution of radioactivity within the animal. A dual nuclear/X-ray projection imaging system which combines nuclear imaging using a parallel hole collimator (PHC) with X-ray radiography, has been designed in our laboratory. A single planar detector (BaFBr plate) captures a gamma-ray image on one side of the plate and an X-ray image on the other side. These images are co-registered and fused after analog to digital conversion is completed. This dual imaging system allows for high throughput imaging, and the low radiation levels permit investigators to obtain functional information more frequently in serial studies. A PHC designed specifically for small animal imaging will be manufactured using micro-fabrication lithography techniques. The optimal design of low energy and high energy parallel hole collimators has been studied using Monte Carlo simulations. A detector array with 50 μm square pixels and a collimator with square apertures have been modeled. The tradeoff between spatial resolution and sensitivity was assessed for PHCs with aspect ratios (height/aperture size) from 10:1 to 50:1.

No. 249

***IN VIVO* OPTICAL IMAGING OF TUMORS EXPRESSING CARCINOEMBRYONIC ANTIGEN (CEA) USING ENGINEERED ANTIBODY-LUCIFERASE FUSION PROTEINS**

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Two novel tumor targeting fusion proteins have been developed which consist of an engineered antibody fused to either an optimized *Renilla* luciferase (RLuc8) or *Gaussia* luciferase (GLuc), allowing for *in vivo* optical detection of the endogenous tumor marker carcinoembryonic antigen (CEA). The genetically engineered anti-CEA T84.66 diabody (Db), a dimer of the single-chain Fv, has previously exhibited high level tumor targeting in biodistribution and microPET imaging studies using a CEA-positive tumor model. The purified Db-RLuc8 and Db-GLuc fusion proteins remain bifunctional: able to bind to the antigen, CEA, and simultaneously emit light in the presence of the substrate, coelenterazine, as shown by bioluminescence ELISA assays. *In vivo* optical imaging of tumor bearing mice demonstrated specific targeting of Db-RLuc8 and Db-GLuc to CEA-positive xenografts. The Db-RLuc8 reached a maximum tumor:background ratio of 6.0 ± 0.8 in CEA-positive tumors at 6 hours after intravenous injection, compared to CEA-negative tumors at 1.0 ± 0.1 ($p < 0.05$, n=7). The Db-GLuc similarly targets only the CEA-positive tumor, although it demonstrates different clearance properties due to the lower molecular weight. Targeting and distribution was also evaluated by microPET imaging using ^{124}I-labeled fusions and confirmed that the optical signal was due to antibody-mediated localization of luciferase. These two luciferases, fused to biospecific sequences such as engineered antibodies, can be administered systemically to provide a novel, sensitive method for optical imaging based on expression of cell surface targets in living organisms.

No. 250

EFFECTS OF MISALIGNMENT BETWEEN TRANSMISSION AND EMISSION SCANS FOR A MACAQUE MONKEY BRAIN WITH MICRO POSITRON EMISSION TOMOGRAPHY P4

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MicroPET P4 (CTI Concorde, TN, USA) is a positron emission tomography (PET) scanner for laboratory small animal study, which has 2 mm FWHM, 19 cm transverse field of view, 7.8 cm axial field of view and has capability to scan macaque monkey head. To obtain quantitative Macaque monkey head images, attenuation correction is essential. To minimize anesthetic effect on 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) accumulation, a monkey keeps awake state 45 minutes after the FDG administration. The anesthesia was started at 45 minutes after the administration and positioned in the microPET and an emission scan was started at 60 minutes after the administration. In this procedure, transmission scan could not be performed before the injection due to no anesthesia before the injection. Post Injection Transmission technique for microPET P4 had not established yet. Then, transmission scan was performed on another day, but there were slight misalignment between transmission and emission data. In this study, a numerical model of a macaque monkey head and a monkey head holder was designed by using a real macaque monkey head and head holder transmission data, and evaluated pixel value changes in brain region caused by linear or rotation misalignment of μ-map data for attenuation correction. The followings were obtained as results; 1 mm linear displacement in transaxial plane induces +/- 1.5 % error, +/- 2.5 % by 1 mm linear displacement along with the Z-axis and 1 degree rotation about all 3 axes induces 1.0 % error.

No. 251

APPLICATION OF CLICK CHEMISTRY FOR THE PREPARATION OF COX-2 AND CA II IMAGING AGENTS

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The *in situ* click chemistry approach to lead discovery uses the biological target itself for synthesizing inhibitors. Equilibrium-controlled sampling of bio-orthogonal, click chemistry enabled fragments by the biological target eventually leads to an irreversible reaction that essentially 'freezes' the fragment pair that best fits the protein's binding pockets. New cyclooxgenase-2 (COX-2) and carbonic anhydrase II (CA-II) imaging agents, containing [F-18]-fluorine, were developed using this target guided synthesis (TGS) approach. Acetylene-bearing fragments that exhibited relatively low binding affinities for the respective targets were incubated with the proteins and a library of azide-bearing fragments, leading to the formation of several hit compounds *in situ*. Traditional enzyme assays revealed these *in situ* generated hits to be potent inhibitors of the enzymes that generated them. In case of COX-2, the best inhibitor displayed an IC_{50} value of 20 nM, and in case of CA-II, IC_{50} values of 0.5 nM were achieved. The COX-2 imaging probe was identified as specific for COX-2 and inhibited endotoxin-induced PGE_2 production in RAW264.7 macrophages. Micro positron emission tomography (PET) imaging of the COX-2 probe in mice revealed uptake primarily in the liver and small intestine. The CA-II imaging agent was identified as specific for CA-II. CA-specific cellular uptake of the CA-II imaging probe was confirmed by in-vitro studies with human erythrocytes (highly expressing CA-II) and the CA-II inhibitor ethoxzolamide. MicroPET studies demonstrated accumulation of the CA-II imaging probe in organs with high CA-II expression, such as lungs and kidneys.

No. 252

A QUANTIFICATION METHOD OF SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY/ COMPUTED TOMOGRAPHY DATA FOR ANALYZING DRUG DISTRIBUTIONS IN TUMORS

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Quantification of single photon emission computed tomography (SPECT) image data is one of the important issues for applications of SPECT imaging in the cancer research. Due to random shapes of tumors under study, a quantitative method is needed for analyzing drug distributions in tumors using SPECT image data. We propose a method to quantify intensity distribution of drugs in tumors based on the iso-distance shells from the tumor surface. The procedure includes the following steps: (1) the tumor is segmented from the neighbor tissues by combining SPECT and computed tomography (CT) images of the tumor; (2) the binary 3-D tumor is created based on segmented SPECT intensity data; (3) a 3-D distance object is created for the binary 3-D tumor image in step 2. The iso-distance shells are created based on the 3-D distance image. The random topological structure of the tumor is maintained when iso-distance shells are constructed; (4) the intensity distribution for each distance shell is calculated; (5) the drug distribution in the tumor can be calculated using information from the step 4 and other information obtained independently from the tumor including the weight of the tumor, the percentage dose left in the tumor, and the volume of tumor. Statistical analysis can be carried out based on results generated from the above quantification procedure. We used Matlab computer software to implement the procedure and use 2LMP tumors with Tc-99m-labeled humanized mTRA-8 as examples to demonstrate the proposed method.

No. 253

IMAGING *IN VIVO* HEPATIC GROWTH HORMONE SIGNALING

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Bioluminescence imaging was applied to study hepatic growth hormone (GH) signaling. GH regulates postnatal growth and metabolism by interacting with cell surface GH receptor (GHR), which signals via the tyrosine kinase, JAK2. Liver is a major GH target organ, and hepatic GH response is mediated by JAK2-dependent activation of a transcription factor called signal transducer and activator of transcription 5b (STAT5b). Methods. Adenoviruses encoding the rabbit GHR (Ad-GHR) and a luciferase reporter driven by STAT5-binding GH response elements (Ad-GHRE-luc) were evaluated. *In vitro* studies tested whether Ad delivery of both genes could allow GH-dependent response. Nude female mice (n=10) were injected with Ad-GHRE-luc and Ad-GHR (1×10^9 pfu each) iv one day apart, and compared with mice injected with Ad-GHRE-luc alone (n=10) or no virus (n=5). Bioluminescence imaging (IVIS-100 system) began after four days with baseline images. Thereafter, images were obtained one, three, five and seven hours after iv GH (1 mcg/gm). Results. Infection of cells with one virus followed one day later by the other allowed a significant ($p<0.05$) GH-induced response by imaging. A substantial GH-induced liver signal was observed in mice receiving both viruses (2.2 ± 0.2-fold at the three hours peak response), which was significantly ($p<0.05$) more robust than in those not receiving Ad-GHR. This pattern was reproduced on three separate days. Conclusions. This new system allowed noninvasive monitoring of *in vivo* GH signaling serially within the same animals. This will allow important studies of GHR, JAK2, and STAT5 mutants in intact animals.

No. 254

MULTIPLE MOUSE SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY

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Large field of view gamma cameras can image several small animals at once, providing high throughput and simultaneous imaging of all members of a cohort. We have developed a device for single photon emission computed tomography (SPECT) of up to six mice using a stationary gamma camera detector (M.Cam, Siemens Medical Solutions, Hoffman Estates, IL). It consists of six acrylic tubes that surround plastic "sleds" to which anesthetized mice are gently affixed. Anesthesia is maintained through a gas inlet at the nose end of each tube and exhausted via a common scavenging system. The tubes stand upright in front of the horizontally-facing detector on a mechanism that rotates them with a common worm gear that is driven by a programmable stepper motor. The stepper motor program is dynamically generated on a small computer from user-input scan parameters and downloaded to the motor. The motion of the mice is continuous because of the absence of gantry motion control signals. Projection images are acquired in a dynamic mode to allow 256x256 matrices. A zoom factor of 2.0 is typically used, yielding a pixel size of 1.2 mm. A custom reconstruction program slices the projections into subimages centered on each tube's center of rotation and reconstructs separate images of each mouse by filtered backprojection. SPECT of capillary tubes, each containing 3.7 MBq of Tc-99m, yields point spread functions of 11 mm full width at half maximum. SPECT of mice administered 3.7 MBq of Tc-99m-MDP demonstrates the major bones, but argues for higher dosages.

No. 255

K-MEANS SEGMENTATION OF DYNAMIC PET DATA: DOES MONTE CARLO SAMPLING MEET THE NEED FOR SPEED?

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Background: Several authors have reported the benefits of using iterative K-means clustering as a means of (1) improving the signal-to-noise ratio and (2) functional segmentation of dynamic positron emission tomography (PET) data. On the characteristically large data sets associated with PET however, clustering can be a lengthy process, thereby reducing its feasibility. Monte Carlo sampling is an established means of lowering computational time, and here we investigate its effects on the functional segmentation of dynamic PET. Methods: Twenty frames of dynamic 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) data were simulated using a 128x128 mathematical phantom (background, torso, heart and tumour) and clinically-derived tissue activity curves (TACs). Motion was incorporated as sinusoidal heart beating. Average mean squared error (MSE), region identifiability and speed of clustering were compared using Monte Carlo sampling of 200, 500, 1000, 2000, 4000, and 16384 TACs. Results: Sampling of TACs prior to clustering results in a considerable increase in speed, but also an increase in MSE. However this is not a linear relationship, and results suggest there's little advantage to be gained in terms of accuracy using sample sizes above 1000. Any improvements are minor and counterbalanced by sharply increasing time penalties. Incorporation of motion leads to inaccurate size estimation and misclassification dependent on size of organs. Conclusion: Whilst there is a trade-off between speed and accuracy when sampling, results here are encouraging. However, we must also consider the potential limitations of applying such clustering techniques in general with respect to clinical data, including the effects of object size and motion.

No. 256

THE BIODISTRIBUTION OF COBALAMIN IN MURINE BRAIN TISSUE: CORRELATION TO A MURINE ALZHEIMER'S MODEL

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Objective: To demonstrate the biodistribution of adenosylcobalamin, the most prominent form of cobalamin in mammalian cerebral parenchyma, in both wild-type (WT) and Alzheimer dementia-type (AD) murine brains. Method: Ten WT and two AD mice were injected intra-peritoneally with 1 mCi of the radiotracer indium 111-Adenosylcobalamin (In111-AC), and sacrificed 24 hours later. Mouse brains were perfused with formalin and sucrose, extracted, and frozen in 2-methyl-butane at -70 degrees celsius. Thirty micron brain slices were obtained using a cryostat, and placed on slides. Every other slide was stained with H&E, and an autoradiographic emulsion was applied to all the slides. The slides were developed after seven days of exposure. Silver grains representing In111-AC activity were counted in multiple brain regions for both brain types, and compared. Results: In111-AC was most avidly concentrated in murine choroid plexus and pituitary gland. The AD mice had increased uptake compared to the WT mice in the thalamus, cerebral cortex, hippocampus, and corpus callosum. Uptake in the choroids plexus of AD mice was decreased compared to the WT mice. No uptake was observed in regions of amyloid plaques in the AD brains. Conclusion: This study depicts for the first time the cerebral biodistribution of adenosylcobalamin in a murine model. The generalized increased uptake of adenosylcobalamin throughout much of the AD brains requires further explanation. The lack of In111-AC uptake in amyloid plaques likely indicates cell death; however the relationship between cellular cobalamin metabolism and plaque formation in Alzheimer dementia remains unclear.

No. 257

ANIMAL MODEL FOR *IN VIVO* INVESTIGATION OF THE ENDOTHELIN SUBTYPE A RECEPTOR WITH PET IN PROSTATE CARCINOMA

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The successes of anti-angiogenesis therapies using endothelin antagonists will depend on the presence of the endothelin receptors. *In vivo* endothelin receptor imaging could make the difference between failure and success when endothelin antagonists are chosen as a treatment option. Our results show that the endothelin subtype A receptor (ETAR) is expressed in a high proportion of human prostate carcinomas. In all 20 human tissues examined ETAR mRNA was easily detected both in cancer and normal specimens. ETBR mRNA was expressed in six of twenty tumors, fifteen of twenty normal specimens. Immunostaining data were consistent with RT-PCR results. This molecular basis suggests that it is feasible to develop an ETAR based *in vivo* imaging method for localizing and characterizing human prostate cancer and its metastases. We developed a mouse prostate cancer model with a well characterized and reproducible endothelin receptor profile. PCR analysis of human prostate cancer cell lines identified that ETAR mRNA was high in PC3 and almost undetectable in 22Rv1 cell lines. SCID mice were implanted subcutaneously in one shoulder with 22Rvl and the other with PC-3. Fifteen days after implantation the animals were sacrificed and subjected to *ex vivo* analyses to ascertain stability of receptor expression. High ETAR protein and mRNA expression were detected in the ETAR positive tumor xenograft by immunohistochemistry, autoradiography and RT-PCR. Low receptor expression was seen in ETAR negative tumor. To assess the feasibility of PET, two radioligands, non-selective [C-11]L-753,037 and ETAR selective [C-11]ABT-627, are being further investigated with this new animal model.

No. 258

POSITRON EMISSION TOMOGRAPHY/ COMPUTED TOMOGRAPHY FLY-THROUGH VIRTUAL BRONCHOSCOPY: A MODEL FOR SYSTEMATIC INVESTIGATION OF 3-D VISUALIZATION

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Objectives: Virtual bronchoscopy using 2-deoxy-2-[F-18]fluoro-D-glucose (FDG)- positron emission tomography (PET)/ computed tomography (CT) is a promising tool relevant to diagnostics, treatment planning, and interventional guidance. In order to realize the clinical utility of this modality, a more thorough understanding of quantitative limitations is required. Here we present a physical model for investigating 3-D visualization of endoluminal lesions of the airways using virtual PET/CT bronchoscopy and demonstrate the application of this model for studying the sensitivity for detection of lesions of different sizes, locations, and activity. Methods: An anatomically accurate plastinated porcine heart-lung phantom was used to simulate the airways and peripheral structures. Capsules of various sizes between 4-10mm were designed and filled with physiologically relevant levels (1-5 μCi) of Fluorine-18 activity and were introduced into the trachea using a flexible bronchoscope and placed at locations along the bronchial tree. PET and CT images were acquired sequentially on a GE Discovery LS PET-CT scanner and reconstructed at 2.5mm and 5mm using the filtered-backprojection and OSEM algorithms respectively. PET and CT images were visualized as a virtual bronchoscopy and as a volume-rendered fly-around image using GE Volume Viewer Plus, a commercial software tool. Fly-around perspective images clearly show focal tracer activity with respect to anatomical landmarks. Conclusions: The anatomical airway model described is a powerful tool for systematic investigation of 3-D fly-through virtual bronchoscopy and fly-around visualization for PET/CT images. Future work may simulate more realistic conditions including background activity, variation in lesion morphology, as well as lesions located in regions peripheral or adjacent to the airways.

No. 259

AN INTERACTIVE WHOLE BODY MOUSE ANATOMY VISUALIZATION AND REALISTIC MICROCT/MICROPET IMAGE SIMULATION TOOL - A KINETIC IMAGING SYSTEM (KIS) EXTENSION

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The Kinetic Imaging System (KIS) developed at UCLA (Huang, 2004) has provided significant functionalities of learning, planning, design, and data analysis of mouse micro positron emission tomography (PET) studies. However, KIS is currently short on 3-D visualization capability and scanner simulation for microCT/microPET image generation that are desirable for research and teaching purposes. In this study, we extend KIS by developing an additional subsystem to address these two major aspects. The underlying data for this system is the 4-D digital mouse phantom data (Segars, 2004). The subsystem is coded in Java for easy consolidation with the main KIS system. The Java 3-D package is exploited to render the 3-D mouse in an interactive way to give a good perspective of the anatomy of major internal organs. With the system, users can use computer mouse to control the viewing angle of the whole mouse, or to pick any organ for its anatomical information and radio-activity level. Users can also implant spherical tumors "virtually" with a selectable size and density into the normal anatomy. After a cross-section in the mouse is selected, the corresponding microCT and microPET images will be simulated immediately. The users can easily change the imaging parameters ("injected dose," reconstruction filter, etc) to simulate different imaging conditions/scanners. The parameter values that set the radio-activity distributions among different organs or tissues can be easily changed and saved to disk. They can be downloaded from or uploaded to a shared repository on the Internet for easy comparison/reference among users in different institutions.

No. 260

***IN VITRO* EVALUATION OF ^{18}F-2,3,5,6-TETRAFLUORO-3'-SULFAMOYLBENZANILIDE AS A POTENTIAL PET PROBE FOR CARBONIC ANHYDRASE IX**

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Carbonic Anhydrase IX (CA IX) is a transmembrane enzyme that is transcriptionally regulated by the hypoxia-inducible factor 1 (HIF-1). CA IX has been found overexpressed in a variety of human solid tumors. The strong relationship between CA IX expression and treatment outcome in the clinic, as well as its easily accessible extracellular active site, make CA IX a promising target for imaging. The CA IX inhibitor, 2,3,5,6-tetrafluoro-3'-sulfamoylbenzanilide was labeled with fluorine-18 (^{18}F-TFSB) and its uptake by HeLa, Panc1, and HT-29 cells was investigated under normoxic (21%O_2) and hypoxic conditions (0.5%O_2), with RCC4 and Caki-1 as positive and negative controls for CA IX respectively. The cell uptake results were compared with western blots and uptake of ^{64}Cu-ATSM under the same conditions. Significant higher uptake of ^{18}F-TFSB was observed under hypoxia conditions in HeLa and HT-29 cells ($p<0.05$), which mirrored the trends in CA IX expression seen in western blots. All three cell lines showed significant increased ^{64}Cu-ATSM uptake after being exposed to hypoxia ($p<0.05$).These results indicate the potential of ^{18}F-TFSB as a PET tracer for CA IX. This molecular-specific probe may complement hypoxia-specific tracers such as ^{64}Cu-ATSM to better understand tumor hypoxia and hypoxia-induced physiology.

Table 1. Uptake of 18F-TFSB and 64Cu-ATSM in cells (% applied radioactivity per 10^6 cells)

Cell Lines	RCC4	Caki-1	HeLa (N)	HeLa (H)	Panc1 (N)	Panc1(H)	HT-29 (N)	HT-29 (H)
^{18}F-TFSB	6.9±1.4	2.0±0.5	1.2±0.6	5.1±2.5	1.7±0.8	1.7±0.6	1.2±0.5	5.5±1.7
^{64}Cu-ATSM	10.2±3.4	7.5±0.5	9.6±0.8	7.9±0.8	1.9±0.4	9.7±1.4	1.8±0.2	3.2±0.3

No. 261

***IN VITRO* CHARACTERIZATION OF PNA-CONJUGATED ANTI-CEA MOUSE MONOCLONAL ANTIBODY T84.66**

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DNA analogs including peptide nucleic acids (PNAs) and morpholino have been tested as antibody conjugates in pretargeting, with DNA analog-antibody conjugation ratios of ~0.2. Higher conjugation ratios may result in improved contrast performance by keeping injected dose of DNA analog constant while decreasing the injected dose of antibody. A concern is the potential for decreased immunoreactivity as conjugation ratios increase. Our group used fluorescent cell-based assays to evaluate the specificity of PNA-antibody (T84.66) against CEA-positive colorectal cancer LS174T cells, with CEA-negative rat glioma C6 cells used as control. The binding affinity of PNA-antibody for CEA was evaluated using surface plasmon resonance (SPR). Equivalent tests were performed on unconjugated T84.66 IgG antibody for positive-control comparison. PNAs were conjugated to the anti-CEA T84.66 antibody with an average conjugation ratio of 1.5:1 as demonstrated by MALDI-MS. The fluorescent cell-based assays showed specific binding of PNA-antibody to LST174T cells, with similar intensity

to that of unconjugated antibody. SPR measurements showed dissociation constants k_D of 10^{-9} M for both conjugated and unconjugated antibodies. These results indicate that PNA-antibodies with high conjugation ratios do not appear to suffer from significant affinity and specificity losses, and should exhibit similar *in vivo* targeting and pharmacokinetic properties as unmodified antibodies.

No. 262

REROUTING LIPOPROTEINS TO SELECTED ALTERNATE RECEPTORS: A NATURE'S NANOPLATFORM FOR TARGETED DELIVERY OF DIVERSE IMAGING AND THERAPEUTIC AGENTS

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We report a new concept, a lipoprotein-based nanoplatform (LBNP) generated by conjugating tumor homing molecules to the protein components of naturally occurring lipoproteins in order to reroute them from their normal lipoprotein receptors to other selected cancer-specific receptors. Multiple copies of these targeting moieties may be attached to the same nanoparticle, or a variety of different targeting moieties can be attached. Such a diverse set of tumor-homing molecules could be utilized to create a variety of conjugated lipoproteins as multifunctional, biocompatible, nanoplatforms with broad application to both cancer imaging and treatment. The same principle can be applied to imaging and treatment of other diseases and for monitoring gene expression and for stem cell tracking. For proof-of-concept, a low-density lipoprotein (LDL)-based folate receptor (FR)-targeted particle was prepared by conjugating folic acid to the apoB-100 protein. The particles were either surface-labeled with the optical reporter DiI or DiR or core-labeled with a lipophilic PDT agent SiPc-BOA. Cellular localization of the LBNP was monitored by confocal microscopy and flow cytometry in FR overexpressing KB cells, FR nonexpressing CHO and HT-1080 cells, and LDL receptor (LDLR) overexpressing $HepG_2$ cells. *In vivo* receptor-specific uptake of the LBNP was confirmed by a Xenogen imager using a nude mouse bearing KB and HT1080 tumors on its opposite flanks. After injection of the LBNP, the fluorescent label ended up in only the folate positive tumor. These studies demonstrate that folic acid conjugation to Lys □-amino groups blocks the LDLR binding and reroutes the functionalized LDL nanoparticles to FR.

No. 263

FUSION OF POSITRON EMISSION TOMOGRAPHY IMAGES TO CHARACTERIZE mGluR5 LIGANDS IN A 6-OHDA INDUCED RAT PD MODEL

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The aim of this study is to perform fusion of positron emission tomography (PET) images to investigate 6-OHDA induced modulation of dopaminergic-glutamatergic receptor functions in different brain areas including striatum, hippocampus and cortex.
A series of PET imaging studies were conducted one month after a unilateral striatal lesioning with 6-OHDA. The lesion severity was investigated with [C-11]CFT (2□-carbomethoxy-3□-4fluorophenyltropane) based on dopamine transporter function. Four different ligands, [C-11]MPEP (2-[C-11]methyl-6-(2-phenylethynyl)pyridine), [C-11]M-MPEP (2-(2-(3-[C-11]methoxyphenyl)ethynyl)pyridine), [C-11]M-PEPy (2-(2-(5-[C-11]methoxypyridin-3-yl)ethynyl)pyridine) and [F-18]MTEP (2-[F-18]fluoro-5-(2-(2-methylthiazol-4-yl)ethynyl)pyridine), were used to investigate striatal lesion induced modulation of metabotropic glutamate subtype 5 receptors (mGluR5). To characterize ligands in different brain areas, it is required that the time activity curves (TACs) are generated from the same anatomical region's of interest (ROIs) such as striatum, hippocampus or cortex. Supposed there is no brain deformation during the series of studies, the PET image of one mGluR5 ligand could be merged/fused manually to the corresponding PET image of [C-11]CFT on a pixel-by-pixel basis on a RAID workstation. The fusion software was ASIPro 6.0. The merging procedures relied on using Harderian glands and olfactory bulbs as internal markers as well as utilizing the whole brain contours. After obtaining TACs, the final data analyses were done using Logan linear model to calculate binding parameters. Manual image fusion is helpful and effective, though time-consuming, in analyzing data generated from the same anatomical area.

Clinical Poster Presentations

No. 264

STANDARDIZED UPTAKE VALUES IN Ga-68-DOTATOC POSITRON EMISSION TOMOGRAPHY

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Purpose: [Ga-68]DOTA-D-Phe^1-Tyr^3-Octreotide (DOTATOC) is a highly specific positron emission tomography (PET) tracer for the detection of somatostatin receptor (SSTR) positive tissues. While it is not problematic to recognize pathological uptake in areas with little background, the uptake in organs with physiologically high density of SSTR is difficult to interpret. Based on our DOTATOC-PET data, maximum of standardized uptake values (SUVs) for different organs were determined. A set of SUV ranges for normal tissues was derived. Methods: Organ related maximum SUVs (SUVmax) were determined in DOTATOC-PET studies of 120 consecutive patients without abnormalities in the organs under consideration, confirmed by histology or follow-up. We calculated tolerance intervals which contain 95% of the population with a probability of 90%. Results: The normal tissue ranges derived from out data are given in the table below. Conclusion: Normal tissues show broad variation of DOTATOC-SUV. The normal ranges here presented provide a useful tool for quantitative assessment of individual organ uptakes.

	Liver	Kidney	Adrenal glands	Spleen	Pituitary gland
Median	8.3	9.5	8.8	24.5	5.4
95% tolerance interval	4.5-13.3	4.9-22.4	4.1-16.7	8.2-48.7	2.2-13.5
	Brain	Orbita	Thyroid	Intestines	
Median	0.3	0.7	2.5	4.0	
95% tolerance interval	0.1-0.6	0.4-1.3	1.2-5.1	1.9-6.9	

No. 265

COMPARISION OF 2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE-POSITRON EMISSION TOMOGRAPHY, COMPUTED TOMOGRAPHY AND POSITRON EMISSION TOMOGRAPHY/ COMPUTED TOMOGRAPHY IN STAGING OF PATIENTS WITH LOW GRADE NON-HODKIN LYMPHOMA

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Objective: The aim of this study was to compare the accuracy of clinical staging with integrated 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) - positron emission topography (PET) / computed tomography to that of positron emission tomography (PET) and computed tomography (CT) alone, in patients with low grade non-Hodgkin lymphoma (LGNHL). Patients and Methods: Thirty four patients (19 f, 13 m; age range 37 - 86 years) with LGNHL underwent initial staging (n=13) or restaging (n=20) with contrast enhanced FGD-PET/CT. PET and CT images were interpreted independently and separately by two experienced readers blinded to any clinical information. PET/CT images were read in a joint session by both readers. Based upon image interpretation, a stage was assigned using the Ann Arbor classification and lymph node region conventions set by the Rye Symposium. PET/CT findings were either confirmed by other imaging modalities, biopsy and/or clinical follow up. Results: Compared to CT,

PET/CT correctly upstaged five patients (16%). PET/CT identified the correct stage in an additional eight patients (25%) compared to PET alone. PET/CT correctly upstaged five patients and correctly downstaged three patients. Overall PET/CT correctly staged 25 patients (78%), PET 19 (59%) and CT 20 (63%). Conclusion: PET/CT improves the staging of LGNHL and would have led to significant changes in staging of patients compared to both PET and CT.

No. 266

COMPARISON BETWEEN 2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE- POSITRON EMISSION TOMOGRAPHY/COMPUTED TOMOGRAPHY AND BODY DIFFUSION WEIGHTED IMAGING FOR TUMOR EVALUATION IN CANCER PATIENTS ON THE SAME DAY

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Purpose: A new way of body diffusion weighted imaging using the short TI inversion recovery-echo planar imaging (STIR-EPI) sequence and free breathing scanning (diffusion weighted whole body imaging with background body signal suppression; DWIBS) is similar to 2-deoxy-2-[F-18]fluoro-D-glucose (FDG)- positron emission tomography (PET) imaging. We compared DWIBS and FDG-PET/ computed tomography (CT) to investigate which modality is more useful for evaluation of malignant tumors. Methods: The 22 malignant and seven benign lesions were evaluated. In the PET/CT study, the patients received FDG 60 minutes prior to image acquisition. The DWIBS were acquired at 1.5T using a single-shot EPI sequence (TR/TE=4222-5009/70ms, 10NEX, b=1000s/mm2). The ability of cancer detection was evaluated in these images visually. The ADC value (□10-3 mm2/sec) and the standardized uptake value (SUV) in the cancer lesions were evaluated and compared for quantitative evaluation of DWIBS and FDG-PET, respectively. Results: Nineteen (88.0%) of the 22 malignant lesions were detected as positive in DWIBS by visual inspection, whereas FDG-PET/CT detected 14 lesions (63.6%), suggesting that DWIBS has a better special resolution than FDG-PET. Our quantitative evaluation study indicated that there were significant differences between the SUV values of benign (n=7, 1.28+/-0.61) and malignant (n=14, 4.64+/-2.54) lesions (p<.01), however, there was no significant correlation between the ADC values of benign (n=7, 1.54+/-0.24) and malignant (n=16, 1.42+/-0.68) lesions, suggesting that the ADC values might not be conclusive in distinguishing between the benign and malignant lesions. Conclusion: DWIBS seems to be feasible in the detection of cancers, but it may be difficult to differentiate between the benign and malignant lesions using the ADC values.

No. 267

VISUALIZATION OF BETA-AMYLOID PLAQUES IN NONDEMENTED ELDERLY WITH [C-11]PIB: EARLY INVOLVEMENT OF PRECUNEUS?

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Background. Alzheimer's disease (AD) is characterized by the widespread brain deposition of beta-amyloid (Abeta) plaques. A positron emission tomography (PET) radiotracer, N-methyl-[C-11]2-(4'-methylaminophenyl)-6-hydroxybenzothiazole, or [C-11]PIB (for "Pittsburgh Compound B"), binds to Abeta *in vivo*, and can be used to image the distribution of plaques. A significant difference in PIB binding has been found between DAT subjects and controls. We used PIB PET imaging in a cross-sectional study of non-demented elderly controls to investigate which regions demonstrate the earliest deposition of Abeta plaques. Methods: Forty-one nondemented subjects (age range 18 to 86 years) underwent PIB PET and magnetic resonance imaging (MRI) scanning. Eight different regions-of-interest were drawn on the MRI, and Logan graphical analysis was used to calculate binding potentials (BPs) for PIB using cerebellum as a reference. BP values from the younger subjects were used to calculate a threshold (> 2 S.D.) for elevated PIB binding in the older subjects. Results. DAT patients had significantly elevated mean cortical BP values (0.633 ± 0.351) compared to nondemented subjects (0.052 ± 0.169), $p < 0.0001$. Four of the non-demented subjects demonstrated elevated mean cortical BP (0.472 ± 0.255). In these four, the precuneus had greatest binding across all regions (0.725 ± 0.243). Conclusions. The precuneus may show Abeta plaques early in development of AD. More subjects and longitudinal follow-up are needed to determine the actual risk of future AD in this population, but the potential identification of the earliest regions of Abeta deposition could lead to a diagnostic and therapeutic target for Alzheimer's disease.

No. 268

2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE AS A MARKER FOR THE EFFICACY OF TUMOR TRATMENT WITH 2-DEOXYGLUCOSE (2-DG)

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Objectives: Accelerated glycolysis is a hallmark of cancer. 2-Deoxyglucose (2-DG) and 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) are glucose mimetics that are transported into the cell via Glut 1, phoshporylated to 2-DG-6-P and trapped intracellularly. We hypothesized that the degree of FDG uptake correlates with increased cell sensitivity to 2-DG. Methods: Baseline FDG uptake was measured in 10 different cell lines. Cells were plated and after two days, media was removed. 2ml of FDG-in media (1□Ci FDG/ml media) was added to each well for one hour. Cells were then washed and trypsinized for gamma counting. The sensitivity of cells to 2-DG was also determined. After cells were plated, a 0.1 mg/ml or 0.4 mg/ml dose of 2-DG was added. The cytostatic effect of 2-DG was determined three days post incubation. Baseline FDG uptake (no treatment) was expressed as the ratio of intracellular over extracellular concentration. Results: HCC4006 cells (lung cancer) exhibited the highest uptake of 10.8 ± 1.19, while LN18 cells (brain cancer) showed the lowest uptake of 2.3 ± 0.23. Sensitivity to 2-DG was highest in HCC4006 cells and lowest in LN18. Doses of 0.1 mg/ml and 0.4 mg/ml 2-DG resulted in a 40% and 66% decreases in HCC4006 cell numbers, respectively, with no effect seen in LN18 cells. Overall, FDG uptake and 2-DG sensitivity tended to correlate (r^2= 0.59; p=0.07). Conclusions: Tumor cell sensitivity is correlated to the degree of FDG uptake *in vitro*. The degree of FDG uptake might therefore be a useful biomarker for predicting the response to 2-DG therapy.

Drug Development Poster Presentations

No. 269

NOVEL INTEGRIN BINDING PEPTIDES FOR CANCER IMAGING AND THERAPY

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We had previously reported on the discovery of novel alpha-3 integrin binding peptides through screening "one- bead one-compound" combinatorial cyclic peptide libraries with live ovarian adenocarcinoma cell lines. We have shown that these ligands can be used as effective probes to image ovarian tumor xenografts both optically and with positron emission tomography. Based on the results obtained from the primary and secondary library screens, we have designed and synthesized four tertiary libraries that can be used to further optimize our lead compounds. These libraries include (a) main chain modification with three diversity points, (b) side chain modification with three diversity points, (c) main chain and N-terminal modifications, and (d) N-terminal extensions with two diversity points. By using this sequential iterative screening approach with a number of different combinatorial libraries, we can rapidly probe the conformational spaces surrounding the initial lead compound. We have also identified other novel peptides from random peptide library screening that have a common motif that bind to ovarian adenocarcinomas as well as transition cell tumors. Preliminary data shows that one of these identified peptides preferentially binds to beta-1 integrin. These peptides could

potentially be used as carrier to delivery radionuclides or cytotoxic drugs to the target tumor.

No. 270

SYNTHESIS AND INITIAL BIODISTRIBUTION OF RADIOIODINATED NAPHTHYLALANINE DERIVATIVES IN NORMAL AND DIABETIC MICE

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Non-invasive monitoring the progression and response to therapy of diabetes by imaging will add to the understanding of the disease. Derivatives of naphthylalanine have been reported to bind to somatostatin receptors (SSTRs) with very high affinities. We report the synthesis and evaluation of □-and □-naphthylalanine derivatives affinities for SSTRs on the pancreatic □-cells. Methods: The synthesis of the two isomers involved condensation of the acid chloride of naphthylalanine to a mono protected hexanediamine derivative. The conjugate was radioiodinated by electrophilic iododestannylation on a tin precursor and purified by chromatography. These radiotracers were then evaluated *in vitro* by radioligand binding assay on CHO cell line expressing SSTR2 and also isolated mouse pancreatic islets. Lastly biodistribution studies were carried out in normal CBA/J as well as NOD mice. Results: The radioligand studies showed that the compound had low affinity for the SSTR2. Approximately 20% of [^{125}I]-Tyr-SS14 could be displaced by the ligand from the islets. However, biodistribution in normal mice showed a significant uptake in the pancreas. This uptake could be reduced by co-injection of excess of the unlabeled compound. The pancreatic uptake of radioactivity in the NOD mice was significantly lower than in the normal mice ($p<0.05$). The □-isomer showed higher retention than the □-isomer. Conclusion: The pancreatic uptake of □-isomer of the radioiodinated peptidomimetic was significantly higher in the normal CBA/J as compared to the NOD at all time points studied. This decrease may be directly related to the destruction of the □-cells in these mice.

No. 271

SURGICAL REMOVAL OF THE HARDERIAN GLANDS: A METHOD TO ELIMINATE CONFOUNDING RADIOACTIVITY IN IMAGING OF THE RAT BRAIN AS ASSESSED BY 2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE MICROPET

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Rat harderian glands accumulate a number of positron emission tomography (PET) radiotracers, including 2-deoxy-2-[F-18]fluoro-D-glucose (FDG). In microPET imaging of the brain, proximity of these glands to the frontal cortex may cause errors in quantification due to limited spatial resolution. Surgical removal of the harderian glands may eliminate interfering radioactivity. Two groups of six male Sprague-Dawley rats were studied by FDG microPET: harderian gland intact (HG) and surgical removal of harderian glands (HGx). Imaging studies commenced two weeks post surgical procedure. Rats were briefly anesthetized under isoflurane during i.v. tail vein injection of FDG, then after a 30 minute uptake period, reanesthetized and scanned in a Concorde Microsystems R4 microPET scanner. Images were scaled to standardized uptake value (SUV) and coregistered to a stereotaxic rat brain atlas. Images between groups were tested using Statistical Parametric Mapping software (SPM2). Whole brain SUV was not significantly different between HG (2.6) and HGx (2.9) groups. SUV over harderian gland site was significantly different between HG and HGx groups (4.9 and 1.7, $p<0.001$). Regionally, t-maps showed significant differences in focal areas bilaterally within the frontal cortex between HG and HGx groups. Given the limited spatial resolution of microPET, harderian gland surgical removal is a practical and simple procedure that may prove useful in microPET imaging of radiopharmaceuticals showing high accumulation in the glands.

No. 272

STRUCTURAL ELUCIDATION OF ANTI AND SYN DIASTEREOMERS IN TARGETED RADIOPHARMACEUTICALS AS IMAGING AGENTS

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Neotect^{TM} (^{99m}Tc depreotide), which is used for imaging deep vein thrombosis and lung tumors, forms two species due to the chirality of its N_3S backbone ligand. Mass spectrometry, infrared spectroscopy and two-dimensional NMR suggest that these two species are diastereomers. However, the data could not identify the diastereomers as the syn or anti species and correlate them to their HPLC profiles. It is critical to identify diastereomers in radiopharmaceutical preparations because the biological behavior may be significantly different, as it is in the case of ^{99m}Tc depreotide. We have recently prepared a number of model tripeptide ^{99}Tc complexes varying the amino acids (L configuration) with cysteine at the carboxylate terminus to anchor and stabilize the complex. We have isolated and crystallized the ^{99}Tc diastereomers. Their color profile, reverse-phase HPLC profile and NMR and Circular Dichroism spectroscopy match exactly with depreotide diastereomers. Thus, now the specific structures of the diastereomers of Tc complexes of N_3S tripeptides can be understood. We discovered that the amino acid residues dictate the dynamic interconversion behavior of the diastereomers. Moreover, the residues have the potential to stabilize one diastereomer over the other. Stabilization of selected diastereomers is important to maximize the biologically active species and to minimize byproducts in radiopharmaceutical kits.

No. 273

***IN VIVO* ULTRAHIGH RESOLUTION ECG GATED ^{99M}TC TETROFOSMIN PINHOLE SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY IMAGING OF MYOCARDIAL FUNCTION IN TRANSVERSE AORTIC CONSTRICTION MURINE MODEL OF HEART FAILURE**

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Purpose: Determine the feasibility of ultrahigh resolution ECG gated ^{99m}Tc tetrofosmin single photon emission computed tomography (SPECT) imaging in transverse aortic constriction (TAC) model of heart failure in mice. Methods: Normal control C57/B6 wild type mice (NL; n=3) and TAC mice (n=4) underwent ultrahigh resolution ECG gated pinhole SPECT. Transverse aortic constriction was produced by surgical banding of the aorta under general anesthesia. After 12 weeks of pressure overload, cardiac function was measured with M-mode echocardiography. Short axis mid-ventricular % fractional shortening (FS) was defined as (end-diastolic diameter-end-systolic diameter)/end-diastolic diameter*100%. All mice received ~12 mCi of ^{99m}Tc tetrofosmin via tail vein or retro-orbital sinus injection. ECG gated SPECT was obtained for 45 minutes under general anesthesia with a 3-detector system. Acquisition parameters included: 1.0 mm tungsten pinholes(3), step and shoot 2 degrees/step, 8 time bins/ECG cycle, 64x128 matrix, radius of rotation 2.7cm. Reconstruction parameters included: iterative reconstruction, 3 iterations, voxel size 0.125□l/voxel. Image analysis was performed with commercially available software (Cedars-Sinai QGS-QPS). Subjective visual interpretation of gated SPECT was performed without knowledge of M-mode echocardiography results. Results: All mice survived the imaging procedure. Quantitative analysis showed significantly higher mean LVEF in NL compared to TAC mice (68%±15% versus 36%±14%, respectively; $p=0.02$). Visual interpretation of SPECT showed the most severely hypokinetic TAC study with the lowest LVEF (21%). This corresponded to the lowest FS by echocardiography (26%). Conclusions: Ultrahigh resolution ECG gated ^{99m}Tc tetrofosmin pinhole SPECT imaging is a feasible method to assess myocardial function in the TAC murine heart failure model.

No. 274

PREPARATION AND *IN VIVO* IMAGING OF SM-153 LABELED HUMANIZED ANTI-TAG72 ANTIBODY IN LS174T TUMOR BEARING NUDE MICE

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Humanized anti-TAG72 antibody is reactive and affinity-maturated with the tumor-associated antigen TAG72. Sm-153 has favorable radiation characteristics (T 1/2=46.7 h, beta and gamma ray emitters) to image during therapy. We investigated the labeling condition and imaging of Sm-153 labeled humanized anti-TAG72 antibody in tumor bearing mice. For the chelation of Sm-153, anti-TAG72 antibody was conjugated with isothiocyanato benzyl diethylene triamine penta-acetic acid (CITC-DTPA) in borate buffer pH 8.5. FITC to antibody conjugation method was used to confirm that CITC-DTPA to antibody conjugation molar ratio was how to affect the affity of antibody. Reaction molar ratio of antibody to FITC was 1:10, 1:20, 1:40, 1:80. reaction mixtures were purified by desalting column. Immunoreactivity of FITC conjugated antibody was confirmed with flow cytometry. For the Sm-153 labeling, Sm-153-Cl_3 (1.9 Ci/mg) was added to CITC-DTPA conjugated anti-TAG72 antibody in acetate buffer pH 5.4.). *In vivo* imaging was performed to confirm the tumor-targeting ability of Sm-153 labeled anti-TAG 72 antibody in nude mice bearing LS174T human colon cancer. Optimal reaction molar ration of antibody/FITC was 1:20. In this condition, 5 FITC was conjugated to a antibody. Antibody/CITC-DTPA=1:20, this conjugate was labeled with Sm-153. The labeling yield of ^{153}Sm labeled antibody was 96% after three hours incubation. In tumor imaging studies with pinhole collimator equipped gamma camera, Images of Sm-153 labeled anti-TAG72 antibody showed selective tumor localization from three hours to four days after injection. These images of Sm-153 labeled anti-TAG72 antibody demonstrate that ^{153}Sm labeled antibody may be useful for imaging and therapy.

No. 275

FBASB: AN F-18 FLUOROBENZYL ANALOGUE OF DASB. ITS SYNTHESIS, *IN VITRO* BINDING, AND *IN VIVO* BIODISTRIBUTION STUDIES

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Purpose: C-11 DASB is widely used positron emission tomography (PET) ligand for SERT imaging. We and others are developing F-18 analogue of DASB to facilitate SERT imaging studies at centers without on-site cyclotron. Recently, we have synthesized FBASB, an N-(4-fluorobenzyl) analog of DASB. Method: The FBASB was synthesized by replacing N-methyl group of DASB with N-(4-fluorobenzyl) group and tested *in vitro* for its affinity to SERT. The F-18 FBASB was obtained via N-alkylation of DASB precursor (MASB) with [F-18]FBI followed by HPLC purification. *In vivo* tissue biodistribution studies were performed in normal mice at 30 and 60 min. Results: *In vitro* binding of FBASB to SERT was 0.51 μM ± 0.113 μM. Radiochemical yield for FBASB was 36 ± 8% with radiochemical purity of 99+%. The percent injected dose (%ID) of F-18 in mouse brain was 0.34 ± 0.02 (C-11 DASB: 0.89 ± 0.39 %ID in rats) at 30 min. F-18 uptake as %ID per gram of tissue in mice at 30 minutes was 0.26 ± 0.19, 0.09 ± 0.02, 0.13 ± 0.1, and 0.05 ± 0.01 in liver, spleen, lung, and blood, respectively. A two to twelve fold lower F-18 uptake was seen in normal tissues for FBASB compared to C-11 DASB, resulting in favorable brain to normal tissue ratios for FBASB. Conclusion: FBASB showed preferential binding to SERT *in vitro*, albeit to lower magnitude than DASB. Favorable brain to normal tissue ratio for FBASB appears attractive. Further studies are required to fully assess the potential of this ligand.

No. 276

SYNTHESIS, *IN VITRO* BINDING, RODENT BIODISTRIBUTION, AND NON-HUMAN PRIMATE PET IMAGING USING F-18 FIPPT

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Purpose: F-18 labeled N-4-(fluorobenzyl)-2□-propanoyl-3□-(4 chlorophenyl)tropane (FCT) was developed to study imaging of dopamine transporters and showed excellent *in vivo* positron emission tomography (PET) images in monkeys. We further modified tropane substitution pattern to develop F-18 labeled SERT imaging agent based on the FCT template. Method: We replaced 4-chlorophenyl group of FCT with a 4-isopropylphenyl group to synthesize FIPPT and tested *in vitro* for DAT and SERT activity. After developing F-18 radiolabeling procedure, *in vivo* biodistribution studies were performed in mice at 30 and 60 minutes. Positron emission tomography images were acquired in rhesus monkey using 4.5 mCi injection of [F-18]FIPPT Results: The calculated logP remained unaltered for the two analogs (FIPPT 5.94; FCT 5.68). FIPPT showed excellent *in vitro* binding to SERT (SERT: 0.29 nM ± 0.08 nM; DAT: 38.7 nM ± 4.9 nM) with SERT to DAT ratio of 133. The F-18 FIPPT was obtained via N-alkylation using [^{18}F]FBI, and desired product purified on HPLC in 28 ± 6% radiochemical yields. Percent injected dose per gram of tissue in mice at 30 min post injection was 0.80 ± 0.11, 1.41 ± 0.39, 1.54 ± 0.05, 0.13 ± 0.18 and 0.16 ± 0.00 in liver, spleen, lung, blood, and brain respectively. The monkey PET images showed no F-18 uptake in the brain. Conclusion: Structure modification to FCT molecule yielded a compound with excellent *in vitro* binding to SERT. Tissue distribution studies showed low F-18 uptake in mouse brain. Further modifications are necessary to yield promising F-18 labeled SERT ligand.

No. 277

THE BIODISTRIBUTION OF ANNEXIN V IN THE HEARTS OF PATIENTS RECEIVING CHEMOTHERAPY FOR BREAST CANCER

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Tc-99m-Annexin-V has been proposed for imaging cardiotoxicity secondary to chemotherapy (CTx). Objective: To determine if there is non-specific uptake of Annexin V in the heart during CTx in the absence of cardiac symptoms. Methods: 6 females who received CTx (group 1) and 4 control (group 2) were imaged. Whole body and static images of the chest were obtained at 30 minutes, two and 24 hours after injection of 25 mCi of Annexin V. Regions of interest (ROI) were drawn around the heart (H), mediastinum (M), lung (L) and background (Bk). Ratios for H/M, H/L, H/Bk between the two groups were compared. Follow-up of the patients' cardiac status was performed for 9-11.5 month after imaging. Results: The mean age was 54.4 years. The mean ratios at the different imaging intervals are as follows:

Imaging (hr)	Group	H/M	H/L	H/Bk
1.1	1	1.08	1.70	3.07
	2	1.05	1.27	3.30
2	1	1.06	1.71	3.08
	2	1.10	1.47	2.77
24	1	1.06	1.66	2.63
	2	1.25	1.72	3.20

There was no significant difference between the two groups except in the H/M ratio at 24 hours (p=0.007). None of the patients had cardiac symptoms except one patient from the control group. Conclusion: Annexin V does not exhibit nonspecific cardiac uptake with CTx.

No. 278

MEASURING OPIATE RECEPTOR OCCUPANCY IN PRESENCE OF LY255582 USING [C-11]-DIPRENORPHINE BY POSITRON EMISSION TOMOGRAPHY

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Introduction: LY255582 [(3R, 4R)-1-((S)-3-hydroxy-3 cyclohexylpropyl)-4-(3-hydroxyphenyl)-3, 4-dimethyl-1-piperidine] is under development for treating obesity. We quantified Opiate Receptor Occupancy of [C-11]-Diprenorphine in presence of opiate antagonist LY255582 in primate brain by positron emission tomography (PET). Methods Two anaesthetized baboons underwent a baseline and a blocking scan (each 90 minutes) per study (n=6)for four doses of LY255582,which was injected five minutes before [C-11]-Diprenorphine administration [Average injected activity 18.1 ±2.6 mCi, specific activity 10259.11 ± 5844.6 mCi/□mole, and mass 0.78 ±0.2 □g]. A two-compartment model with arterial plasma input function was used for tracer kinetic analysis. The parametric images of *Distribution Volume* (DV) and K_1 images were generated with a linear regression approach. *Binding Potential* (BP) was calculated by (DV_{Tissue} / $DV_{Cerebellum}$)-1and *Percent Opiate Receptor Occupancy* by [($BP_{Baseline}$- $BP_{DrugLY255582}$)/ $BP_{Baseline}$] * 100. Results: The caudate, putamen and thalamus showed the highest DV at baseline. DV decreased with blocking scan. Cerebellum showing the least change was used as reference. LY255582 showed a dose-dependent response and completely saturated opioid receptors at 0.3 mg/kg dose. The test-retest variability in same animal was less than 10% at 0.03mg/kg dose. Conclusion: Opiate receptor occupancy by LY255582 can be measured by [^{11}C]-Diprenorphine and PET, which contributes towards new drug development for obesity.

Percent Opiate Receptor Occupancy

	(LY255582 Drug Dose)	Frontal Cortex	Temporal Cortex	Parietal Cortex	Occipital Cortex	Cortex	Pons	Putamen	Caudate	Thalamus
Baboon-A	Dose **0.0003mg/kg** (n=1)	5.5	20.7	9.3	10.8	11	28.7	10.0	22.4	4.9
Baboon-B	Dose **0.003mg/kg**(n=2 Mean)	38.4	41.4	42.9	9.0	38.0	48.0	34.4	36.7	40.0
Baboon-A	Dose **0.03mg/kg**(n =2 Mean)	85.8	90.4	93.8	82.5	88.7	89.4	82.8	88.7	87.4
Baboon-B	Dose **0.3mg/kg**(n= 1)	100	100	100	97.1	100	100	95.0	99.5	92.7

No. 279

SAFETY ASSESSMENT OF C-11-CARFENTANIL USE IN HUMAN SUBJECTS

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BACKGROUND/AIMS: C-11-carfentanil is a mu-opiate receptor agonist used in positron emission tomography (PET) imaging. At 10,000 times the potency of morphine, carfentanil offers a narrow range between tracer and pharmacologically active doses. A mass of 2.1 micrograms is thought to be below the level at which carfentanil has a measurable pharmacologic effect. The objective of this study was to assess the hemodynamic, respiratory, and cardiac conduction effects following administration of 2.1 micrograms of carfentanil. METHODS: Healthy adult male volunteers (n=24) received five intravenous boluses of C-11-carfentanil over seven days. Electrocardiograms were obtained at baseline, immediately following injection, and one hour following injection. BP, RR, HR, body temperature, and SaO2 were recorded at five-minute intervals for twenty minutes and then at ten minute intervals to sixty minutes post-injection. RESULTS: The means of baseline parameters measured prior to dosing were compared with the means of parameters measured during and after C-11-carfentanil administration. No significant differences were measured in any objective parameter (alpha=0.05, Student's t-test). CONCLUSION: Doses of carfentanil up to 2.1mcg had no measurable affects on the hemodynamic, respiratory, and cardiac conduction systems of subjects. Results confirm that imaging doses of carfentanil from 0.01 to 0.03 mcg per kg are well tolerated and safe.

No. 280

HIGH- THROUGHPUT SMALL ANIMAL MAGNETIC RESONANCE IMAGING AT 3 TESLA

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Introduction and Purpose: Multi-coil probes capable of imaging many animals at one time will reduce the cost of magnetic resonance imaging (MRI) testing and shorten the turn around time for data presentation. This can be implemented on both large and smaller sized magnets, irrespective of field strength. The purpose of this work: To implement high-throughput (HTP) capabilities on a clinical 3 T system and to assess the time to acquire images with good conspicuity and signal-to-noise. Some distinct advantages of a large magnet at 3T are: a large volume of homogeneity and reduced susceptibility artifacts. Methods: A four coil 'multi-mouse array'(MMA) was constructed using quadrature birdcage coils having a 38 mm i.d. and 70 mm o.d.; each coil is 100 mm long. These coils are large enough to image the entire mouse. In order to 'unwrap' the images from each coil due to the field-of-view mis-registration, a software tool was developed to accomplish this. Results: High resolution images, in-plane resolution <200 microns, obtained simultaneously from four mice will be presented. All the image data were obtained in 30 minutes or less of scan time and without cardiac gating. Discussion and Conclusions: HTP MRI of rodents at 3T is demonstrated using multiple quadrature small volume coils. The larger bore space of a human clinical unit is of benefit for providing access for larger animals to be imaged when necessary, anesthesia delivery, vital signs monitoring and interventions during imaging. 3T offers a wide range of research uses in animals including drug development and toxicity testing.

No. 281

SIMPLIFIED METHOD FOR NK1 RECEPTOR QUANTIFICATION IN HUMAN BRAIN

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Neurokinin-1 receptor (NK1-R) antagonists have been proposed as potential novel treatments for a variety of disorders including mood regulation disorders, anxiety, nausea/emesis, motor control and pain. Central NK1-R imaging with the NK1 antagonist [F-18]SPA-RQ has been shown to be a valuable tool for basic biomedical research, as well as a tool for facilitating the discovery and development of central nervous system (CNS) drugs. We applied a simplified method for NK1-R quantification in human brain using [F-18]SPA-RQ. Positron emission tomography (PET) kinetic data were obtained in six male healthy subjects on a HR+ camera (Siemens) and consisted of two sequential emissions segments: 0-90 min (starting with tracer administration) and ~215-275 min. Both PET segments and an anatomical MRI were aligned and target regions of interest were defined on the caudate, putamen, thalamus, hippocampus, amygdala, frontal, parietal, temporal and occipital cortices. The cerebellum, devoid of

NK1-R, was also defined. The Reference Tissue Model which has been shown to provide robust [F-18]SPA-RQ binding potential (BP_{RTM}) estimates was used as gold standard. The proposed simplified method estimates SPA-RQ specific binding using the area under the time activity curves during the second scanning segment: BP_{AUC} = AUC(target)/AUC(cerebellum)-1. Good agreement was found between the BP_{RTM} and BP_{AUC} for all subjects. Both receptor indexes were highly correlated (r^2~1.0). The linear regression slope values were in the interval [0.94, 1.01]. Thus, the simplified method is suitable for brain NK1-R quantification using [^{18}F]SPA-RQ with a 60-minute scanning time. Additionally, generation of specific binding parametric images to perform voxel-by-voxel analysis is straightforward.

No. 282

MICRO POSITRON EMISSION TOMOGRAPHY IMAGING OF HSV-TK ACTIVITY IN CELL CULTURES AND ANIMAL MODELS WITH THE SUBSTRATE F-18-PENCICLOVIR AS A MEANS OF ASSESSING ADENOVIRAL VECTORS FOR GENE DELIVERY AND EXPRESSION

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Current cancer gene therapies are hindered by poor gene delivery and lack of tumor specificity. In order to enhance tumor specificity, the therapeutic suicide gene HSV-1 thymidine kinase (TK) sequence was modified with a 5' upstream-untranslated region (5'-UTR) from the rat bFGF mRNA. This modification restricts protein translation of the suicide gene and cytotoxicity to cancer cells previously shown to express high levels of the translation initiation factor eIF4E. Micro positron emission tomography (PET) scans utilizing a MicroPET rodent four-ring system (modelR4) from CTI Concord Microsystems, LLC (Knoxville, TN) imaging the radiolabled HSV1-TK substrate F-18-penciclovir were used to test suicide gene delivery and selective expression in tumor cells with *in vitro* cell cultures and *in vivo* animal models. A non-lytic adenovirus vector containing the HSV1-TK gene (Ad-TK) or the HSV1-TK gene modified with a 5'-UTR (Ad-UTK) was used to deliver the suicide gene in both model systems. *In vitro* model studies compared TK expression from the two vectors in human breast epithelial cells MCF-10A, and MCF-10A cells stably transfected to express high levels of eIF4E (MCF-10A-4E). Administration of Ad-TK and Ad-UTK to non-tumor bearing nu/nu mice and nu/nu mice bearing human breast cancer tumors was used to compare *in vivo* expression of TK in non-tumor and tumor tissues, respectively. Analysis of emission data from these studies showed that Ad-TK administration resulted in broad expression of TK activity in normal and tumor tissues, whereas expression of suicide gene activity from Ad-UTK vector administration was restricted to cells expressing high levels of eIF4E and tumor tissues.

No. 283

***IN VIVO* MICROCT IMAGING CHARACTERISTICS OF A LONG-ACTING BLOOD POOL AGENT IN NORMAL AND TUMOR-BEARING MICE**

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Objective: Micro computed tomography (CT) scanners capable of sub 20-micron isotropic spatial resolution have recently been introduced that afford potential to monitor tumor growth and development in preclinical mouse models. However, long acquisition times associated with microCT preclude the use of conventional water-soluble contrast agents. The goal of this study was to assess the utility of a new long-lasting contract agent for high-resolution imaging of vascular network in normal and tumor-bearing mice. Material and Methods: A pegylated chylomicron-like vehicle containing a polyiodinated triglyceride packaged within its lipophilic core (approx. 52 mg I/mL) was obtained from Alerion Biomedical Inc. (San Diego, CA). Anesthetized female Balb/c bearing colon-51 xenografts and controls (n=3) were scanned using a CTI microCAT II (Knoxville, TN) prior to and at predetermined time intervals after intravenous administration (15-20 mL/kg bw). Reconstructed CT images were displayed and analyzed using Amira (TGS, Inc., San Diego, CA). Relative vascular and liver enhancement profiles were obtained for comparison between each group. Results: Following intravenous injection of the agent, relative vascular density was significantly enhanced in all studies. Tumor-induced angiogenesis characterized by the degree of branching and tortuosity, as well as normal intermediate and large vessels were readily observed. Moreover, quantitative relative tissue density measurements and soft tissue identification could both be achieved following a single injection of the vascular contrast agent. Conclusions: The *in vivo* imaging properties of the long-acting blood-pool contrast agent permits visualization and quantitative vascular characterization, as well as soft tissue identification, for both normal and tumor-bearing animals using microCT.

No. 284

SYNTHESIS OF N-SUBSTITUTED 3-PHENYL-8-AZA-BICYCLO[3.2.1]OCTANE ANALOGS AS SIGMA-2 RECEPTOR LIGANDS

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Sigma (□) receptors are a distinct class of receptors that are expressed in many normal tissues, including liver, kidneys, endocrine glands and the central nervous system. There are two types of □ receptors, □$_1$ and □$_2$. It has reported an over expression of □$_2$ receptors in both human and murine tumors. Additional studies have shown the expression of □$_2$ to be a reliable biomarker for the proliferative status of solid tumors. Therefore, radioligands having a high affinity and high selectivity for □$_2$ vs. □$_1$ receptors should be good tracers for the non-invasive assessment of the proliferative status of solid tumors using positron emission tomography (PET) or single photon emission computed tomography (SPECT). We have recently reported a series of C-11-labeled and F-18-labeled conformationally-flexible benzamide analogs having a high affinity and outstanding selectivity for □$_2$ receptors. *In vivo* evaluation in mice has shown promise as PET radiotracers for imaging breast tumors. However, it is not clear if these compounds will be successful for imaging □$_2$ receptors in patients. The function of the current study was to continue structure-activity relationship studies to identify lead compounds for imaging the □$_2$ receptor status of breast tumors. Therefore, a series of substituted 3-phenyl-8-aza-bicyclo[3.2.1]octane compounds were synthesized and their affinities □$_1$ and □$_2$ receptors were measured. The compound, 1-(3-Bromophenyl)-2-(3-phenyl-8-aza-bicyclo[3.2.1]oct-8-yl)ethanol, had the best affinity and selectivity □$_2$ receptors (□$_1$ = 554.2 ± 35.6 nM; □$_2$ = 12.4 ± 1.8 nM). This compound has the potential to be labeled with Br-76 for tumor imaging studies with PET. Acknowledgment. This research was supported by NIH grant CA102869.

No. 285

REPRODUCIBILITY OF 2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE AND FLT UPTAKE IN PRECLINICAL TUMOR MODELS

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2-Deoxy-2-[F-18]fluoro-D-glucose (FDG) and FLT are used as tracers for positron emission tomography (PET) imaging of tumors in a clinical setting. The use of microPET for preclinical animal models of oncology is less well established. We therefore examined the reproducibility of FDG and FLT uptake in a human tumor xenograft model in nude mice. Two groups of six animals with advanced human Colo205 tumors were studied. Tumors were implanted subcutaneously and allowed to grow for two weeks before imaging. The studies lasted for five days and the animals were scanned on days 1, 2, 3 and 5. Around 200 μCi of FDG,]FLT was

administered. TACs, %ID/g, SUV, ANOVA, and reference region analysis methods were used to analyze the data. The mean±SD of %ID/g for FDG on days 1 and 5 were 5.51±0.48% and 4.16±0.54 respectively, The equivalent values for [^{18}F]FLT were 3.67±1.24% and 4.22±0.34% respectively. Similar changes were found for standardized uptake value (SUV). Regions of brain, a transverse slice on the shoulder (TSlice), heart, lung, liver, and kidney, were used to select the best reference region. The TSlice showed the smallest variations and was therefore used as the reference region to determine the ratio of tracer uptake in the tumor relative to normal tissue. The ratios on days 1 and 5 were 3.97±0.26 and 3.04±0.18 for FDG, and 1.79±0.22 and 1.86±0.22 for FLT. FDG uptake showed a tendency of decrease during all five days scans, while FLT keeps a constant value. These initial measurements will form a basis to monitor drug efficacy in preclinical tumor models.

ICP Addendum

Oral Presentations

No. 286

2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE- POSITRON EMISSION TOMOGRAPHY/ COMPUTED TOMOGRAPHY IS MORE ACCURATE AND SENSITIVE DETECTING AXILLARY METASTASES IN PATIENTS WITH LYMPHOVASCULAR INVASION AND/OR LARGE BREAST TUMOR

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Introduction: Axillary node metastasis is the most important prognostic factor in early stage breast cancer patients. It is suggested that in early - stage breast cancer, 2-deoxy-2-[F-18]fluoro-D-glucose (FDG)- positron emission tomography (PET)/ computed tomography (CT) has a limited ability to detect small-volume axillary-disease. This study was done to determine if FDG-PET/CT is more accurate and sensitive detecting axillary-metastases in patients with lymphovascular invasion and large breast tumor. Methods: The sensitivity and accuracy of FDG-PET/CT was determined in 19 consecutive early-stage breast cancer patients who had subsequent staging of their axilla by sentinel lymph node scintigraphy (SLN) in 11, and/or axillary lymph node dissection in eight. The results for all patients n=19 (Group-I); patients with lymphovascular invasion of the tumor n=12 (Group-II); and patients with tumor size> 3.0 cm n=9 (Group-III) were compared. Results: The positive predictive value, negative predictive value, accuracy and sensitivities were: 100%, 54%, 68%, and 50% for Group-I; 100%, 57%, 75%, and 63% for Group-II; 100%, 50%, 78% and 71% for Group-III respectively. In two out of four patients SLN were nonvisualized with lymphoscintigraphy although PET scan was positive for axillary LN. Those two patients had a primary tumor with lymphovascular invasion and sentinel lymph nodes were proven to have extensive axillary metastatic involvement. Conclusion: Although the sensitivity and accuracy of FDG-PET/CT are relatively low in early-stage breast cancer patients, FDG-PET/CT is more accurate and sensitive in detecting axillary metastases in patients with lymphovascular invasion and/or large tumors. Sentinel lymph node scintigraphy may not be helpful in patients with large axillary nodes and lymphovascular invasion in their primary tumor.

No. 287

POSITRON EMISSION TOMOGRAPHY/ COMPUTED TOMOGRAPHY IMAGING IN THE CHEST: BENIGN AND INFLAMMATORY CAUSES OF 2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE UPTAKE

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Purpose: Our goal was to develop an atlas of the most common and atypical benign causes of 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) uptake in the chest using combined positron emission tomography (PET)/ computed tomography (CT) scanners. Materials & Methods: All anatomical (CT), functional (PET) and fused anatomical/functional (PET/CT) images were acquired and selected from combined PET/CT scans of approximately 15,000 patients scanned at our institution on five different PET/CT scanners since 1998. Patients were referred for a variety of malignancies. All abnormal findings were confirmed by histopathology or follow-up imaging. Results: Examples of benign lesions in the chest include radiation pneumonitis, inflammatory pseudotumor, sarcoidosis, silicosis, focal retrocrural brown fat, tuberculosis, hamartomas and calcified lymph nodes causing attenuation correction artifacts. Several patients included in this atlas were misdiagnosed as having primary or recurrent malignancy. Some patterns mimicked malignant patterns of FDG uptake, while others mimicked physiologic or benign patterns. Conclusion: We present an atlas of common and atypical non-malignant causes of FDG uptake in the chest using combined PET/CT. Recognition of these patterns and an awareness of the overlap between benign and malignant causes of FDG uptake is essential for interpreting physicians to avoid misdiagnosis.

No. 288

RADIATION DOSIMETRY OF CHOLLINERGIC IMAGING AGENT F-18 FLUOROBENZYLTROZAMICOL

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Background/Objective: [F-18](+)-4-fluorobenzyltrozamicol (FBT) is a useful ligand to image and quantify pre-synaptic vesicular acetylcholine transporter using positron emission tomography (PET). We obtained biodistribution and human dosimetry estimates of FBT based on PET imaging performed in humans and cynomolgus monkeys. Methods: The biodistribution data was obtained from whole-body PET scans acquired for humans (n=2; 180 min) and monkeys (n=2; 250 min) after 5.3 $\pm$ 0.76 mCi bolus injections of FBT. Regions of interest were drawn to generate tissue time-activity curves and radiation dosimetery estimates were calculated using OLINDA software. Results: Human biodistribution studies showed FBT localized in cholinergic rich regions and highest accumulation was seen in lungs, liver, brain, kidneys, and pancreas. Peak percent injected dose in humans was observed at 1.5 minutes (52.7%), 3.5 minutes (17.0%), 7.5 minutes (6.3%), and 26 minutes (5.9%) in the lungs, liver, kidneys, and brain, respectively. In humans, the organs receiving the highest radiation dose (mSv/MBq) were pancreas (0.213), lungs (0.129), kidneys (0.111), and liver (0.0907). In monkeys, the four organs with highest radiation dose were the gallbladder wall (0.356), pancreas (0.339), kidneys (0.113), and bladder wall (0.073). Effective dose obtained from human studies was 0.036 mSv/MBq. Estimated effective dose in humans calculated from monkey studies was 0.030 mSv/MBq. Conclusions: The dosimetery of FBT is favorable for human studies. Absorbed radiation dose based on a 10 mCi FBT injection is within regulatory limits. FBT is a safe and potentially useful PET ligand for human studies. Supported in part by WFU-GCRC (M01-RR07122).

No. 289

EVALUATING TREATMENT RESPONSES IN PATIENTS WITH BONE AND SOFT TISSUE SARCOMA WITH 2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE - POSITRON EMISSION TOMOGRAPHY/ COMPUTED TOMOGRAPHY

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Background: Neoadjuvant therapy prior to surgery is the standard of care in sarcoma patients. There is a need for non-invasively assessing tumor

responses to therapy. The goal of this study was to determine whether treatment induced changes in glucose metabolic activity correlate with histological amount of tumor necrosis in patients with sarcomas. Methods: Forty-seven patients were studied with positron emission tomography (PET)/ computed tomography (CT) at baseline and then again 11 ± 3.7 weeks later. The time from follow up scan to surgery was 3.8 ± 2.7 weeks. There were eight bone and 39 soft tissue sarcomas. Tumor glucose metabolic treatment response was estimated by changes in standardized uptake values (SUV). The extent of tumor necrosis was expressed as fraction of residual viable tumor. Results: All 47 sarcomas had visible 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) uptake with mean SUVs ranging from one to 19. Complete data are available in 14 patients. Tumor mean SUV decreased by 55 ± 33 % from 4.9 ± 3.0 to 2.0 ± 1.1 after therapy (p=0.0007). Relative changes in FDG-uptake as well as residual FDG-uptake after therapy were significantly correlated with the amount of tumor necrosis in the resected specimens (r^2=0.66, p=0.007 and r^2=0.70, p=0.005, respectively). There was no significant correlation between pre-therapeutic FDG-uptake and percentage of tumor necrosis (r^2=0.28, p=0.15). Conclusions: This preliminary analysis suggests that there is a close correlation between changes in tumor glycolysis and histopathologic tumor regression following neoadjuvant chemotherapy. Thus, FDG-PET is a promising tool for non-invasively identifying those sarcoma patients who respond to neoadjuvant chemotherapy.

No. 290

DETERMINATION OF POST FILTERING PARAMETERS FOR ACCURATE DEFECT SIZE DETERMINATION IN A CARDIAC PHANTOM USING TC-99M, F-18, AND I-124

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Background: Tc-99m agents, 2-deoxy-2-[F-18]fluoro-D-glucose (FDG), and Rb-82 can all be used for cardiac imaging, however counts rate, energy and camera differences yield image differences that are independent of the underlying biological distribution. Methods: Due to the short halflife of Rb-82 (76s), I-124 was used as a surrogate high energy positron emission tomography (PET) tracer. We determined post filtering parameters which resulted in the correct quantification of defect size for each tracer, and then applied the resultant I-124 post-filtering parameters to Rb-82 patient data. By imaging a cardiac phantom with and with out a 5% defect for each tracer, the measured %defect was calculated from the images. Results: Application of a 45% maximum pixel threshold to the Tc-99m single photon emission computed tomography (SPECT) images with a Butterworth filter order 7, cutoff 0.55 resulted in an accurate calculation of defect size. Maintaining the 45% maximum pixel threshold, it was shown that filtering the F-18 data with a Butterworth order 5, cutoff 0.7, and the I-124 data with a Butterworth order 5, cutoff 0.8, resulted in accurate defect size estimation. When the calculated I-124 parameters were applied to Rb-82 patient data, a difference in the estimated change in defect size between rest and post-DIP was found compared with those obtained with the routine PET filtering parameters. Conclusion: When assessing myocardial defect size, physical properties need to be taken into consideration, particularly when comparing images obtained using different nuclides (ie. Rb-82 or Tc-99m agent perfusion and FDG viability)

No. 291

TUMOR PROLIFERATION AND HYPOXIA POSITRON EMISSION TOMOGRAPHY IMAGING DURING RADIATION THERAPY

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Molecular imaging has a potential to significantly improve cancer treatment planning, treatment adaptation and assessment of treatment response. A distinct feature of many tumors is rapid cell proliferation, which is altered in response to antineoplastic therapies. However, reduced proliferation in hypoxic areas is a serious complicating factor because intratumoral oxygen levels may influence a series of biologic parameters that also affect the malignant potential of a neoplasm. In order to concurrently assess cell proliferation and hypoxia during radiation therapy, two positron emission tomography (PET) imaging markers were used - 3'-Deoxy-3'-[F-18] fluorothymidine (FLT) and [61Cu]-diacetyl-bis(N4-methylthiosemicarbazone) (Cu-ATSM). Several canine subjects with soft tissue sarcomas were repeatedly imaged with both markers before, during and after radiation treatment. The CT data between the imaging sessions was co-registered and corresponding PET data compared. Approximately 200 MBq of FLT or Cu-ATSM activity was administered per scan. Standardized uptake values (SUV) were calculated to evaluate the uptake and its change through the course of radiation therapy. The tumors were treated with 60Co, typically with four fractions of 8Gy. Tumor response varied significantly between the subjects. Heterogeneity of both, cell proliferation and hypoxia marker distributions (up to 50% in SUV) of the tumor was observed, typically more significant in larger tumors. Distributions of proliferation and hypoxia markers were often found to be complementary, with the increased uptake of Cu-ATSM observed in the vicinity of the increased uptake of FLT; however, the complementarity was not exclusive.

No. 292

COMPARISON OF MAMMOGRAPHY, BREAST MRI AND F-18 2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE- POSITRON EMISSION TOMOGRAPHY/ COMPUTED TOMOGRAPHY FOR BREAST CANCER DETECTION

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Objective: 2-Deoxy-2-[F-18]fluoro-D-glucose (FDG)- positron emission tomography (PET)/ computed tomography (CT) is used for cancer diagnosis, staging and therapy monitoring, including breast cancer (BC). Magnetic resonance imaging (MRI) gains major role for high risk BC. The role of PET/CT with mammography and MRI in BC is not defined. We were therefore prompted to review our experience with mammography, MRI and PET/CT in BC. Methods: This is a retrospective study of 27 patients with BC, 31-82 years old (average: 52±12.9), with mammography, MRI and PET/CT at our institution from Jan 2003 to Jun 2005. Four patients had studies for initial staging, 23 for restaging. Reinterpretation of the studies and data analysis were performed. Results: The tumor types were: 23 ductal (14 infiltrative, eight invasive, one in situ), two lobular (one infiltrative, one invasive), two mucinous carcinomas. Tumor size at cancer presentation ranged 0.9-7.5 cm (average: 2.68±1.6). FDG doses were 10.3-20 mCi (average: 15.3±2). Sensitivities and specificities for BC detection were 69.2% (95% CI: 42.3-87.3) and 77.8% (95% CI: 45.3-93.7) for mammography, 80% (95% CI: 54.8-92.9) and 91.7% (95% CI: 64.6-98.5) for MRI, 71.4% (95% CI: 45.3-88.3) and 92.3% (95% CI: 66.7-98.6) for PET/CT. PET/CT showed axillary metastases in five patients (62.5% sensitive, 100% specific) and distant metastases in four patients (75% sensitive, 91.3% specific). Conclusion: Breast MRI appears more sensitive than PET/CT and mammography for BC detection. The specificities of MRI and PET/CT are similar. PET/CT detected axillary and distant metastases in nine patients (33.3%). PET/CT and MRI should be considered as complimentary tests for locally advanced or non-favorable histology BC.

No. 293

ADDED VALUE OF TRUE WHOLE-BODY POSITRON EMISSION TOMOGRAPHY/ COMPUTED TOMOGRAPHY IMAGING IN PATIENTS WITH MELANOMA

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Objectives: The most commonly used axial co-scan range selected for the arms-up 2-deoxy-2-[F-18]fluoro-D-glucose (FDG)-positron emission tomography (PET)/ computed tomography (CT) whole-body protocols only covers a field-of-view (FOV) from the base of skull to the upper thighs.

The routinely used FOV may underestimate the true extent of the disease by missing metastases to areas outside the typical Limited whole-body FOV. The aim of this study was to evaluate the incremental added value of true whole-body over limited whole-body FDG-PET/CT in melanoma patients. Methods: True whole-body FDG-PET/CT scans from the top of the skull to the bottom of the feet were performed in 70 consecutive melanoma patients. Images were acquired on a PET/CT scanner (Phillips, Gemini with an axial co-scan range of 193 cm). Images were retrospectively evaluated, and a log was kept to record cases of suspected metastases outside the typical limited whole-body FOV. Suspected lesions were verified by correlation with surgical pathology, CT, magnetic resonance imaging (MRI) or clinical follow-up. Results: PET/CT studies suggested the presence of metastases outside the typical limited whole-body in 12 out of 70 patients. Of these 12 patients, one had false positive findings, whereas malignancy was confirmed in 11 patients (15.7%). Examples of lesions outside limited whole-body were metastases to Brain (one patient), Scalp (four patients), Upper (six patients) and Lower extremities (six patients). Of these 11 true positive patients, three out of 70 (4.2%) had their only metastatic site outside limited whole-body FOV. Conclusion: TWB PET/CT provided a more accurate staging/restaging exam in 15.7% of our studied patients and should therefore be the standard of care for melanoma.

No. 294

INCREASED RESOLUTION AND CONTRAST FOR HIGH-RESOLUTION 3-D BRAIN POSITRON EMISSION TOMOGRAPHY IMAGING

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Purpose: Although iterative reconstructions have become the standard in whole-body positron emission tomography (PET) imaging, 3-D reprojection (3-DRP) is still routinely used in 3-D brain scanning. Our current clinical practice is to acquire high-statistic (15 minute duration) 3-D brain scans and reconstruct using 3-DRP with a Hann window cut off at the Nyquist frequency. A new enhanced Hann filter has been developed that better preserves the high frequency spectrum. Our goal was to assess the efficacy of this filter for improving image quality in clinical brain imaging. Methods: Several 3-D brain scans of 15 minutes duration have been acquired with listmode data on a GE Discovery STE scanner. The listmode files were retrospectively processed into scans of 15, 10, five and two minutes duration. Images for each duration were reconstructed with 3-DRP using the 4.8 mm Hann window and 4.8, 6.0, 8.0 enhanced Hann windows. All images were assessed for image quality relative to the 15 minutes 4.8 mm traditional Hann reconstruction. Results: The five and 10 min scans reconstructed with the 4.8 mm Hann were comparable to the 15 minutes data, implying that the statistics of the 15 minutes scan can support higher spatial resolution. The 15 minutes image generated with a 6.0 mm enhanced Hann did show noticeably improved resolution and contrast with comparable noise properties. Conclusion: Improved spatial resolution and contrast in brain imaging can be achieved with the enhanced Hann filter applied to 3-D data of sufficient statistics.

No. 295

CY5.5-LABELED ANTI-EGFR ANTIBODY DETECTS HUMAN SQUAMOUS CELL CARCINOMA *IN VIVO*

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Objectives. Current methods for detecting local and regional spread of head and neck cancer are limited to anatomic and metabolic imaging. We sought to exploit epidermal growth factor (EGFR) overexpression in head and neck squamous cell carcinoma to image human tumors *in vivo*. Methods. SCID mice were injected with tumor cell line (UM-SCC-1) on the right flank and either implanted human skin (n= 2) or human skin grafted onto the left flank (n= 4). Anti-EGFR antibody (cetuximab) labeled with Cy5.5 was injected systemically into six mice and five uninjected mice served as a control group. The mice underwent *in vivo* fluorescence imaging (time domain-Explore Optix and stereomicroscopy) at day 0, 2, 7, and 9. Samples were then processed for histology to assess binding of the Cy5.5-cetuximab by confocal microscopy. Results. Mice injected with the Cy5.5-cetuximab showed significantly higher tumor fluorescence compared with implanted skin (p=.0072) at all time points when compared to uninjected controls. The largest difference occurred at day 2. SCC-1 tumors showed significantly higher fluorescence (35.1 million vs.10.7 million photons) (p=.0258) when compared to skin graft in the same mice, the greatest difference was two hours post-injection. The fluorescence stereomicroscopy detected tumor margins better than visual inspection, and when compared to background fluorescence. Conclusion. The Cy5.5-cetuximab showed higher binding to human tumors *in vivo* than to human skin xenografts. Fluorescent imaging using an endoscope or stereomicroscope may improve assessment and guide resection of local and regional disease in head and neck cancer patients.

No. 296

2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE UPTAKE AND GLUCOSE TRANSPORTER TYPE 1 EXPRESSION IN MEDIASTINAL LYMPH NODE OF NON-SMALL-CELL LUNG CANCER

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Purpose: 2-Deoxy-2-[F-18]fluoro-D-glucose (FDG) uptake in NSCLC is related with glucose transporter type 1 (Glut-1) expression. We investigated the correlation of FDG uptake and Glut-1 expression of mediastinal lymph node (LN) of NSCLC. Methods: Fifty-five curative resections of 53 NSCLC patients (male:female=36:17, age=62.0± 11.8 years) were included. History of neo-adjuvant chemotherapy or diabetes was exclusion criteria. After 6 hours of fasting, preoperative FDG- positron emission tomography (PET) was performed (mean 5.18 MBq/kg). Maximum standardized uptake value (maxSUV) and Glut-1 immunostaining results for mediastinal LNs were compared. Results: Of 316 LNs pathologically confirmed, 12.3% (39/316) were malignant LNs. In malignant LN analysis, FDG uptake positive LNs were no different to FDG uptake negative LNs in the size (15.0±6.1mm vs 10.0±6.1mm, p>0.05), and the tumor proportion in LNs (39.2±28.4% vs 39.2±38.4%, p>0.05) but FDG positive LNs were greater than FDG negative LNs in the percentage of Glut-1 positive cells in tumor (22.7±31.8% vs 22.7±18.7%, p<0.01), and the Glut-1 staining intensity (2.4±0.9 vs 1.8±1.3, p<0.01). All FDG negative metastatic LNs featured cytoplasmic pattern of Glut-1 expression and adenocarcinoma. Glut-1 staining intensity had significant correlation with maxSUV of metastatic LNs (rho=0.516, p<0.05) but the percentage of Glut-1 positive cells in tumor did not (r=0.2072, p>0.05). In benign LN analysis, FDG positive LNs did not reveal correlation between maxSUV and degree of follicular hyperplasia or its Glut-1 expression (p>0.05). Conclusions: Glut-1 expression as an intensity of immunostaining had a significant correlation with FDG uptake of malignant LNs. Follicular hyperplasia and its Glut-1 expression were not correlated with FDG uptake of benign LNs.

No. 297

UTILITY OF 2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE-POSITRON EMISSION TOMOGRAPHY/ COMPUTED TOMOGRAPHY SCANNING FOR MONITORING STEREOTACTIC RADIOSURGERY RESPONSE IN PATIENTS WITH LOCALLY ADVANCED PANCREATIC CANCER

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Introduction: The aim of this study was to evaluate 2-deoxy-2-[F-18]fluoro-D-glucose (FDG)- positron emission tomography (PET)/ computed tomography (CT) for monitoring response to stereotactic

radiosurgery (SRS) in patients with locally advanced pancreatic cancer. Methods: Sixteen patients with an ECOG performance status of two or less and locally advanced pancreatic cancer were prospectively scanned and treated with SRS to the primary site. All patients underwent a PET/CT and a contrast enhanced pancreatic CT one month prior and one month after SRS. To assess response, the change in standardized uptake value (SUV) on PET was measured and compared to change in size on pancreatic CT. Follow-up contrast CT and interval clinical evaluations for at least two months after therapy were used to assess local tumor control. Results: On PET, 16 out of 16 (100%) patients had a decrease in SUV of the pancreatic tumor after SRS with an average decrease of 2.9 (range 0.6-8.6). On CT, there was no change in pancreatic tumor size in 12 out of 16 patients, a slight size *increase* in four out of 16 patients, and a size decrease in zero out of 16 patients. Follow-up confirmed that all 16 patients had stable disease shown by interval CT scanning (14/16 patients) at greater than two months (range two to eight months) post treatment or by clinical evaluation (two out of 16 patients) at two to six months post treatment. Conclusions: Using SUV analysis, FDG-PET/CT can accurately monitor response of locally advanced pancreatic cancer to SRS and is more accurate than contrast enhanced CT.

No. 298

EFFICIENT MAGNETIC LABELING OF CELLS WITH NEW USPIO AGENT "COMBIDEX"

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The purpose of this study was to investigate effectiveness of a new ultra small superparamagnetic iron oxide (USPIO), ferumoxtran-10 (Combidex®, Advanced Magnetics) to label cells for cellular magnetic resonance imaging (MRI). Different dose ratios of ferumoxtran-10 and transfection agents [poly-l-lysine (PLL, MW = >300K and 150K) and protamine sulfate] were used. Labeling efficiency and toxicity were determined. For this study, gliosarcoma cells (9L) were used. Ferumoxtran-10 was used at concentrations of 100 and 200 □g/ml and the concentration of transfection agent varied from 2 to 100 □g/ml. Intracellular iron was determined by Prussian blue staining and iron concentration was determined by UV/VIS spectrophotometric method using hydrochloric acid and potassium ferrocyanide. Labeled cells at different concentration were suspended in gelatin in NMR tubes and magnetic resonance imaging (MRI) was performed using a 3T clinical system to get T2-weighted images and R2 maps. Ferumoxtran-10-protamine sulfate complexes failed to label cells even with highest ferumoxtran-10-protamine sulfate ratio. However, PLL complexed with ferumoxtran-10 labeled the cells efficiently. Most effective labeling was achieved at a ratio of 100:15-25 (ferumoxtran-10:PLL) without loss of cell viability. PLL at a dose higher than 25 □g/ml resulted in loss of cellular viability. In these experiments, no extracellular iron was observed. Iron concentration in cells varied from 0.5 to 7.2 pg/cell depending on the PLL dose. MRI showed a linear correlation between the number of cells in the NMR tubes and their R2 values. This study concludes that ferumoxtran-10 is an effective iron oxide nanoparticle for MRI cell labeling besides feridex and MION.

No. 299

POSITRON EMISSION TOMOGRAPHY/ COMPUTED TOMOGRAPHY WITH DIAGNOSTIC CT (PET/DCT) FOR EVALUATION OF ENDOMETRIAL CANCER

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Endometrial cancer is traditionally staged surgically, and little information is available regarding the role of positron emission tomography (PET)/ computed tomography (CT) imaging in evaluating this disease. A combined PET/CT protocol has been developed which allows PET with 2-deoxy-2-[F-18]fluoro-D-glucose (FDG-PET) and diagnostic CT (DCT) to be performed in a single imaging session (PET/DCT). In order to determine the potential value of PET/DCT for evaluating endometrial cancer, a prospective study was performed to correlate findings on PET/DCT with findings at staging laparotomy. Ten patients underwent PET/DCT as part of this pilot study. All patients received oral contrast for the CT study, and 8 out of 10 patients received intravenous contrast. One patient did not receive intravenous contrast due to allergy, and a second patient had undergone a separate contrast-enhanced CT scan on the previous day. Surgical staging was performed within 16 days of PET/DCT. The primary tumor site was evident on PET/DCT in all 10 cases. For nodal disease (n=39 nodal regions), PET/DCT had a sensitivity of 100% and a specificity of 97%. PET/DCT was less accurate for distant metastases. In particular, omental disease present in two patients at surgery was not identified on PET/DCT. An inflammatory mediastinal lymph node resulted in false positive activity in one patient. These results suggest that PET/DCT has high accuracy for nodal staging of endometrial cancer, which may provide important prognostic information and may aid in planning therapy in patients who are at high operative risk. PET/DCT may have decreased sensitivity for detecting omental disease in the subset of patients with papillary serous adenocarcinoma.

No. 300

NUCLEOFECTION OF IMAGING REPORTER GENES INTO HUMAN PERIPHERAL BLOOD MONOCYTES

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Human Dendritic cells (DC) have potential applications in cellular gene therapy. We have investigated the application of Amaxa's nucleofection technique (for direct insertion of transgenes into cell nucleus) for achieving long-term expression of imaging reporter genes (RG) in DCs, so as to monitor their trafficking non-invasively in living subjects. CD14 enriched human monocytes were nucleofected with triple fusion (TF) plasmids carrying Firefly Luciferase (fLuc: bioluminescent RG), Green Fluorescent Protein (GFP) RG and a truncated mutant Herpes Simplex Virus 1 thymidine kinase (HSV1-tsr39tk: PET RG) and MaxGFP plasmid using Amaxa's human monocyte nucleofector kit. Monocytes were then cultured with IL-4 and GM-CSF to differentiate into DCs. Nucleofected cells were assayed for fLuc activity and for viability and GFP expression by flow cytometry. TF nucleofected monocytes were subcutaneously implanted with Matrigel in immunodeficient mice and imaged for bioluminescence up to 21 days. Flow cytometry data and fLuc assay results are listed in the table. Bioluminescent signals were detected for up to five days at the site of cell implantation in mice. Our data indicate that human monocytes can maintain gene expression as they differentiate into DCs. However, long-term viability was compromised by TF nucleofection and fLuc activity per microgram protein declined.

Reporter Gene Expression and Viability of Nucleofected Human Monocytes

Type of Plasmid	Microgram Plasmid	Number of Cells Nucleofected	Days after nucleofection	fLuc Activity per microgram protein	Percent viable cells	Percent GFP Positive
None	0	5,000,000	2	5	80	0
None	0	5,000,000	6	1	75	0
MaxGFP	1	10,000,000	1	2	92	65
MaxGFP	1	10,000,000	6	4	82	53
TF	2	10,000,000	1	154,463	87	7
TF	2	10,000,000	6	6.434	7	4

Poster Presentations

No. 301

2-DEOXY -2-[F-18]FLUORO-D-GLUCOSE- POSITRON EMISSION TOMOGRAPHY IS AN EFFECTIVE IMAGING MODALITY FOR DETECTING AGE RELATED ATHEROSCLEROTIC CHANGES IN THE LARGE ARTERIES

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Aim: Accumulation of 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) due to atherosclerosis can be seen in the walls of large arteries in many patients. Our aim was to determine and quantify the changes in FDG uptake in large arteries with aging. Methods: We evaluated the presence of FDG vascular uptake in 115 non diabetic subjects (50 men, 65 women; age 5-80 years) who underwent positron emission tomography (PET) scans for the assessment of non cardiovascular disorders. We divided the patients into eight groups, each group representing a decade, with at least 11 patients per group. We recorded the presence of atherosclerotic FDG uptake in total 920 segments (ascending, arch, descending and abdominal aorta, left and right iliac, left and right femoral arteries) for each patient. We detected the % visible segment uptake for all decades. We scored the uptake in segments as 0: no uptake, 1 mild uptake, 2: moderate and 3: severe uptake. We calculated the mean FDG uptake score for each decade. Results: The percentage visible segment uptake was 27% for the first decade. It was 39%, 65%, 73%, 74 %, 78%, 78%, and 79% for the following decades respectively. Mean of the FDG uptake score for the first decade was 2.45. It was 3.0, 6.2, 8.5, 8.7, 10, 10, and 12.3 for the following decades respectively. Conclusion: These data indicate that percent visible FDG segment uptake and degree of uptake in segments increases in large arteries with age. This is probably due to the increase in extent and the severity atherosclerotic process in large arteries.

No. 302

AUTOMATED 2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE-POSITRON EMISSION TOMOGRAPHY IMAGE-ANALYSIS SOFTWARE PACKAGE FOR THE DIFFERENTIAL DIAGNOSIS OF ALZHEIMER DISEASE

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The Center for Medicare and Medicaid Services has approved the use of 2-deoxy-2-[F-18]fluoro-D-glucose (FDG)-positron emission tomography (PET) for the differential diagnosis of Alzheimer's disease (AD) in certain patients with dementia, and has recommended the development of improved image-analysis techniques for this indication. We have developed an automated software package to characterize a patient's pattern of regional hypometabolism relative to a cognitively normal control group and compare this pattern to that found in patients with probable AD. Our streamlined software package is based on SPM99 (http://www.fil.ion.ucl.ac.uk/spm/). With one mouse click, the program spatially deforms the patient's image to a standard template, computes a statistical map of significant regional/whole brain metabolic reductions relative to a control group, and superimposes this map onto the pattern of metabolic reductions in previously studied patients in the moderate stage of probable AD. Software users have the option of matching the patient to normal control subjects for their gender, age, educational level, and apolipoprotein E. Features of the package include image pre-processing quality control, interactive inspection of brain regions for metabolic reduction pattern, a text window to record relevant findings, and the generation of jpg files, which may be helpful to physicians, their patients and patient families. The software package uses FDG-PET images from 82 well characterized, cognitively normal control subjects, 62±6 years of age. Additional studies are needed to demonstrate this algorithm's added value in predicting the clinical course and neuropathological diagnosis of patients with suspected AD and frontotemporal dementia.

No. 303

EFFICACY OF WHOLE-BODY 2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE -POSITRON EMISSION TOMOGRAPHY IN TUMOR DETECTION FOR PATIENTS WITH SUSPECTED PARANEOPLASTIC SYNDROMES

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Objectives: Assess the usefulness of 2-deoxy-2-[F-18]fluoro-D-glucose (FDG)-positron emission tomography (PET) in the search for a tumoral source of patients with suspected Paraneoplastic syndromes (PNS). Methods: We retrospectively studied 17 patients with clinical suspicion of PNS who underwent a whole-body FDG-PET scan following negative/inconclusive investigations. Final diagnosis was made according to subsequent conventional imaging, and/or pathologic examination. Results: Three patients were lost to follow-up (three out of 17). Among the remaining 14 patients (14 out of 17), FDG-PET scan detected two out of three pathologically proven malignancies. One was a Hürthle cell carcinoma, and the other was a non-small cell lung cancer. The tumor that was not detected by FDG-PET was an adenocarcinoma of the prostate. There were two false positive PET scans that suspected a focal colonic activity, including the patient who had a falsely negative FDG-PET for localized prostate cancer. One patient, who had abnormal FDG uptake in the lung, was found to have pneumonia. None of remaining eight patients with negative FDG-PET were found to have a tumor on clinical follow-up. Conclusions: FDG-PET has a potential role in detecting the tumor source of PNS, but is limited in the abdomen and pelvis due to colonic and urinary activity. The advantage of FDG-PET is the scanning of the whole body in one study, for the search of any suspicious metabolic activity, which can be followed by a more focused work up. In the patients who have a negative FDG-PET scan, it is unlikely that the PNS is caused by a malignancy and other causes should be considered.

No. 304

VALUE OF WHOLE-BODY POSITRON EMISSION TOMOGRAPHY/ COMPUTED TOMOGRAPHY SCAN AND CLINICAL UTILITY FOR EVALUATION OF CANCER OF UNKNOWN PRIMARY

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Diagnostic management of patients with unknown primary or suspected metastases of unknown origin represents a clinical challenge. Conventional imaging (computed tomography (CT), magnetic resonance imaging (MRI) or ultrasounds) usually has a very low sensitivity in the correct identification of the primary site of cancer. Positron emission tomography (PET) imaging provides limited information about correct localization of primary lesions, with poor results in its pathological confirmation. We evaluated the performance of whole-body hybrid technology (PET/CT) in order to detect and localize primary lesions. Patients and methods: 90 patients were evaluated on the basis of capacity of PET/CT to localize a probable primary lesion for its pathological confirmation. Thirty-nine patients presented lymph node metastases (LNM), 26 patients presented signs of involved visceral metastatic tumor spread (VM), six paraneoplasic neurological syndromes (PNS), 10 patients malignant pleural effusion (MPE) and 9 had elevated tumor markers (ETM). All patients underwent whole-body FDG imaging (GE Healthcare, PET/CT Discovery ST) in a standard protocol (50 minutes after 260-530 MBq 18FDG i.v. PET data reconstructed using iterative algorithm and attenuation correction CT) Results: 12 out of 39 patients (31%) with LNM, 12 out of 26 patients (46%) with VM, three out of six patients (50%) with PNS, seven out of 10 patients (70%) of MPE and one out of nine patients (11%) with ETM)

could be oriented in order to pathological confirmation of primary tumor. Conclusion: PET/CT in highly efficient in detecting cancer of unknown primaries. This hybrid technology improves anatomical localization of suspected lesions in order its pathological confirmation.

No. 305

PERFORMING DIAGNOSTIC CT IN A CLINICAL POSITRON EMISSION TOMOGRAPHY SETTING

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We began performing diagnostic CT (dCT) at our positron emission tomography (PET) facility in January 2004. Since that time we have performed 3121 dCTs in conjunction with our clinical PET imaging. During 2005 26% of patients received dCT and clinical PET imaging in one visit. As we increased the number of patients receiving dCT, we have modified our normal clinical procedures to accommodate this additional imaging. When scheduling for dCT, we screen for contrast allergies, renal function, age restrictions and past chemotherapy treatments. We also insure physician coverage and allow an additional 15 minutes of scanner time. Upon patient arrival but before FDG injection, we confirm the assessment form and achieve IV access. Oral contrast is begun prior to FDG injection and continues during the uptake phase. Immediately before scanning the patient is asked to void, remove any metal objects and the IV is rechecked for potency. Routine scout and attenuation correction CTs are obtained. Individual patient parameters (contrast volume, delivery rate) are programmed before beginning the dCT. After successful dCT acquisition, the PET is acquired. Post scanning, the patient has an exit interview to assess any allergic reaction, possible dose infiltration, and is given follow-up instructions. Following this procedure we estimate we will perform 3300 PET and 3100 dCT studies in 2005.

No. 306

SINGLE CELL IMAGING BY USING 1.5 TELSA MAGNETIC RESONANCE IMAGING *IN VITRO*

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Technologies of single cell imaging are critical for evaluating the efficacy and accuracy of cell targeting imaging. This technology could also be applied for cell trafficking. Macrophage imaging has currently drawn more and more attention because of its important role in atherosclerotic plaque formation, inflammatory process and cancer cell interaction. We demonstrated the feasibility of using clinical 1.5 Tesla MR for *in vitro* imaging of macrophage which has been labeled with iron oxide particles. The amount of iron oxide been labeled has been estimated and the MR pulse sequence was optimized for single cell imaging. Briefly, 3-D GRE pulse sequence was used and the scan time was 54 minutes. The voxel size was 60um*60um*700 um. The single cell image was proved by matching the MR images with fluorescent images. The minimal iron oxide content has been also evaluated. Our effort proved that clinical 1.5 Tesla MR System has the potential for developing molecular and cellular imaging *in vivo*.

No. 307

MERKEL CELL CARCINOMA: IS THERE A ROLE FOR 2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE POSITRON EMISSION TOMOGRAPHY/ COMPUTED TOMOGRAPHY?

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Objectives: 2-Deoxy-2-[F-18]fluoro-D-glucose (FDG)-positron emission tomography (PET)/ computed tomography (CT) is available as a powerful imaging modality for detection of active metabolic processes and their morphologic features. Its role is proven in lymphoma, melanoma, colorectal carcinoma, and other cancers. Rare malignancies like merkel cell carcinoma (MCC) can potentially be evaluated with PET/CT. We were therefore prompted to review our PET/CT experience in patients with MCC. Methods: This is a retrospective case series of six patients with MCC, 58-81 years old (average: 69±8.3), who had PET/CT at our institution from Jan, 2003 to Aug, 2005. Two patients were women and four were men. Reinterpretation of imaging studies and data analysis from medical records were performed. Results: Twelve examinations were acquired for 6 patients (one had six PET/CT, one had two PET/CT and four had one PET/CT). The FDG doses ranged 10.3-18.1 mCi (average: 15.5±1.9). Four patients had PET/CT for initial staging and two for restaging. Six lesions (pancreas, adrenal, lip, submandibular/cervical lymph nodes and parapharyngeal) were identified in three patients and confirmed with histopathology. Glucose uptake in these areas was intense, with maximum standardized uptake values (maxSUV) of 5-14 (average: 10.4±3.8). In one patient PET/CT identified focal rectal uptake, diagnosed adenocarcinoma on biopsy. Two patients had negative scans, without clinical evidence of disease. Conclusions: Our study suggests a significant role for PET/CT in the management of patients with MCC. These results need to be confirmed in larger, prospective trials. However, this is an extremely rare cancer and patient enrollment will be difficult outside the setting of a multicenter project.

No. 308

POSITRON EMISSION TOMOGRAPHY/ COMPUTED TOMOGRAPHY MISREGISTRATION ATTENUATION CORRECTION ARTIFACT ELIMINATED BY AUTOMATED LEAST SQUARES CARDIAC ALIGNMENT

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Alignment of positron emission tomography (PET) and computed tomography (CT) images is essential for accurate measurements of cardiac perfusion. Misalignment can produce an erroneous attenuation map that projects lung attenuation parameters onto the heart wall, thereby underestimating the attenuation, and creating artifactual areas of hypoperfusion which may be misinterpreted as myocardial ischemia or infarction. The main cause of misregistration between CT and PET images is the respiratory motion of the patient. CT images were obtained in multiple different phases of respiration: normal tidal end expiration, halfway through normal tidal end expiration, and forced end expiration. To compare these methods, an automated least squares cardiac software alignment method was implemented to determine which phase most closely matches with PET imaging. In this approach, the heart is extracted from the PET data through windowing and c-mean clustering, and the CT scans are segmented to obtain the corresponding heart geometry. From this processed data, the heart geometries are registered, and a motion correction vector is calculated such that the alignment error of the two modalities is minimized by a least squares approach. Results of this optimization procedure have been evaluated on 24 patient PET/CT cardiac data sets producing accurate cardiac alignment which eliminated PET/CT misregistration attenuation correction artifact. Normal tidal end expiration is the most optimal CT dataset (mean Z-axis shift -4.3 mm +/- 3.3 mm) for alignment followed by forced end expiration (-7.1 +/- 7.85 mm) , and halfway through normal tidal end expiration (-9.49 +/- 3.24 mm).

No. 309

EVALUATION OF DUAL TIME POINT 2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE- POSITRON EMISSION TOMOGRAPHY IMAGING FOR FOCAL PULMONARY LESIONS

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Objectives: This study compares the accuracy of the early and delayed maximal standardized uptake values (eSUV and dSUV, respectively) and RI for differentiation of malignant tumors. Methods: In a retrospective study, 64 patients (14 benign, 50malignant lesions) exhibiting a solitary pulmonary 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) uptake, underwent a dual time point positron emission tomography (PET) imaging, and eSUV and dSUV measurements and RI calculation were included. The RI was calculated using the following formula: [(dSUV - eSUV) / eSUV] □ 100. The lesions whose SUV-RI showed a positive value were considered malignant. Results: The eSUV and dSUV of the malignant lesions were significantly higher than those of the benign lesions (eSUV: 4.94 +/- 2.73 vs. 1.87 +/- 0.42, $p<0.0001$; dSUV: 5.78 +/- 3.15 vs. 2.10 +/- 0.51, $p<0.0001$). The RI was not significantly different between the benign and malignant lesions (18.77 +/- 13.75 vs. 13.35 +/- 16.50). The lesion-based sensitivity, specificity, accuracy, positive predictive values, and negative predictive values were calculated when the cut-off value of SUV for differentiating benign from malignant lesions was 2.5 on the early and delayed images. High specificity was observed in both eSUV (92.9% (13 out of 14) and dSUV 85.7% (12 out of 14). However, the specificity on a positive RI value was only 28.6% (four out of 14), although a high sensitivity 96% (48 out of 50) was observed. The overall results are shown in the table. Conclusion: This study showed that SUV measured from both early and delayed images was an accurate parameter for differential diagnosis in patients with pulmonary nodules.

No. 310

IMAGING TUMOR ANGIOGENESIS USING IODINE-123 LABELED VASCULAR ENDOTHELIAL GROWTH FACTOR-165 (123I-VEGF165)

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Aim: Recent studies have shown that vascular endothelial growth factor (VEGF) plays an important role in the process of tumor angiogenesis and that VEGF receptor is over-expressed in vascular endothelial cells of human tumors and in human tumor cells. The aim of this study was to evaluate the usefulness of imaging tumor angiogenesis with iodine-123 labeled VEGF165 in patients with solid tumors. Materials and Methods: Human recombinant VEGF165 was radiolabeled with I-123 by electrophilic radioiodination using the chloramine T-method. I-123-VEGF165 was administered intravenously (mean dose 189±14 MBq (<130 pmole (< 5 g) VEGF165 per patient) to thirty patients with solid tumor (gastrointestinal tumors, n=20; differentiated thyroid cancers with negative radioidodine scan, n=8; osteosarcomas, n=2) . Dynamic acquisition was initiated after administration and carried out until 30 minutes post injection. Whole-body images were done at various time points. All patients underwent single photon emission computed tomography (SPECT) imaging 1.5 hours post injection. Scanning with I-123-VEGF165 was compared with computed tomography (CT) and magnetic resonance imaging (MRI). Results: Intravenous injection of I-123-VEGF165 did not cause any side-effects. Thirteen of 20 (65 %) patients with gastrointestinal tumor had positive I-123-VEGF165 scan results. I-123-VEGF165 was positive in five of eight (63 %) patients with differentiated thyroid cancer, and showed abnormal findings in two of two (100%) patients with osteosarcoma. Conclusion: These results indicate that scanning with I-123-VEGF165 can visualize various solid tumors and their metastases expressing receptors for VEGF165. I-123-VEGF165 receptor scintigraphy may be useful for visualization of tumor angiogenesis.

No. 311

WHOLE-BODY 2-DEOXY-2-[F-18]FLUORO-D-GLUCOSE-POSITRON EMISSION TOMOGRAPHY CAN PROVIDE MORE INFORMATION THAN TC-99M MDP BONE SCINTIGRAPHY FOR DETECTION OF BONE METASTASES IN PATIENTS WITH LUNG CANCER

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Purpose: Lung caner is the most common cancer in the world accounts for 12.3% of all new cancer cases with millions of deaths per year. For the prognosis and the therapy strategies, the evaluation of tumor spread is important. Recently, 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) positron emission tomography (PET) is used to detect local and distant metastases. The aim of this study was to evaluate the effect of adding whole body FDG PET to conventional bone scan for staging and detection bone metastasis in patients with lung cancer. Materials and Methods: One hundred and thirty-two lung cancer patients who had suspected local or distance metastases before treatment have been evaluated. Bone scan and FDG-PET were performed blinded to the findings of history and physical examination. The combined results of CT or magnetic resonance imaging (MRI) and the subsequent clinical course served as the gold standard for the identification of bone metastases. Results: Bone scan showed a sensitivity of 87% and FDG-PET was 96% in detection of bone metastases. Without the findings of bone scintigraphy and the gold standard methods, 19% of patients would have undergone unnecessary surgery or neoadjuvant therapy. The addition of a PET scan to a bone scintigraphy allowed for more precise localization of the lesions in some patients. Conclusion: The addition of FDG-PET to the standard bone scan is useful to detect the unexpected distant bone metastases, and give more information to the local and distant metastases. Abnormal PET findings should be confirmed to prevent patients from being denied appropriate treatment.

No. 312

ANALYSIS OF MALIGNANT AND BENIGN THYROID NODULES IN UPTAKE OF FDG

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The aim of this study was to analyze 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) uptake in malignant and benign lesions found by positron emission tomography (PET)/ computed tomography (CT). MATERIAL AND METHOD: From July 2002 ~ September 2005, 32 focal thyroid lesions noted on FDG-PET in 30 patients (female= 23; male=7, mean age=51.7y/o) were collected for study. All of these focal lesions were proved by pathology. The pathology revealed 16 malignant thyroid lesions and 16 benign thyroid lesions. Both early and delayed studies were performed in 24 patients. Visual uptake intensity ranging from Grade I, II and III and maximum standardized uptake value (SUV) were recorded for analysis. RESULT: Fourteen of 16 malignant lesions showed grade III uptake and two of 16 showed grade II uptake. None of lesions with Grade I uptake was malignant. Six of 16 benign lesions showed grade III uptake, four benign lesions showed grade II uptake, and six of benign lesions showed grade I uptake. There was significant statistically in visual uptake intensity between malignant and benign lesions (P value <0.001). The maximum SUV of malignant lesions were 7.63± 3.91and that of benign lesions were 5.08± 2.64. There was not significant statistically. The change in SUV of early and delayed studies between these two groups was also not significant statistically. CONCLUSION: Malignant thyroid lesion demonstrated significantly higher FDG uptake by visual interpretation. Although standard uptake value and delayed imaging failed to distinguish malignant thyroid lesions in our study, focal thyroid lesion with high FGD uptake should not be overlooked.