AACR/SNMMI State-of-the-Art Molecular Imaging in Cancer Biology and Therapy: Abstracts

The Society of Nuclear Medicine and Molecular Imaging (SNMMI) and the American Association for Cancer Research (AACR) have reconvened to cosponsor State-of-the-Art Molecular Imaging in Cancer Biology and Therapy, a conference to be held February 14–17, 2018, at the Manchester Grand Hyatt in San Diego, California. The conference will focus on cutting-edge research in molecular imaging research as applied to cancer biology and therapy.

In a focused, small-meeting setting, this event brings together imaging scientists with physicians and cancer biologists in basic, translational, and clinical research to discuss, promote, and support the application and use of molecular imaging in cancer biology and therapy. The conference also offers a unique, collaborative structure of didactic lectures, poster presentations, and opportunities for participation from junior scientists, as short talks have been selected from the most highly rated abstracts.

The conference features two education sessions on Wednesday, "Emerging Advances and New Imaging Technologies" and "Novel Ligands and Target Identification." Wednesday evening will include the welcome reception and a special keynote address from Chi Van Dang of the Ludwig Institute for Cancer and The Wistar Institute: "Targeting the tumor metabolic microenvironment: Imaging challenges and opportunities." The conference includes eight plenary sessions, Thursday–Saturday, on imaging cancer immunotherapy, imaging and liquid biopsies, novel agents, modeling cancer, optics, and more.

New this year will be a session for early-career investigators from Jason Lewis on "How to set up a lab and compete for grant funding" and collaboration with the NIH/NCI Quantitative Imaging Network for a one-hour session.

In addition to the didactic lectures, the meeting will include poster presentations. The abstracts in this issue of the *JNM* are listed alphabetically by the last name of the presenting author. Submitted abstracts from attendees are followed by the abstracts from the invited speakers. Please note that the presenting author at the conference is not always the first author of the abstract.

We hope that many of our members will be able to attend this exciting conference.

Conference Chairs
Todd E. Peterson, PhD, Vanderbilt University Medical Center, Nashville, TN
David R. Piwnica-Worms, MD, PhD, The University of Texas MD Anderson Cancer Center, Houston, TX

Program Committee and Abstract Reviewers

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MAIN ABSTRACTS

1

Detection of early-stage pancreatic cancer in Kras–mutated transgenic mice by [18F]FEL-PET: Comparison with pancreatitis. Sandun Perera, Frederick S. Robinson, Giannicola Genovese, Louis DePalatis, Seth Gammon, David Piwnica-Worms, Mian M. Alauddin; The University of Texas MD Anderson Cancer Center, Houston, TX

Introduction: The inability to diagnose pancreatic cancer at its early stage contributes to the high rate of mortality. We previously demonstrated the potential of [18F]FEL, which targets HIP/PAP, a biomarker that is over-expressed in peritumoral regions of the pancreas, as a tracer for PET imaging of pancreatic cancer. Herein we used [18F]FEL to identify early-stage pancreatic cancer in Kras–mutated mice and compared the results with those in a mouse pancreatitis model. **Methods:** Pancreatic cancer was induced in reporter transgenic mice bred for activation of a mutant Kras allele in a p53-deficient background in pancreatic epithelial cells (KRPΔ/Δ). Pancreatitis was induced in nude mice by injection of caerulien (50 mg/kg). Tumor growth was monitored by bioluminescence imaging (BLI). Animals were injected with [18F]FEL (3.7 MBq) and underwent static PET/CT scans 30-40 min after tracer injection. Following imaging, animals were sacrificed and pancreases were collected, weighed and radioactivity quantified. HIP/PAP expression was detected in sections of each pancreas by immunohistochemical staining. **Results:** Accumulation of [18F]FEL in the pancreases of normal mice, mice with pancreatitis, and 3- and 5-week-old mice with pancreatic cancer were 0.18±0.07, 0.49±0.11, 4.22±0.24, and 0.70±0.13 %ID/g, respectively. Accumulation of [18F]FEL in pancreatic cancer of 3-week-old mice was 8.6-fold higher than that in pancreatitis tissues. Immunostaining results showed a positive correlation between [18F]FEL accumulation and HIP/PAP expression. **Conclusion:** Spontaneous pancreatic cancer can be detected at an early stage by [18F]FEL-PET targeting HIP/PAP, but expression of HIP/PAP in pancreatitis is insufficient for visualization by [18F]FEL-PET. [18F]FEL-PET may be useful for detection of early stage pancreatic cancer and distinguishing from pancreatitis.

2

The quantum paradigm and its importance for understanding the real mechanisms of operation for PET and MRI imaging and the use of PET and MRI imaging for cancer detection. Keith Allen; Society of Brain Mapping, Los Angeles, CA

The quantum paradigm allows us to appreciate some new scientific possibilities that are beyond the reach of Newton's mechanics. The basis for both PET and MRI imaging is cogent and solid according to the logic of quantum mechanics. We can better understand the physics for PET imaging according to a Feynman diagram. The diagram demonstrates that a positron and an electron interact in an annihilation interaction where two particles are converted into two waves. This is also consistent with the concept of wave-particle duality in quantum physics. We can better understand the physics of MRI imaging according to quantum spin states. This indicates that, consistent with Planck's famous equation E=hf, the protons in the human body are able to absorb a radio wave photon in the presence of a magnetic field. This radio wave absorption is also described by the Larmor equation. In fact, quantum jumps occur when the proton absorbs a radio wave and jumps up into the higher energy spin state. A consequent relaxation is the source of signal in MRI. We can also extend the logic to quantum field theory. Quantum field theory is a successful integration of quantum mechanics and special relativity. We can add the Schrodinger equation together with four dimensional space-time in special relativity. Using Dirac's method, we arrive at the basis for the magnetic vectors in MRI. Using the Klein-Gordon method, we arrive at the positron and the basis for PET. PET and MRI are both very important technologies for cancer detection. PET provides important metabolic information. Basic MRI scans can provide important anatomical information, however, we can also use more specialized MRI methods like contrast enhanced MRI or diffusion weighted MRI to improve cancer detection. As provided in my protocol summary, we can use both PET and MRI for early cancer detection. We can also improve early detection methods using health information from existing databases to select "high risk" groups that should be scanned. This is a system that is available using computerized medical record systems. If cancer is caught early using imaging, then we can improve the probability for successful outcomes. This works well for cancers that form tumors like lung cancer, breast cancer, prostate cancer, and many brain tumors. If the cancer is caught very early, then it may be possible to remove the tumor using surgery. Radiation will also be most effective at this phase. If the cancer is caught at a more intermediate stage, then the information from PET and/or MRI will still provide important information for treatment planning using surgery, radiation therapy, and/or chemotherapy. The clinical outcome can be improved. In summary, early cancer detection using imaging can improve the probability of success for many treatment interventions.

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Characterization of a dual-labeled somatostatin analog for fluorescence-guided surgery. Servando Hernandez Vargas, Julie Voss, Sukhen C. Ghosh, Jo Simien, Ali Azhdarinia; Institute of Molecular Medicine, McGovern Medical School, Houston, TX

Objectives: Surgery is the primary treatment option for most solid tumors and can be curative if all cancer cells are removed. For several types of neuroendocrine tumors (NETs), surgery is indicated not only for localized lesions but also for metastatic disease to control excessive hormone production. Clinical evidence has shown that fluorescent agents can improve intraoperative detection of tumors compared to visual observation. An ideal approach for developing an intraoperative imaging agent for NETs would be to build upon the clinically established imaging utility of radiolabeled peptides that target somatostatin receptor-2 (SSTR2) overexpression. Using a customized multimodality chelator (MMC), we introduced a fluorescent label onto the radiolabeled somatostatin analog, 68Ga-DOTA-TOC, and produced the bioactive dual-labeled analog, 68Ga-MMC(IR800)-TOC. Here, we evaluate the imaging properties of 68Ga-MMC(IR800)-TOC in SSTR2-expressing xenografts. **Methods:** The macrocyclic compound, DO2A, was used to synthesize the customized MMC and permit site specific conjugation of Tyr3-octreotide (TOC) and IR800 using solid-phase and solution-phase synthesis, respectively. 68Ga labeling was performed using cation exchange chromatography based on 68Ga-DOTA-TOC methods. Nude mice were implanted subcutaneously with SSTR2-overexpressing HCT116 cells (HCT116-SSTR2) and used for in vivo studies. Mice underwent PET/CT and near-infrared fluorescence (NIRF) imaging at 3 h post-injection to determine tracer distribution, followed by biodistribution studies to quantify tissue uptake. Delayed imaging effects were then examined by NIRF imaging at 24 h along with ex vivo imaging on resected tissues to determine image contrast at sites associated with metastatic disease in NET patients. **Results:** MMC-TOC was synthesized using standard solid-phase techniques and dual labeling with IR800 and 68Ga was confirmed by HPLC. At 3 h post-injection, imaging studies demonstrated that 68Ga-MMC(IR800)-TOC tumor uptake could be well-visualized by both modalities along with prominent kidney signal indicating renal excretion. Biodistribution data were in agreement with these findings as shown by %ID/g values of 3.8±1.8 (tumor) and 20.0±3.1 (kidneys). Low muscle uptake afforded a tumor-to-muscle ratio of 5.1±1.2. On delayed NIRF imaging, the combination of increased tumor uptake and washout from normal tissues resulted in excellent image contrast. Accordingly, we obtained tumor-to-nontumor ratios of 5.4±1.8 (pancreas), 9.9±3.9 (intestine), and 9.6±3.1 (muscle) from ex vivo tissue analysis (n=5). **Conclusions:** SSTR2-expressing tumors could be visualized by multimodality imaging with a fluorescent 68Ga-DOTA-TOC analog. Further characterization of this compound could potentially improve real-time surgical guidance for patients with NETs.

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Cherenkov and bioluminescence imaging with the LightPath Imaging System. Kvar C.L. Black[1], Xuefeng Gao[1], Lynne Marsala[1], Gail P. Sudlow[1], Kunal Vyas[2], David Tuch[2], Samuel Achilefu[1]; [1]Washington University School of Medicine, St. Louis, MO, [2]Light Point Medical, Chesam, England

Designed to image Cherenkov radiation in a clinical setting, the LightPath Imaging System was explored as a preclinical tool to image targeted radionuclides and cancer cells with bioluminescent reporters. The LightPath Imaging System consists of a radiation-shielded, Peltier-cooled emCCD with fast optics. For the first application, the prostate specific membrane

antigen (PSMA) was targeted with a ^{64}Cu-labeled small molecule in a bilateral PC3 prostate cancer xenograft model wherein one tumor was genetically modified to overexpress PSMA. Upon injection of the ^{64}Cu radiotracer, Cherenkov radiation was observed in tumors, and importantly, significantly higher signal was observed in PSMA-positive tumors compared to control tumors (1.1×10^5 vs. 7.1×10^4 counts, $p = 0.015$). In the second application, 4T1-Luc breast cancer cells with a luciferase reporter were plated with decreasing concentrations in a 96 well plate and the LightPath sensitivity was compared to standard IVIS equipment. Interestingly, bioluminescent signal was detected by the LightPath system in wells where the IVIS was unable to detect signal from background (625 cells per well). In a further study, PYMT tumors were grown in vivo, and again detection sensitivities were compared between the 2 systems. While bioluminescent signal observed from primary tumors was visible in both systems, LightPath was able to identify signal from metastatic cells not visible with IVIS. Taken together, the enhanced sensitivity of the LightPath system to more conventional systems has significant potential in identifying low concentrations of Cherenkov emitters as well as bioluminescent micrometastases in preclinical in vivo models.

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Preclinical evaluation of a new radiolabeled peptide for PET imaging of GPC3 expression in hepatocellular carcinoma. Kai Chen, Hubing Wu, Jun Zhang, Peter S. Conti; University of Southern California, Los Angeles, CA

Objectives: Target-specific imaging of hepatocellular carcinoma (HCC) holds considerable clinical significance for early diagnosis and development of targeted therapies for patients with HCC. Among the numerous proteins and receptors expressed on HCC cell membrane, Glypican-3 (GPC3) has attracted substantial attention because its expression is correlated with HCC tumorigenesis and prognosis. In this study, we radiolabeled a new GPC3-targeted peptide (GTP) with ^{64}Cu, and the resulting PET tracer was subsequently subjected to biological evaluations in a subcutaneous HCC mouse model. **Methods:** The immunofluorescence assay was used to identify the expression of GPC3 and determine the binding of GTP in tumor cells. A hydrophilic linker was incorporated into the GTP with an aim of improving pharmacokinetics (PK) of the PET tracer. The GTP was conjugated with NOTA chelator and radiolabeled with ^{64}Cu in ammonium acetate buffer. The HCC targeting efficacy of ^{64}Cu-labeled GTP was evaluated by cellular uptake, small animal PET, and ex vivo biodistribution. **Results:** The immunofluorescence assay showed that GPC3 is highly overexpressed on the membrane of HepG2 cells, and HepG2 cell uptake of GTP is strongly associated with the GPC3 expression. The ^{64}Cu radiolabeling was achieved in 85% decay-corrected yield with radiochemical purity of >98%. The specific activity of ^{64}Cu-GTP was estimated to be ~40 MBq/nmol. The octanol–water partition coefficient measurement determined the high hydrophilicity of ^{64}Cu-GTP. PET study showed ^{64}Cu-GTP has preferential tumor uptake in HepG2 xenografts. Significantly low liver uptake at 2 h post-injection of ^{64}Cu-GTP (2.16 ± 0.62%ID/g) was observed in the subcutaneous mouse tumor model, suggesting that the tracer excretion is predominated via the renal system. The biodistribution results were consistent with the quantitative analysis of PET imaging. **Conclusions:** We have successfully synthesized a new ^{64}Cu-labeled GTP PET tracer and evaluated its efficacy for PET imaging of GPC3 expression in HCC. The incorporation of a hydrophilic linker into the GTP significantly reduces the tracer uptake in liver. Favorable target specificity and PK of ^{64}Cu-GTP show the potential for further translational studies.

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Evaluation of therapeutic effect using molecular imaging technologies for orthotopic brain cancer mouse model. Jang Woo Park, Eun Sang Lee, Ok-sun Kim, Hye Kyung Chung, Chi Soo Kang; Korea Institute of Radiological & Medical Sciences, Seoul, Korea

Cancer is the second leading cause of death in the U.S. following heart disease (1). Among the types of cancer, glioblastoma mutiform is the most aggressive and invasive brain tumor with high recurrence rate and low survival rate after diagnosis (2,3). Animal models have been utilized as an essential tool to develop effective cancer therapeutics and diagnostic probes, and it has been recognized validation of an appropriate tumor model is critical to translate preclinical studies into clinical settings. Subcutaneous tumor models have been predominantly exploited due to their convenience in monitoring disease progress. Although cancer genetics and growth behavior would be the same independent of location of the xenograft, a subcutaneous model does not reflect the tumor micro-environment and is not able to predict the outcome in the clinic properly (4,5). Therefore, orthotopic xenograft models, which provide appropriate organ-specific tumor micro-environment, have been identified as a better tool for studying cancer biology and therapeutics (6,7). In the current study, we reported (8) validation of orthotopic brain cancer model development in Balb/c-nu/nu mouse using bioluminescence imaging (BLI), positron emission tomography/computed tomography (PET/CT) imaging, and magnetic resonance imaging (MRI). We investigated whether therapeutic effect could be evaluated by imaging with temozolomide using an established brain tumor model. It was confirmed that BLI signal and FLT uptake decreased significantly compared to the control group according to the treatment of the temozolomide. Similar results were obtained with MR imaging in that the size of the brain tumor caused by the temozolomide was reduced. Based on these results, it was confirmed that the therapeutic efficacy of the temozolomide can be fully evaluated by noninvasive methods. Using this method, we expect to be able to evaluate the efficacy of drug candidates using a brain orthotopic tumor model. **References:** 1. Heron M., Natl Vital Stat Rep, 2016, 65(5). 2. Berens M.E. and Giese A., Neoplasia, 1999, 1(3), 208-219. 3. Xie Q. et al., Neuro-Oncology, 2014, 16(12), 1575-1584. 4. Tavera-Mendoza L.E. and Brown M., Lab Anim, 2016, pii: 0023677216640706. 5. Teicher B.A., Expert Opin Drug Discov, 2009, 4(12), 1295-1305. 6. Kalra J. et al., Cancer Biol Ther, 2011, 11(9), 826-838. 7. Talmadge J.E. et al., Am J Pathol, 2007, 170(3), 793-804. 8. Kang C.S. et al., WMIC, 2017, P274.

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Targeting glutamine metabolism through inhibition of GLS1 enhances therapeutic efficacy of EGFR-targeted antibodies in colorectal cancer. Allison S. Cohen, Ling Geng, Ping Zhao, Allie Fu, Michael L. Schulte, Ramona G. Deal, Robert J. Coffey, Michael L. Nickels, H. Charles Manning; Vanderbilt University Medical Center, Nashville, TN

Colorectal cancer (CRC) is a leading cause of cancer-related deaths worldwide. Epidermal growth factor receptor (EGFR) monoclonal antibodies (i.e., cetuximab [CTX] and panitumumab) are approved for patients with advanced wild-type (WT) RAS CRC and can be used in first-line or late-line therapy. A large proportion of patients do not respond to anti-EGFR monotherapy, and patients commonly, and often rapidly, acquire resistance. Thus, novel therapeutic combinations are needed that enhance the efficacy of these agents and/or overcome resistance. A hallmark of cancer is altered metabolism. Cancer cells utilize glutamine (Gln) as a carbon source for ATP production, biosynthesis, and as a defense against reactive oxygen species. We hypothesized that Gln metabolism contributes to resistance to EGFR-targeted therapy in CRC and hence, targeting glutaminolysis may represent a promising therapeutic avenue. Glutaminase 1 (GLS1) is a mitochondrial enzyme responsible for catalyzing the conversion of Gln to glutamate (Glu). GLS1 has higher expression in CRC tumors compared to normal colonic tissue. The goal of this work was to combine CTX with a GLS1 inhibitor, CB-839, for the treatment of CRC. We tested the combination treatment using in vitro and in vivo experiments in models of WT RAS CRC. Using 2D cell models, we showed that CB-839/CTX significantly decreased cell viability when compared to vehicle or single agent controls. In addition, in 3D cell culture CB-839/CTX overcame resistance to EGFR mAb treatment and resulted in significantly reduced colony counts. Finally, mice bearing xenograft tumors were longitudinally treated with either vehicle, CB-839, CTX, or CB-839/CTX. Mice bearing SW48 xenografts progressed on single agent CB-839 or CTX yet exhibited significantly reduced tumor volumes when treated with CB-839/CTX. Mice bearing HCA-7 CC-CR, a CTX-resistant model, were treated for 21 days and tumor volumes were monitored to day 57. CTX-treated xenografts, which had regressed during treatment, recurred, while CB-839/CTX xenografts did not. Immunohistochemistry was used to evaluate molecular determinants of response. CB-839/CTX treatment led to significantly reduced pS6 and Ki67 compared with single agents. In conclusion, combining CB-839 with anti-EGFR mAbs represents a new translational approach for the treatment of patients with CRC. We currently have a clinical trial testing the combination of panitumumab with CB-839 in patients with metastatic WT RAS CRC. In addition, we are interested in developing biomarkers to non-invasively predict response to treatment by

using positron emission tomography (PET) imaging of Gln metabolism. We are developing two radiotracers ^{11}C-Gln and ^{18}F-FSPG that report on Gln influx and Glu efflux, respectively. We have a clinical trial to perform the first-in-human studies of ^{11}C-Gln and are one of a few sites using ^{18}F-FSPG clinically. We are also developing magnetic resonance spectroscopy (MRS) of Gln and Glu concentration as a preclinical correlate to PET imaging.

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[^{18}F]HX4 PET demonstrates the hypoxia-modulating capacities of metformin and acts as a prognostic biomarker for survival in a NSCLC xenograft mouse model. Sven De Bruycker[1], Christel Vangestel[2], Tim Van den Wyngaert[2], Steven Deleye[1], Leonie Wyffels[2], Steven Staelens[1], Sigrid Stroobants[2]; [1]University of Antwerp, Antwerp, Belgium, [2]Antwerp University Hospital, Antwerp, Belgium

Introduction: Metformin (MET) may improve tumor oxygenation and thus radiotherapy (RT) response. However, appropriate imaging biomarkers for patient selection are critically needed to advance to clinical trials with MET as a radiosensitizer. In this study, we first assessed the effect of acute MET administration on NSCLC xenograft tumor hypoxia using PET imaging with the hypoxia tracer [^{18}F]HX4. Second, we verified the effect of a single dose of MET prior to RT on long-term treatment outcome. Third, we examined the potential of baseline [^{18}F]HX4 as a prognostic and/or predictive biomarker for treatment response. **Experimental procedures:** A549-tumor bearing mice (n=21; inoculated in both hind legs) underwent a [^{18}F]HX4 PET/CT scan to determine baseline tumor hypoxia. The next day, mice received an IV injection of 100 mg/kg MET. [^{18}F]HX4 was administered IV 30 min. later and a second PET/CT scan was performed to assess changes in tumor hypoxia. Two days later, mice were divided into three groups with comparable tumor volumes: a control group (1), an RT group (2), and a MET+RT group (3). Animals received saline (groups 1-2) or 100 mg/kg MET (group 3) IV, followed by a single dose of 10 Gy 30 min. later (groups 2-3). Control mice (group 1) underwent sham RT without dose. Tumor growth was monitored 3x/week by caliper measurements. Calculation of the relative tumor volumes ($RTV=V_{time\ x}/V_{baseline}$) started when tumors reached 100 mm^3 on average. The tumor doubling time (TDT), i.e., the time to reach 2x the pre-irradiation tumor volume, was used as a proxy for progression-free survival (PFS) and was defined as the end-point. **Results:** Thirty min. post-MET treatment, [^{18}F]HX4 could demonstrate a significant change in A549 tumor hypoxia with a mean intratumoral reduction in [^{18}F]HX4 tumor-to-background ratio (TBR) from 3.21±0.13 to 2.87±0.13 ($p=0.0001$). Overall, RTV over time differed across treatment groups ($p<0.0001$), with the MET+RT group having significantly lower RTV than controls from day 3 post-therapy onwards (2.00±0.21 vs. 3.13±0.29 resp.; $p=0.008$), and MET+RT-treated tumors from day 10 post-therapy onwards compared to RT-treated tumors (1.98±0.19 vs. 3.67±0.59, resp.; $p=0.03$). Similarly, the median TDT was significantly different between the treatment groups (log-rank $p<0.0001$), with a median TDT of 19, 34 and 52 days in the control group, the RT-group and the MET+RT-group, respectively. Baseline [^{18}F]HX4 TBR was a prognostic biomarker for TDT (HR 2.0; 95% CI 1.2-3.2; $p=0.004$) accross treatment groups and adjusting for baseline tumor volume. We could not demonstrate baseline [^{18}F]HX4 to be a predictive biomarker for therapy response. **Conclusions:** Using [^{18}F]HX4 PET imaging in a NSCLC xenograft model, we showed that MET may act as radiosensitizer by decreasing tumor hypoxia and that baseline [^{18}F]HX4 shows promise as a prognostic biomarker for PFS.

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Revolutionary role of FDG-PET and FDG-PET/CT in the evaluation of ascites. Sahra Emamzadehfard[1], Vahid Eslami[2], Farima Kahe[3], Tom Werner[4], Abass Alavi[4]; [1]University of Texas Health Science Center, San Antonio, TX, [2]University of Texas Medical Branch, Galveston, TX, [3]Beth Israel Deaconess Medical Center, Harvard University, Boston, MA, [4]University of Pennsylvania, Philadelphia, PA

Introduction: Ascites is the imbalance of fluid between the vascular bed and extra-vascular tissues. One of the most important causes of ascites is malignancy, which accounts for 10% of cases. Differential diagnosis of malignant ascites can be confusing and needs to exclude many of the potential causes. Advanced molecular imaging through Positron emission tomography/computed tomography (PET/CT) is an imperative tool for accurate tumor diagnosis, pre-treatment management and post treatment surveillance. PET/CT could accurately and early locate both the primary disease site and tumor expansion. Quantitative imaging through Minimum standardized uptake value (SUVmin) is a frequently used tool, because partial volume effects least affect it. SUVmin measurement provides an accurate technique for assessing tumor pathology and its metastases. Role of FDG-PET and FDG-PET/ CT in detecting primary lesions that cause ascites: FDG PET can identify the primary tumor in almost 50% of patients with metastatic carcinoma. The sensitivity of PET/CT for perceiving the primary malignancies causing ascites was 64%. Differentiation of malignant and benign ascite SUVmax analysis has separate implantation, which may help in distinguishing malignant causes of ascites from benign conditions. The SUVmax of malignant causes of ascites is shown to be significantly higher than physiologic peritoneal uptake and benign causes of ascites. The SUVmax can also be high in infectious and inflammatory causes of ascites such as peritoneal tuberculosis. Malignant ascites secondary to ovarian cancer: An ovarian mass, enormous ascites and elevated serum CA-125 levels in postmenopausal women propose a malignant ovarian tumor, especially advanced epithelial ovarian cancer. PET/CT has high accuracy (92%) to differentiate malignant ascites caused by ovarian tumors from benign causes of ascites. Malignant ovarian tumors have a SUVmax >3. Also, the mean SUVmax of malignant ovarian tumor was 9.32±4.58. **Conclusion:** Although PET/CT has limitations such as a high cost, the method may facilitate differential diagnosis and detection of the underlying cause of ascites. The use of PET/CT allowed for accurate diagnosis and early treatment of patients with malignancy, in the long course, will result in lower costs and benefit for the patients. The results of PET/CT investigations, which are appraised by SUVmax analysis, may help in the differentiation of malignant ascites from benign ascites, because the SUVmax of malignant ascites is significantly higher than that of physiologic peritoneal uptake and benign ascites. **Clinical relevance:** Despite a relatively small number of PET/CT studies in ascites, there is evidence for its value in locating the primary tumor and in distinguishing benign from malignant causes.

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The evolving role of molecular imaging in the diagnosis, staging and treatment of breast cancer. Sahra Emamzadehfard[1], Vahid Eslami[2], Koosha Paydary[3], Farima Kahe[4], Tom Werner[3], Abass Alavi[3]; [1]University of Texas Health Science Center, San Antonio, TX, [2]University of Texas Medical Branch, Galveston, TX, [3]University of Pennsylvania, Philadelphia, PA, [4]Beth Israel Deaconess Medical Center, Harvard University, Boston, MA

Objectives: PET/CT is not routinely performed for the diagnosis of primary breast cancer; its findings in specific subtypes of breast cancer correlate with some histopathologic features of the primary tumor. PET/CT can detect lymph node metastases accurately. Enhanced glycolytic activity of tumor cells and increased uptake of FDG allow for the visualization of malignant lesions by FDG-PET/CT. In this review, we will discuss the applications of FDG-PET/CT in the diagnosis, staging, prognosis assessment, recurrence and restaging as well as monitoring treatment response of patients with breast cancer. Application of PET/CT in primary staging of breast cancer: PET/CT is useful only in the primary staging of patients who are at a considerable risk of metastasis. Application of PET/CT in evaluating of lymph nodes metastasis: A sentinel node biopsy (SNB) positive finding warrants further investigations with axillary lymph node (ALN) dissection. Accuracy of PET/CT in diagnosing lymph node metastasis in patients with breast cancer revealed high specificity. Evidence for the diagnostic accuracy of PET/CT for ALN involvement has been shown compared to other non-invasive methods such as MRI and US. Application of PET/CT in evaluating distant metastasis: PET/CT is a valuable alternative when conventional MRI shows indeterminate or benign lesions. The functional nature of this modality gives it the advantage for detecting early metastasis to the bone marrow, the most common site of metastasis. Application of PET/CT in management modification of breast cancer: The diagnostic accuracy of PET/CT has an important role in modification of management plan in more advanced stages, specially in stage IIB and III. Pretreatment PET/CT provides important information about involvement of loco-regional and mediastinal lymph nodes, as well as unsuspected sites of distant metastasis, which are vital in the design of radiation therapy fields in inflammatory breast cancer. Application of PET/CT in prognosis of breast cancer: PET/CT can determine prognosis by providing quantitative measures such as SUVmax before and following neo-adjuvant chemotherapy, whole-body

total lesion glycolysis and whole-body metabolic tumor volume. Texture analysis for assessment of tumor heterogeneity has been recommended to assess tumor aggressiveness. Such evidence further highlights the future implications of PET/CT as a prognostic tool in advanced-stage breast cancer. Application of PET/CT in recurrence of breast cancer: Suspected cases of recurrence that have equivocal conventional studies would be the best candidates for PET/CT investigations. PET/CT has been regarded as a "one-stop shop" imaging modality that can define the extent of disease burden in cases of loco-regional recurrence and distant metastasis. **Conclusion:** The reported accuracy of PET/CT is comparable to that of conventional imaging modalities. It is clear that PET/CT imaging has a role in the staging, restaging and management of advanced-stage breast cancer.

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Revolutionary role of advanced molecular imaging: PET/CT and PET/MRI in the early diagnosis and accurate management of gynecologic malignancies. Sahra Emamzadehfard[1], Vahid Eslami[2], Farima Kahe[3]; [1]University of Texas Health Science Center, San Antonio, TX, [2]University of Texas Medical Branches, Galveston, TX, [3]Beth Israel Deaconess Medical Center, Harvard University, Boston, MA

Purpose: Cervical, ovarian and endometrial cancers are among the most common malignancies in females. As a result, early recognition would significantly improve the management of patients. Through this review, we try to present the roles of PET/CT and PET/MRI in earlier diagnosis and accurate management of patients with gynecologic malignancies. Cervical cancer (CC): Early detection of CC, as one of the most common leading causes of female cancer-related death, is mandatory and could be easily achieved with advanced molecular imaging. PET/CT is favorable in pretreatment evaluation and post-treatment surveillance as well as in post treatment response assessment and predicting prognosis. PET/MRI has great significance for its ability to early detect local tumor extension, due to the high soft tissue contrast declaration of MRI, which allows for regional nodal and distant metastatic discovery in pretreatment planning. Ovarian cancer (OC): Unfortunately, OC is one of the most common gynecologic malignancies which may go undetected for an extended period of time. Patients are often diagnosed with extensive disease. PET/CT can detect lymph node and distant metastasis in ovarian cancer with high precision, which can consequently impact patient management and result in better survival and patient outcome. The role of PET/CT staging is superior for N and M staging of ovarian cancer, however its role is limited for T staging. Additionally, PET/CT is of great benefit in estimating treatment response and has prognostic value in patients with ovarian cancer. PET/MRI may be valuable for tumor staging because MRI has higher soft tissue contrast with no ionizing radiation exposure. DWI/MRI with contrast administration is particularly useful to consider the local extent of tumor, and to improve assessment of regional nodal and distant sites of metastatic diseases. Endometrial cancer (EC): EC is the most common gynecologic cancer, which tends to be aggressive. Unlike OC, up to 25% of cases appear in premenopausal patients. Ultrasonography and MRI are the prime modalities for the diagnosis of EC. CT and MRI are typically used to assess lesions in the abdomen and the pelvis, and as such, miss the extra-peritoneal invasion. The major role of PET/CT for the initial treatment strategy in EC is exclusion of lymph node aggregation and classifying distant metastases. This allows the avoidance of surgical lymph node dissection in patients who are poor surgical candidates, and this drastically decreases the mortality rate in this population. PET/CT is useful for the early assessment of disease recurrence following therapy. PET/MRI evaluates the extent of involvement while accurately staging nodes and assessing the distant metastases. **Conclusion:** Although the diagnostic value of molecular imaging for gynecologic malignancies has been limited in the past, some trials have proved its diagnostic role in these cancers. Thus, molecular imaging with PET should be considered as an alternative to the conventional diagnostic imaging.

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Radiolabeled caspase-3 substrates for non-invasive imaging of apoptosis by PET/CT. Brian J. Engel, Argentina Omelas, Zhen Lu, Federica Pisaneschi, Rajan Chaudhari, Seth T. Gammon, Hailing Yang, Victoria Yan, Lindsay Kelderhouse, Amer M. Najjar, William P. Tong, David Piwnica-Worms, Shuxing Zang, Robert C. Bast, Steven M. Millward; University of Texas MD Anderson Cancer Center, Houston, TX

Treatment of cancer with chemotherapy or radiation requires a prolonged waiting period to determine efficacy. As these treatments typically result in apoptotic cell death, imaging-based readout of cellular apoptosis could rapidly verify treatment efficacy and be used to monitor off-target cytotoxicity in real-time. To this end, we developed novel radiolabeled caspase-3 substrates for non-invasive imaging of cell death using PET/CT. Structure optimization based on the M808 irreversible caspase-3 inhibitor yielded the fluorinated substrate 2MP-TbD-MeTE[19F] which showed a 14-fold improvement in substrate activity and significantly enhanced caspase selectivity relative to the initial compound. Molecular modeling suggested that these improvements resulted from favorable interactions mediated by both the side chain of O-benzyl-threonine and the linker functionality between the scissile amide bond and the triazole. Radiosynthesis using a modified [18F]fluoroethylazide protocol implemented on a GE Tracerlab produced 2MP-TbD-MeTE[18F] at multi-mCi yield with specific activities between 22-149 GBq/μmol. The resulting radiotracer accumulated in cisplatin-treated ovarian cancer cells in a caspase- and cisplatin-dependent fashion. Using the Jo2 antibody-induced hepatotoxicity mouse model, 2MP-TbD-MeTE[18F] showed 1.5-fold increased liver uptake in Jo2-treated mice compared with untreated controls. These data suggest that 2MP-TbD-MeTE[18F] PET imaging could provide an immediate pharmacodynamic readout of tumor apoptosis in vivo and enable rapid evaluation of treatment efficacy.

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Transferrin-based PET measures MYC activity in prostate cancer: From bench to bedside. Rahul R. Aggarwal, Spencer C. Behr, Pamela Paris, Charles Truillet, Charles Ryan, Eric J. Small, Michael J. Evans; UCSF, San Francisco, CA

Noninvasive measurement of MYC activity with quantitative imaging modalities could substantially increase our understanding of the role of MYC signaling in clinical settings for which invasive techniques are challenging to implement or do not characterize the biology of all tumors in a patient. Moreover, measuring MYC activity noninvasively opens the opportunity to study changes in MYC signaling in patients under targeted therapeutic conditions thought to indirectly inhibit MYC. The goal of this study was to determine whether ^{68}Ga-citrate (which avidly binds to circulating transferrin) can detect MYC-positive prostate cancer tumors, as the transferrin receptor is a direct MYC target gene. PET imaging paired with ^{68}Ga-citrate and molecular analysis of preclinical models, human cell-free DNA (cfDNA), and clinical biopsies were conducted to determine whether ^{68}Ga-citrate can detect MYC-positive prostate cancer. Importantly, ^{68}Ga-citrate detected human prostate cancer models in a MYC-dependent fashion. In patients with castration-resistant prostate cancer, analysis of cfDNA revealed that all patients with ^{68}Ga-citrate avid tumors had a gain of at least one MYC copy number. Moreover, biopsy of two PET avid metastases showed molecular or histologic features characteristic of MYC hyperactivity. These data demonstrate that ^{68}Ga-citrate targets prostate cancer tumors with MYC hyperactivity. A larger prospective study is ongoing to demonstrate the specificity of ^{68}Ga-citrate for tumors with hyperactive MYC.

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Imaging PD-L1 expression levels with zirconium-89 immunoPET. Charles Truillet[1], Lawrence Fong[1], Charles S. Craik[1], Trever G. Bivona[1], Cheng-I Wang[2], Michael J. Evans[1]; [1]UCSF, San Francisco, CA, [2]Singapore Immunology Network, Biopolis, Singapore

High sensitivity imaging tools could provide a more holistic view of target antigen expression to improve the prospective identification of patients who might benefit from cancer immunotherapy. We developed for immunoPET a novel recombinant human IgG (termed C4) that potently binds an extracellular epitope on human and mouse PD-L1 and radiolabeled the antibody with zirconium-89. Small animal PET studies showed that ^{89}Zr-C4 detected antigen levels on a patient derived xenograft (PDX) established from a non-small cell lung cancer (NSCLC) patient before an 8 month response to anti-PD-1 and anti-CTLA4 therapy. Importantly, this concentration is beneath the detection limit of previously developed anti-PD-L1 radiotracers,

including radiolabeled atezolizumab. We also show that ^{89}Zr-C4 can specifically detect its antigen in human NSCLC and prostate cancer models endogenously expressing a broad range of PD-L1. ^{89}Zr-C4 detects mouse PD-L1 expression changes in immunocompetent mice, suggesting that endogenous PD-1 will not confound human imaging. Lastly, we found that ^{89}Zr-C4 could detect acute changes in tumor expression of PD-L1 due to standard of care chemotherapies. In summary, we show evidence that low levels of PD-L1 can be imaged with immunoPET using a novel human antibody.

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Assessment of murine colorectal cancer by micro-ultrasound using three-dimensional reconstruction and non-linear contrast imaging. Jessica L. Freeling, Khosrow Rezvani; The University of South Dakota, Vermillion, SD

The relatively low success rates of current colorectal cancer therapies have led investigators to search for more specific treatments. Vertebrate models of colorectal cancer are essential tools for the verification of new therapeutic avenues such as gene therapy. The evaluation of colorectal cancer in mouse models has been limited due to the lack of an accurate quantitative and longitudinal non-invasive method. The current work introduces a method of three-dimensional micro-ultrasound reconstruction and microbubble administration for the comprehensive and longitudinal evaluation of colorectal cancer progression. This approach enabled quantification of both tumor volume and relative vascularity using a well-established inducible murine model of colon carcinogenesis. This inducible model recapitulated the adeno-carcinoma sequence that occurs in human colorectal cancer allowing systematic in situ evaluation of the ultrasound technique. The administration of intravenous microbubbles facilitated enhancement of colon vascular contrast and quantification of relative vascularity of the mid and distal colon of the mouse in three dimensions. In addition, two-dimensional imaging in the sagittal orientation of the colon using Non-Linear Contrast Mode enabled calculation of relative blood volume and perfusion as the microbubbles entered the colon micro-vasculature. Quantitative results provided by the outlined protocol represent a non-invasive tool that can more accurately define colorectal cancer development and progression. This ultrasound technique will allow the practical and economical longitudinal study of murine colorectal cancer in both basic and pre-clinical studies.

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Nucleoside diphosphate kinase-3 (NME3) enhances TLR5-induced NF-κB activation in tumor cells. Caleb Gonzalez[1], Kelly Flentie[2], Brandon Kocher[2], Yue Wang[1], Hongtu Zhu[1], Jayne Marasa[2], David Piwnica-Worms[1]; [1]U.T. M.D. Anderson Cancer Center, Houston, TX, [2]Washington University School of Medicine, St. Louis, MO

Bacterial flagellin is a potent activator of NF-κB signaling, inflammation and host innate immunity, and recent data indicate that flagellin may represent a novel anti-tumor ligand acting through TLR5 and the NF-κB pathway to induce host immunity and aid in the clearance of tumor xenografts. To identify innate signaling components of TLR5 responsible for the pro-inflammatory program necessary for these anti-tumor effects, we employed a loss-of-function high-throughput screen utilizing carcinoma cells expressing a dynamic NF-κB bioluminescent reporter stimulated by *Salmonella typhimurium* expressing flagellin. A live cell screen of a siRNA library targeting 691 known and predicted human kinases to identify novel tumor cell modulators of TLR5-induced NF-κB activation uncovered several candidates, including nucleoside diphosphate kinase-3 (*NME3*), as an enhancer of signaling responses to flagellin. Targeted knockdown and overexpression assays confirmed the regulatory contribution of NME3 to TLR5-mediated NF-κB signaling, mechanistically downstream of MyD88. Furthermore, Kaplan-Meier survival analysis for breast, lung and ovarian cancer patients showed that high level expression of NME3 correlated with increased overall survival. Together, these data identify a previously unrecognized pro-inflammatory role for NME3 in signaling downstream of TLR5 that may potentiate cancer immunotherapy.

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Directed evolution of imaging agents and therapeutics targeting LC3 and autophagy. Joshua P. Gray, Lindsay E. Kelderhouse, Zhen Lu, Robert C. Bast, Jr., Steven W. Millward; The University of Texas M.D. Anderson Cancer Center, Houston, TX

Autophagy is a homeostatic cellular process highly conserved among eukaryotes in which damaged proteins and organelles in the cytosol are sequestered by double-membraned autophagosomes and shepherded to the lysosome for degradation. Dysregulation of autophagy has been observed in cancer both as a mechanism to avoid programmed cell death and as a means to survive the hypoxic, nutrient deprived conditions often found in the tumor microenvironment. Study of the role of autophagy on tumorigenesis has been hampered by the lack of selective autophagy inhibitors. Chloroquine and hydroxychloroquine, the only autophagy inhibitors to enter clinical trials, are non-specific lysosomotropic agents and have generally shown poor efficacy and high toxicity. LC3, an 18 kDa ubiquitin-like protein, plays a critical role in the maturation of autophagosomes and the selective recruitment of cargo to the autophagosome interior. Novel ligands that selectively bind to LC3 could be of immense value for tracking autophagy in living cells and for disrupting protein-protein interactions critical for autophagosome function. We have utilized SUPR peptide mRNA display to design LC3-targeted macrocyclic peptides for inhibition and molecular imaging of autophagy. SUPR peptide mRNA display is a directed evolution process in which translated peptides are covalently bound to their encoding mRNA allowing trillions of unique peptide sequences to be iteratively sieved for binding to a target protein. By incorporating unnatural, N-methyl amino acids and post-translational cyclization, peptides with extraordinary protease resistance and nanomolar affinities can be obtained. These Scanning Unnatural Protease Resistant (SUPR) peptides bind target proteins with antibody-like affinities while potentially maintaining the tumor- and cell-penetrating properties of small molecules. SUPR peptide mRNA display selections incorporating N-methyl alanine were performed against recombinant LC3. After 7 rounds of selection, library convergence was observed by binding and PCR analysis. Subsequent sequencing of the library revealed that the final pool was dominated by two families of peptides which each show a consensus amino acid sequence analogous to the LC3 Interacting Motif (LIM) observed in natural LC3 adaptor proteins. Moreover, N-methyl alanine was found to be incorporated at a single position within the macrocycle in all sequences, suggesting that this unnatural residue plays a key role in enforcing cyclic peptide conformation and function. Binding analysis of individual clones confirmed the presence of LC3-binding sequences and indicated several residues within and surrounding the modified LIM that play a significant role in peptide affinity. The most promising LC3-binding SUPR peptides have been chemically synthesized and tested for cell-permeability, binding affinity, and serum-stability to identify candidates for further testing in starvation- and DIRAS3-induced models of ovarian cancer autophagy.

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An advanced paradigm for molecular imaging and radionuclide therapy of cancer. Bennett S. Greenspan; Augusta University, Augusta, GA

We are on the brink of a new, advanced paradigm of detection, diagnosis, characterization and therapy in oncology that will provide far better patient outcomes, with far less morbidity and mortality. This paradigm utilizes positron-emitting tracers for imaging malignancies with PET/CT to diagnose, stage and characterize various tumors, including clonal variations. Imaging will take into account the hallmarks of cancer, as described by Hanahan and Weinberg. Especially important will be evaluation of tumor angiogenesis, evading immune detection and destruction, evading growth suppressors and apoptosis, reprogramming of energy metabolism and tissue invasion and metastasis. Another feature of cancer cells to evaluate will be the tumor microenvironment. In addition to standard imaging with F-18 FDG to evaluate glucose metabolism, a number of novel PET tracers will be used, such as tracers to evaluate proliferation (DNA synthesis), evaluation of amino acid metabolism, such as F-18 glutamine, evaluation of various cell-surface receptors, evaluation of tumor angiogenesis and hypoxia, and

evaluation of clonal variations. Tracers targeting osteoclastic activity may be useful. Advanced versions of PERCIST criteria will be utilized to evaluate the metabolic response to therapy. These studies will provide solid evidence of efficacy, quality and value, and will become incorporated into clinical guidelines, such as NCCN. [Cancers will be classified by molecular phenotypes, and the site of origin will become secondary. Molecular phenotypes will be determined by molecular pathology and various molecular imaging studies using highly specific tracers. - Richard Baum, MD] Once a malignancy is fully characterized, we can utilize radionuclide therapies, most likely in various combinations of alpha- and beta-emitters, many as theranostic pairs, and probably in combination with other modalities, such as immune modulation, radiation therapy, chemotherapy or viral therapy. Treatment will be specifically targeted against each malignancy. These therapies will identify and disrupt various enzymatic pathways, as well as signaling pathways and chemical mediators. These therapies will also rely on precise dosimetry. It will likely be beneficial for patients to receive whole body radiation from Radiation Oncology for immune stimulation prior to initiation of these radionuclide therapies. These therapies will disrupt the tumor microenvironment, which includes the tumor support structure and the acidic intratumoral environment, and especially the inflammatory component. This should be effective against primary tumors as well as metastatic deposits.This comprehensive, combinatorial, targeted approach will lead to more specific and more comprehensive precision targeted therapy for each patient, and should result in much better patient outcomes, and with far less morbidity. With successful therapies, we will be able to achieve the goal of improving survival and quality of life.

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Molecular imaging of physiological random processes for in silico prediction of treatment efficacy. Nick Henscheid[1], Eric Clarkson[1], Kyle J. Myers[2], Harrison H. Barrett[1]; [1]University of Arizona, Tucson, AZ, [2]Food and Drug Administration, Silver Spring, MD

In silico prediction of cancer treatment efficacy for individual patients is a promising direction towards achieving the goal of personalized oncology through mathematical modeling, patient-specific data collection and simulation. While such strategies have begun to gain traction in a limited number of cases, the approach overall continues to face several challenges. First, the inherent physiological and genetic complexity of the disease and the presence of multiple spatial and time scales has led to a lack of sufficiently validated mathematical efficacy models for many treatments. Additionally, while mathematical and computational models are theoretically capable of resolving behavior across a continuum of spatial and time scales, the data available to the clinician has finite spatiotemporal resolution and is furthermore noisy, incomplete, and usually only indirectly related to the parameters which would otherwise be necessary to constrain a highly resolved in silico model. Thus for the in silico paradigm to become clinically relevant across oncology, uncertainty must be addressed in a fundamental way. It is our view that any figure of merit, such as log cell kill, which is used to predict patient-specific treatment efficacy and hence optimize treatment parameters, should be considered as a random quantity so that confidence can be provided. Because both spatiotemporal heterogeneity and uncertainty play key roles, we make the fundamental mathematical modeling assumption that all physiological processes relevant to cancer modeling are spatiotemporal random processes. Furthermore, because multiple interacting physiological processes, such as normal and neoplastic cell density, vasculature, oxygen saturation and drug concentration are required to fully describe the dynamics of treatment delivery, response and ultimately efficacy, we consider coupled physiological random processes. Using the tools of probability and stochastic process theory, we have derived mathematical expressions relating patient-specific parameters to simple, interpretable quantities of interest which can be used to make patient-specific treatment decisions while seamlessly providing quantification of uncertainty. In this work, we discuss specifically how molecular imaging will play a unique role in measuring the spatiotemporal behavior of coupled physiological random processes both in vitro and in vivo, allowing many of the patient-specific parameters necessary to perform in silico prediction of efficacy to be estimated.

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Novel targeted radionuclide therapy achieves complete responses in a syngeneic model of T-cell NHL. Reinier Hernandez, Joseph J. Grudzinski, Kirsti Walker, Jamey Weichert; University of Wisconsin-Madison, Madison, WI

The progress attained in the treatment of B-cell non-Hodgkin's lymphoma (NHL) has not been translated to the treatment of T-cell NHL, which presents a poor average 5-year OS rate of 32%. The current standard of care consisting of combination chemotherapy regimens is largely ineffective against this disease, and does not address concerns regarding patient phenotype heterogeneity, toxicity, and chemotherapy drug resistance. External beam radiation therapy has produced impressive responses and curative rates in localized disease, but its implementation is obviated at later stages when disseminated disease is present. Hence the pressing need to finding improved therapeutic strategies against T-cell NHL. Herein, we developed a theranostic alkylphosphocholine (NM600) radiolabeled with ^{86}Y for PET imaging and ^{90}Y for systemic targeted radionuclide therapy (TRT), in a syngeneic mouse model of T-cell NHL (EL-4). NM600, which features a DO3A chelating moiety, allowed the coordination of ^{86}Y/^{90}Y with quantitative yields and excellent radiochemical purity. Initially, longitudinal PET imaging was performed at 3, 24 and 48 h after injection of 18.5 MBq ^{86}Y-NM600, to corroborate the selective and persistent accumulation of the agent in EL-4 tumors, which peaked at a value of 5.65 ± 0.15 %ID/g (n=3), at 24 h p.i. of ^{86}Y-NM600. During TRT studies, four groups of EL-4 tumor-bearing mice (n=5-7) were administered a single dose of 2.2, 4.6, 9.3, or 18.5 MBq of ^{90}Y-NM600, and a control group (n=6) received excipient injections. Planar phosphor imaging was carried out at the same time points of PET imaging to qualitatively confirm ^{90}Y-NM600 tumor uptake. Tumor progression was monitored by caliper measurements, and overall survival was determined using a maximum tumor volume of 3500 mm3 and mice general well-being status as humane end-points. Survival curves were plotted using the Kaplan-Meier method and compared using Log-rank test. After an observation period of 90 days, significant tumor regression ($P < 0.001$) was observed in the groups administered 4.6, 9.3, and 18.5 MBq; however, a survival advantage was not achieved in the 18.5 MBq due to acute radiotoxicity. Median survival was 8, 16, 30, and 7 days for the control, 2.2, 4.6, and 18.5 MBq groups, respectively. Median survival was not achieved in the group treated with 9.3 MBq due to 80% of the subjects being disease free after the 90-day period. Except for the higher dose group, treated subjects did not present obvious signs of toxicity (e.g., weight loss, lack of appetite, lethargy). Overall, our results demonstrated the potential of TRT using ^{90}Y-NM600 to elicit durable responses in mice bearing radiosensitive T-cell NHL tumors. Further studies investigating the targeting properties and therapeutic efficacy of ^{90}Y-NM600 in the more clinically relevant setting of spontaneous T-cell NHL in companion canines are currently underway in our laboratory.

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Development of a PET/NIRF smart probe for selectively imaging astrocytic gliomas. Kenneth S. Hettie, Eben L. Rosenthal, Frederick E. Chin; Stanford University, Stanford, CA

The standard of care for treating brain tumors typically entails unguided surgical resection followed by a tailored radio- and/or chemotherapy regimen that depends on the type and grade of the tumor. As such, the extent of surgical resection largely governs the survival time. Fluorescence-guided surgery (FGS) is one way to improve maximal resection while preserving eloquent brain tissue. Currently, only 5-aminolevulinic acid is utilized toward FGS of brain tumors. However, its fluorescent product emits at short wavelengths and operates via nonselective signal accumulation, which reduces tissue penetration and provides false-positive demarcations, respectively. A targeted near-infrared smart probe that could overcome these limitations could afford the direct visualization of diffuse and deeply-embedded tumor tissue, thereby allowing for complete resection and preventing recurrence from residual tumor tissue. Here, we have worked towards developing a smart probe for use in the near-infrared fluorescence (NIRF) imaging of intracranial astrocytic gliomas to provide diagnostic information regarding tumor progression and tumor margin delineation upon resection. The smart probe utilizes a molecular logic gate design

strategy that selectively targets select active cathepsin enzyme, which serves as a validated prognostic factor for intracranial astrocytic gliomas with increasing levels of select active cathepsin linearly corresponding to higher grades of glioma.

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Neurotensin receptor-1 expression in human prostate cancer and lymph node metastases. Clément Morgat[1,2,3], Vincent Molinié[4], Henri de Clermont Gallerande[3], Gaétan Macgrogan[5,6], Valérie Vélasco[5,6], Grégoire Robert[3], Bernard Malavaud[2,7], Philippe Fernandez[1,2,3], Elif Hindié[1,2,3]; [1]CNRS, INCIA, UMR 5287, F-33000 Bordeaux, France, [2]University of Bordeaux, INCIA, UMR 5287, F-33000 Bordeaux, France, [3]University Hospital of Bordeaux, F-33000 Bordeaux, France, [4]University Hospital of Fort de France, Fort de France, France, [5]Institut Bergonié, F-33076 Bordeaux, France, [6]INSERM, ACTION U1218, F-33076 Bordeaux, France, [7]University Hospital of Toulouse, F-31000, Toulouse, France

Background: Neurotensin and its receptor NTR1 are involved in the growth of various tumors. No data are available regarding NTR1expression in normal and tumoral human prostate tissues. **Methods:** NTR1 expression was assessed using immunohistochemistry in samples of 12 normal prostate tissues, 11 benign prostatic hyperplasia (BPH), 34 prostate cancers and five related metastatic lymph nodes. **Results:** NTR1-staining was negative in normal prostate and BPH samples. NTR1-overexpression was seen in 11.8% (4/34) of primary tumors. Primary tumors from node-positive patients expressed more frequently NTR1 (3/7; 42.9%) than those from pN0-X patients (1/27; 3.7%; P = 0.021). NTR1-overexpression was more frequent in metastatic lymph nodes (4/5; 80%) than in primary tumors (P = 0.004). **Conclusions:** NTR1-overexpression in primary prostate cancer is associated with the risk of lymph node invasion. The presence of this target in metastatic lymph nodes may open new perspectives for imaging and radionuclide therapy of prostate cancer.

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Monitoring preclinical cancer models: Multilateral evaluation of innovating the discovery of therapeutics. Peng Huang[1], Peng Xu[2], Naijin Xu[1], Masami Watanabe[1], Yasutomo Nasu[1], Chunxiao Liu[2]; [1]Okayama University, Okayama, Japan, [2]Southern Medical University, Guangzhou, China

Background: Prostate cancer is a major cause of death in men around the world. Despite a variety of treatments, disease progression and metastases still occur in most cases. Given the promising effect of combination with immunotherapy for prostate cancer, the construction of an immunocompetent mouse model for simultaneous monitoring of tumor volume, tumor biomarker and immune cell functions, would be useful for further understanding the mechanism of tumor progression and immune regulation. **Methods:** Through genetic engineering techniques, a new cell line, RM9-Luc-pIRES-KLK3 was constructed. The cells were inoculated into immunocompetent mice of strain C57BL/6 via dorsal flank, dorsolateral prostate and tail vein to obtained subcutaneous model, orthotopic model and metastasis model, respectively. Tumor volumes, non-invasive imaging and prostate-specific antigen (PSA) were evaluated. In the metastasis models, either anti-CTLA-4 antibody or PBS was administered to the tumor bearing mice, and the status of circulating immune cells was assessed by flow cytometry. **Results:** The new cell line, RM9-Luc-pIRES-KLK3 was successfully constructed and steadily expressed PSA and Luc, which were confirmed by Western blotting and bioluminescence detection in vitro. The level of expression was positively correlated with cell counts. Three days after injection, RM9-Luc-pIRES-KLK3 cells grew readily in the mice and the tumors could be detected by the IVIS imaging system from then on. Four days later, PET scan was conducted to confirm the lesions. The intensity of bioluminescence imaging in coronal section and FDG uptake in sagittal slices of PET imaging were totally overlay. Comparing with PBS treated mice, MDSCs and T regs in peripheral blood were significantly decreased in the tumor bearing mice treated with anti-CTLA-4. Meanwhile, the proportion of CD44+CD62− effector and memory T cells on CD3+CD8+ cells were significantly increased by >2–3 times after CTLA-4 blockade compared with the control treatment, as well as IFNγ and TNFα. **Conclusion:** The presented models were ideally suited for real-time tracking of drug response and imaging of tumor progression and immune function. In comparison with traditional methodologies, this biomarker/imaging-based approach could lead to improved, early, and sensitive assessment of tumor status.

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Association between dedicated breast PET and MR imaging textural features in primary invasive breast cancers. Ella F. Jones, Mimi Poon, Wen Li, Kimberly M. Ray, Youngho See, Benjamin L. Franc, Laura J. Esserman, Miguel H. Pampaloni, Bonnie N. Joe, Nola M. Hylton; UCSF, San Francisco, CA

Introduction: Dedicated breast PET (dbPET) is an emerging PET technology specially designed for imaging of the breast. DbPET imaging with [F-18]fluorodeoxyglucose (FDG) is a direct measurement of active glucose metabolism that reflects tumor aggressiveness. In breast MRI, contrast kinetics with rapid early enhancement and delayed contrast washout reflect the robust angiogenic property of high grade tumors. In the past, we observed complementary imaging patterns between dynamic contrast-enhanced (DCE) MRI and FDG-dbPET of a breast cancer patient presented with an ER+/HER2- and a triple negative (TN) tumor. The concordance of MRI and PET measurements suggests that tumor angiogenic/metabolic properties are highly coupled. In this study, we performed textural analysis and evaluated the relationship between dbPET and MR imaging features in invasive breast cancers. **Materials and Methods:** In an IRB-approved protocol, patients with biopsy confirmed stage II/III locally advanced breast cancers were imaged with breast MRI (1.5 T Signa LX, GE Healthcare, WI) and dbPET (MAMMI, OncoVision, Valencia, Spain). Standard DCE-MRI was obtained using a dedicated breast coil. Patients also underwent dbPET imaging with 5 mCi of FDG at 45 min post-injection. Image texture analysis was performed using a 3D Slicer with the Heterogeneity CAD plug-in module. Spearman correlation was used to assess the relationship of each dbPET and MR imaging feature. P-value <0.05 was considered statistically significant. **Results:** Eight unique primary tumors from four patients with invasive breast cancers were analyzed. First-order statistics, based on a discrete pixel value and histogram analysis, yielded features such as mean intensity, uniformity, entropy, skewness and kurtosis. Second-order statistics using the gray-level (or intensity) co-occurrence matrix (GLCM) and gray-level run length method (GLRL) yielded features including contrast, energy (also known as angular second moment, pixel repetition/orderliness), entropy, homogeneity and correlation. Among the 57 imaging features obtained from first order (16) and second order statistics (32), and morphology and shapes (9), 30 features showed statistically significant correlation between dbPET and MRI. Features measuring regional variations within the tumor (such as GLCM energy and entropy) have the strongest Spearman correlation (rho>0.95, p=0.003). **Conclusions:** The high degree of concordance of tumor glucose metabolism and angiogenic properties allows more biologic synergy, and together confer a more invasive phenotype. Our findings support the hypothesis that angiogenic properties and glucose metabolism are highly coupled and when increased reflect greater tumor aggressiveness. This work warrants further studies with a larger cohort to verify the metabolism/angiogenicity concordance and to investigate the sensitivity of this relationship in response to treatment. Persistence of either or both may be an early marker of emerging drug resistance.

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Initial experience of dedicated breast PET imaging of ER+ breast cancers using [F-18]fluoroestradiol. Ella F. Jones, Kimberly M. Ray, Rita Mukhtar, Wen Li, Benjamin L. Franc, Laura J. Esserman, Miguel H. Pampaloni, Bonnie N. Joe, Nola M. Hylton; UCSF, San Francisco, CA

Introduction: Breast cancer is a heterogeneous disease encompassing distinct subtypes with variable treatment response, relapse risk and overall prognosis. The majority of breast cancers are estrogen receptor-positive (ER+). While neoadjuvant endocrine therapy trials have been proposed to better identify therapeutic approaches for ER+ breast cancer, accurate quantification of the ER biomarker is necessary to assess the primary tumor and its likelihood of response to treatment. Dedicated breast positron emission tomography (dbPET) is an emerging technology with high spatial

resolution that enables detection of sub-centimeter lesions and depiction of intratumoral heterogeneity. In this study, we report our initial experience with [F-18]fluoroestradiol (FES) dbPET in assessing ER+ primary breast cancers. **Materials and Methods:** In an IRB-approved protocol, patients with biopsy confirmed ER+ breast cancers were imaged with dbPET (MAMMI, OncoVision, Valencia, Spain) as a companion diagnostic tool to standard breast MRI. A dose of 5 mCi of FES was administered and patients were imaged in the prone position at 45 min post-injection. As part of routine clinical care, MR images were reviewed by a certified breast radiologist experienced in breast MRI. DbPET was reviewed by a radiologist specialized in nuclear imaging. **Results:** Five patients with ER+ breast cancers were imaged. Patient ages ranged from 33 to 64. Two patients with infiltrating lobular carcinomas measuring up to 6.7 cm and 5.3 cm at MRI demonstrated corresponding FES tumor-to-normal maximum standard uptake value (SUVmax) ratio at 4.81 and 2.49 respectively. A third patient demonstrated multifocal FES uptake corresponding to multifocal invasive ductal carcinoma (IDC) and ductal carcinoma in situ (DCIS) with disease foci ranging from 9-13 mm. In this patient, the more posterior disease foci seen on MRI were excluded from the field of view of dbPET. One patient demonstrated absence of FES uptake in her 3.4 cm infiltrating ductal carcinoma, which was due to estrogen receptor blockade from recent administration of tamoxifen for a fertility preservation procedure. The final patient had metastatic cervical and axillary lymphadenopathy secondary to a breast primary that was occult on mammography and MRI. FES-dbPET also showed no corresponding uptake in the ipsilateral breast, possibly due to the small size of the primary lesion and/or low tumor to background uptake ratio. **Conclusions:** FES-dbPET imaging has potential as a diagnostic tool that is complementary to MRI in characterizing ER+ primary breast cancers. Limitations include variation of FES uptake in different ER+ breast cancer diseases and exclusion of posterior breast tissue near the chest wall and the axillary regions. However, FES-dbPET has high potential for clinical utility, especially in measuring response to neoadjuvant endocrine treatment. Further development to improve the dPET field-of-view and studies with a larger cohort of ER+ breast cancer patients are therefore warranted.

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Preclinical assessment of estrogen receptor suppression by SAR439859—a new SERD therapy—using 18F-fluoroestradiol positron emission tomography (FES-PET). Erwan Jouannot[1], Laurent Besret[1], Sébastien d'Heilly[1], Cathy Aubert[1], Sébastien Roy[1], Anne Caron[1], Françoise Le-Gall[1], Carole Voland[2], Monsif Bouaboula[3], Chantal Carrez[1]; [1]Sanofi, Vitry S/Seine, France, [2]Sanofi, Montpellier, France, [3]Sanofi, Cambridge, MA

The steroid hormone estradiol plays an important role in the progression of breast cancer; the estrogen receptor (ER) is expressed in more than 70% of breast tumors enabling them to respond to the mitogenic actions of estrogens. SAR439859 is a selective estrogen receptor degrader (SERD), which antagonizes the binding of estradiol and accelerates the proteasomal degradation of ER. 16α-[^{18}F]-Fluoro-17β-estradiol ([^{18}F]FES) is a PET radiotracer that exhibits a high binding affinity and selectivity for the ERα subtype. In the present study, we investigated the use of [^{18}F]FES-PET for non-invasive monitoring of SAR439859 effect on ER expression in a subcutaneous human breast cancer MCF7-Y537S xenograft model in female SCID mice. [^{18}F]FES tumoral uptake was measured at baseline and after 4 consecutive days of treatments with SAR439859 or vehicle. SAR439859 was administered orally to 3 groups of mice (N=9) at doses of 5 and 12.5 mg/kg (bid) and at 25 mg/kg (qd). In the vehicle group the tracer uptake in the tumor was comparable at baseline and terminal time points (SUV baseline=0.40, SUV terminal=0.43), whereas a decrease in tumor uptake was observed in groups treated at 5 mg/kg (SUV baseline=0.48, SUV terminal=0.20), 12.5 mg/kg (SUV baseline=0.41, SUV terminal= 0.17) and 25 mg/kg (SUV baseline=0.41, SUV terminal=0.24). Terminal ER immunohistochemistry confirmed the receptor degradation in the treatment groups compared to the vehicle group: 90% of the nuclei had a 3+ score in the vehicle group, compared to 62%, 51% and 64% for groups treated at 5, 12.5 and 25 mg/kg, respectively. This study demonstrates the potential of [^{18}F]FES-PET as a direct biomarker of target engagement of SAR439859 in a preclinical breast cancer model in mice. [^{18}F]FES-PET currently supports the phase I dose escalation of SAR439859 where it is used as a biomarker of ER occupancy and/or downregulation to help determine the recommended dose in humans.

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Let there be light: Variability in bioluminescent response of luciferase substrates in brain tumor imaging. Minjee Kim[1], Shiv Gupta[2], Shuangling Zhang[1], Janice Laramy[1], Jann Sarkaria[2], William F. Elmquist[1]; [1]University of Minnesota, Minneapolis, MN, [2]Mayo Clinic, Rochester, MN

Bioluminescence imaging (BLI) is useful to measure tumor growth in live animals. D-luciferin is a commonly used substrate, however, there is a limitation in imaging brain tumors, because the delivery of this hydrophilic compound across the blood-brain barrier is restricted. It has been reported that D-luciferin is a substrate of BCRP, an efflux transporter at the BBB, which may limit the CNS delivery of D-luciferin. Recently, a synthetic luciferin analog, CycLuc1, was developed as a possible alternative with better bioluminescent signal production for brain tumors. The goal of the current study is to examine the factors influencing the CNS distribution of both D-luciferin and CycLuc1. Chromatographic methods were developed to quantify the concentration of D-luciferin and CycLuc1. MDCKII wildtype and BCRP-overexpressing cells were used to examine the role of BCRP-mediated efflux. Tissue distribution of D-luciferin and CycLuc1 was examined in wild-type and BCRP knock-out FVB mice. Patient derived glioblastoma (PDX) cell lines (GBM6 and GBM39) with expression of luciferase were created. PDX glioblastoma cells were injected subcutaneously on the flank of athymic nude mice (Hsd:athymic Nude-Foxn1nu, ages 6-7 weeks; Envigo, Indianapolis, IN). An orthotopic (intracranial) model was created by injection of tumor cells into the right hemisphere. Cross-over imaging was done in both flank and intracranial tumor models.The brain distribution of D-luciferin and CycLuc1 was low in both wild-type and Bcrp knockout FVB mice when compared to other tissues. The co-administration of Bcrp inhibitor, ko-143, in wild-type mice did not change distribution to the brain for both D-luciferin and CycLuc1. The bioluminescence signal of CycLuc1 was more consistent and stable when compared to D-luciferin, even at a significantly lower dose than D-luciferin in the intracerebral tumor model. However, there was no difference in the bioluminescence signal between D-luciferin and CyLuc1 with flank tumor model. The half-life of CycLuc1 is significantly longer than that of D-luciferin in both plasma and brain. The BBB efflux transporter, BCRP, may not play a significant role on the delivery of luciferin substrates, D-luciferin and CycLuc1, across the BBB. There was no difference in brain distribution of D-luciferin and CycLuc1 in mice, but the bioluminescence signal produced by CycLuc1 was more stable and stronger than D-luciferin with a much lower dose. This may indicate that the enzyme, firefly luciferase, may have a greater affinity for CycLuc1. Moreover, CycLuc1 has a distributional advantage compared to D-luciferin, with a longer half-life and longer mean transit time in the brain. In further studies, we will examine the correlation between the absolute substrate concentration measured by LC-MS/MS, and the bioluminescence signal. Understanding the mechanisms of bio-distribution of luciferase substrates will inform the development of better methods to obtain more reliable and quantitative imaging in preclinical models of brain tumors.

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An activatable NIR fluorescent rhodol for hypoxia imaging. Jessica L. Klockow[1], Kenneth S. Hettie[1], Timothy E. Glass[2], Frederick E. Chin[1]; [1]Stanford University, Stanford, CA, [2]University of Missouri, Columbia, MO

Hypoxic tumor tissues exhibit considerable resistance to radiation therapy. Identification of hypoxic regions could help guide therapy decisions and facilitate subsequent surgical removal of cancerous tissue. Here, we have developed a small molecular smart probe that is activated by nitroreductase, an enzyme whose activity is elevated in hypoxic tumors. The probe consists of a finely-tuned rhodol scaffold that demonstrates 28-fold fluorescence enhancement in the near-infrared (NIR) spectral region upon activation. We evaluated the molecule's spectroscopic properties, effect on cell viability, cellular uptake, and sub-cellular localization as well as correlative hypoxic markers in various cell lines of glioblastoma. Notable optical properties include a significant Stokes shift (c.a., >150 nm) and fluorescence emission at 711 nm, both of which are well-suited for deep-tissue penetration. The activatable nature of the probe reduces background signal and enhances contrast. Future plans include radiolabeling the structure with a positron-emitting isotope to permit in vivo multimodal imaging using positron emission tomography (PET), both of which can facilitate image-guided surgery.

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Rapid, molecularly targeted ex vivo tumor delineation on preclinical and clinical oral and esophageal cancer samples using a fluorescent PARP inhibitor. Susanne Kossatz, Arianna Strome, Wolfgang A. Weber, Snehal Patel, Thomas Reiner; Memorial Sloan Kettering Cancer Center, New York, NY

Diagnosis and surgical removal of tumors that arise close to the tissue surface, e.g., oral and esophageal cancer, could be improved by introducing a tumor specific optical contrast that facilitates delineation of tumor from healthy tissue. H&E; histopathology, the current standard-of-care, is time-consuming and expensive. Currently available imaging techniques are label-free or use non-specific dyes, and their clinical utility remains to be shown. Here, we tested if the highly cell permeable, fluorescent small molecule PARPi-FL, that targets the DNA repair enzyme PARP1, can be used to identify tumor cells in freshly excised preclinical and clinical samples of squamous cell carcinomas and adenocarcinomas. PARPi-FL is a fluorescently labeled PARP inhibitor (MW: 640 g/mol; excitation/emission max.: 503 nm/515 nm). In this study, we evaluated its capability for ex vivo staining in fresh and frozen tumor samples. First, we characterized PARPi-FL uptake after systemic injection in xenografts of oral and esophageal cancer (FaDu, OE19, OE33, SKGT4, ESO51) and probed PARP1 levels with western blots. Then, we optimized the ex vivo staining on cryosections and fresh tissue samples of FaDu and OE19 tumors towards high staining intensity in nuclei and low cytoplasmic background using confocal microscopy. Different staining times (1-10 min), concentrations (50-1000 μM) and washing protocols were tested. The ex vivo staining quality was validated against tumors that received a systemic PARPi-FL injection (75 nmol, 2 h post injection). Translatability of the approach was tested on fresh samples and cryosections of human biospecimens of oral squamous cell carcinoma. For all experiments, specific localization of PARPi-FL to PARP1 expressing cells was confirmed by anti-PARP1 immunofluorescence (IF), immunohistochemistry (IHC) and H&E; staining for morphological reference. FaDu and OE19 cells showed the highest PARP1 expression levels, while SKGT4 and ESO51 showed lower expression, which corresponded to systemic PARPi-FL uptake. Optimized ex vivo staining was achieved with a 5 min staining at 100 nM PARPi-FL, followed by a 10 min wash on fresh tissues, and a 5 min staining without the necessity of a wash on cryosections. Intensity and specificity of the ex vivo cryosection staining was comparable to systemic injection of PARPi-FL. We were able to translate the protocol to human biospecimen and achieved similar staining results in fresh tissues and cryosections. PARPi-FL staining clearly outlined tumor foci against healthy tissue using staining protocols that are completed in minutes. Importantly, the tissue will be available to be used for other purposes after PARPi-FL imaging (Histology, DNA/RNA sequencing, etc.). PARPi-FL ex vivo staining shows great potential for rapid identification of tumor cells in live samples with molecular specificity, which will help improve early detection and surgical removal of tumors, leading to an increase in survival and quality of life in oral and esophageal cancer patients.

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^{18}F-fluoroestradiol imaging of estrogen receptor alpha gene mutation Y537S in breast cancer. Manoj Kumar, Kelley Salem, Ciara Michel, Justin Jeffrey, Yongjun Yan, Amy M. Fowler; University of Wisconsin-Madison, Madison, WI

Objective: Mutations in the estrogen receptor (ER) alpha gene (ESR1) have been shown to be one of the drivers of resistance to endocrine therapy in metastatic breast cancer patients, resulting in reduced survival. These mutations in ESR1 have been shown to have estrogen-independent receptor activation and impaired ligand binding affinity for ER antagonists resulting in decreased efficacy of endocrine therapy. The purpose of this research was to investigate the effect of the most prevalent ESR1 mutant, Y537S-ER, on the binding and in vivo imaging parameters of ^{18}F-fluoroestradiol (FES). We hypothesized that Y537S-ER will have reduced FES uptake compared to wild-type (WT)-ER due to the altered conformation of the Y537S-ER ligand binding domain. **Methods:** Stable cell lines were generated using ER-negative MDA-MB-231 breast cancer cells that express either WT-ER or Y537S-ER. ER function was measured using an estrogen response element (ERE)-luciferase reporter gene assay and quantitative polymerase chain reaction analysis of expression of two downstream ER-regulated endogenous target genes, progesterone receptor (PGR) and trefoil factor-1 (TFF1). Cell uptake FES saturation binding assay (0.06-6 μCi FES) and nonlinear regression (one site-total and nonspecific binding) were performed to determine the FES equilibrium dissociation constant, K_D, and the total receptor density, B_{max}. In vivo FES uptake was measured in tumor xenografts grown in female athymic nude mice by microPET/CT imaging using 150 μCi FES. Statistical significance was determined using ANOVA. **Results:** Y537S-ER demonstrated a 10-fold increase in constitutive ER activity in the absence of estrogen compared to WT-ER. Constitutive receptor activation of endogenous ER target gene expression in the absence of estrogen was confirmed (8- and 10-fold increase in PGR and TFF1 compared to WT-ER). Y537S-ER had significantly decreased FES binding affinity, K_D values were 0.52 ± 0.18 nM versus 0.076 ± 0.026 nM and B_{max} values were 584 ± 98.9 versus 110 ± 10.7 fmol/mg protein for Y537S-ER compared to WT-ER. Tumor xenografts of Y537S-ER trended to a reduced FES uptake compared to WT-ER via PET/CT imaging; however, the results were not significant. Uptake was 0.011 ± 0.002 and 0.0176 ± 0.003 %injected dose per gram per fmol/mg ER protein in Y537S-ER and WT-ER, respectively ($p=0.17$). **Conclusion:** Y537S-ER demonstrated increased constitutive ER function with decreased FES binding affinity compared to WT-ER. However, at saturating doses of FES used for PET/CT imaging, there was no significant difference in FES uptake between tumors expressing WT-ER versus Y537S-ER. These preclinical results suggest that while Y537S-ER has lower binding affinity for FES, tumor uptake of FES assessed via PET/CT imaging is not significantly impacted. **Future directions:** Further studies are focused on investigating the effect of other clinically reported ESR1 mutations on FES uptake.

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^{18}F-Fluoroestradiol (FES) PET: A case study of quantitative imaging biomarker development. Brenda F. Kurland[1], Lanell M. Peterson[2], Alena Novakova-Jiresova[2], Jennifer M. Specht[2], David A. Mankoff[3], Hannah M. Linden[2]; [1]University of Pittsburgh, Pittsburgh, PA, [2]University of Washington, Seattle, WA, [3]University of Pennsylvania, Philadelphia, PA

Assessment of a breast cancer tumor as estrogen receptor (ER) positive or negative is a crucial factor in recommendations for breast cancer therapy. ^{18}F-Fluoroestradiol (FES) is an estrogen analogue developed as a PET tracer for in vivo measurement of ER expression. While biopsy-based assessment as ER negative indicates that endocrine therapy is unlikely to be effective, an ER positive biopsy does not guarantee response to endocrine therapy. Resistance mechanisms in tumors of patients with prior endocrine therapy are a topic of intense interest. A parallel consideration is evaluation of ER expression that may show within-patient (between-lesion) heterogeneity. Information from these two paths (in vivo imaging and in vitro testing) may both be needed in order to identify an optimal treatment course for patients with widespread but relatively indolent disease. Patients with metastatic ER-positive breast cancer may live for many years with their disease, but often require sequences of endocrine therapy, chemotherapy, and/or other targeted therapy, for which general guidelines are limited. FES imaging can play an important role in precision medicine and personalized sequencing, which is highly desirable in breast cancer treatment. In addition to potential use to guide choice of therapy, FES PET shows promise as a biomarker for effective ER blockade by novel therapies or doses. This presentation reviews the status of clinical development of FES PET biomarkers, and examines gaps in knowledge with regard to technical performance assessment (agreement with ground truth; repeatability; reproducibility) and clinical validation. Strategies for developing both quantitative ^{18}F-fluorodeoxyglucose (FDG) PET and FES PET measures to direct therapy of metastatic breast cancer will be discussed.

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Identification of gene signatures corresponding to 18F-fluorocholine uptake in hepatocellular carcinoma. Sandi A. Kwee[1], Maarit Tiirikainen[2], Miles M. Sato[1], Linda L. Wong[2]; [1]The Queen's Medical Center, Honolulu, HI, [2]University of Hawaii Cancer Center, Honolulu, HI

Background: The level of uptake exhibited by hepatocellular carcinoma (HCC) on ^{18}F-fluoro-D-deoxyglucose PET/CT is often indistinguishable

from surrounding liver. In contrast, HCC frequently shows very high uptake, and occasionally low uptake, compared to liver background on ^{18}F-fluorocholine PET/CT. Functional enrichment analysis techniques may aid in identifying changes at the gene signature level corresponding to these PET phenotypes. **Methods:** With informed consent, 40 patients with HCC underwent ^{18}F-fluorocholine PET/CT followed by tumor resection. Whole-transcriptomic array data derived from tumor samples were then functionally profiled for coordinated gene expression using signatures from 3 well-annotated collections: 1) a collection of 50 sets of coordinately expressed genes reflecting distinct biological hallmarks, 2) a HCC-specific collection of 76 gene signatures from Medline-indexed journal publications, and 3) an extended library of 2675 gene signatures covering a broad range of diseases and biological conditions. Each signature was tested for significance by permutation testing with PET phenotype with high uptake defined by a tumor-to-liver ratio > 1. Assessments of significance were adjusted by false discovery rate (FDR). **Results:** The level of uptake of ^{18}F-fluorocholine was high in 30 tumors and low in 10 tumors. No tumors showed isointense uptake. Low tumor uptake was significantly associated with higher serum alpha-fetoprotein level (190.2 ng/mL vs. 21.3 ng/mL, p = 0.016). There were no significant associations between tumor uptake and Edmondson-Steiner tumor grade or tumor size. Tumors showing high ^{18}F-fluorocholine uptake were significantly enriched for 6/50, 24/76, 15/2675 gene sets from the three respective gene signature collections. High ^{18}F-fluorocholine uptake was associated with significant enrichment of genes related to oxidative phosphorylation (FDR 0.061), fatty-acid metabolism (FDR 0.083), bile acid metabolism (FDR 0.085), and adipogenesis (FDR 0.088). From the liver-specific and extended gene signature collections, significant enrichments for genes comprising multiple HCC-related gene signatures were noted, including 13 molecular sub-classification signatures (e.g., Hoshida_liver_cancer_subclass_S3 signature at FDR 0.056) and 4 prognostic signatures associated with more favorable clinical outcomes (such as Lee_liver_cancer_survival_up at FDR 0.071). **Conclusion:** Tumors that exhibit high uptake of ^{18}F-fluorocholine show coherent enrichment by sets of genes associated with differentiated molecular sub-classes of HCC and better clinical prognosis. ^{18}F-fluorocholine PET/CT may aid in the molecular sub-classification of HCC and in identifying patients less likely to experience tumor recurrence following liver resection.

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Improved MC1R-targeted molecular imaging for metastatic melanoma by up-regulation of MC1R expression with MAPK pathway inhibitors and epigenetic modulators. Mengshi Li[1], Dijie Liu[1], Dongyoul Lee[1], Somya Kapoor[1], Thomas P. Quinn[2], Frances L. Johnson[1], Michael K. Schultz[1]; [1]University of Iowa, Iowa City, IA, [2]University of Missouri, Columbia, MO

Background: Melanocortin subtype-1 receptor (MC1R) has been long investigated as a potential target to deliver radiation dose to melanoma for molecular imaging and radionuclide therapy. Despite the development of numerous MC1R-targeted peptide ligands in the past few decades, efforts have been largely restricted to B16 murine melanoma cells and tumors that highly express the MCR1 receptor. However, heterogeneous (and often low) MC1R expression in human melanoma cells has stalled clinical translation of the approach. **Hypothesis:** FDA-approved MAPK pathway inhibitors (BRAFi and MEKi) and histone deacetylase inhibitors (HDACi) can be used to pharmacologically up-regulate MC1R expression in human metastatic melanoma cells and tumors to improve imaging. **Methods:** BRAF V600E cells (A375, A2058, SK-MEL-3), BRAF wild-type cells (MEWO) and BRAFi-resistant cells (451LUBR) were exposed to BRAFi, MEKi or HDACi before analysis of MC1R expression by qRT-PCR, immunoblotting, flow cytometry and [125I]Nle4,D-Phe7,α-MSH binding. In vivo melanoma tumor imaging was performed in athymic nu/nu mice bearing A375, A2058 and 451LUBR tumors that were subjected to BRAFi (vemurafenib) and/or HDACi (4-phenylbutyrate; PBA). Imaging was performed using a Re-cyclized MC1R-targeted peptide that was labeled with 203Pb for SPECT and 68Ga for PET. Images were analyzed using Inveon Research Workplace (Siemens Healthcare). **Results:** Up-regulated MC1R expression and increased binding with [125I]Nle4D,Phe7,α-MSH were found in human melanoma cells following exposure to MAPK inhibitors and HDAC inhibitors. Enhanced accumulation of MC1R-targeted imaging tracer resulted in improved SPECT and PET images of human melanoma tumor xenografts in mice. **Conclusion:** Clinically approved MAPKi and HDACi can be used to pharmacologically up-regulate MC1R in human melanoma cells and tumors—and significantly improve MC1R-tageted molecular imaging of human melanoma tumor xenografts in mice.

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Interim- and posttreatment response to neoadjuvant chemotherapy assessed by F-18 FDG PET/CT can predict the outcome in osteosarcoma of the extremities. Sangmoo Lim, Byeonghyeon Byeon, Kyochul Lee; Korea Institute of Radiological and Medical Sciences, Seoul, Republic of Korea

Purpose: We assessed whether sequential F-18 FDG PET/CT (PET/CT) could predict the outcome of patients with osteosarcoma of the extremities after one cycle and two cycles of neoadjevant chemotherapy. **Methods:** A total of 73 patients with American Joint Committee on Cancer (AJCC) stage II extremity osteosarcoma treated with two cycles of neoadjuvant chemotherapy, surgery and adjuvant chemotherapy were prospectively enrolled in this study. All patients underwent PET/CT before (PET1), after one cycle (PET2), and after the completion of neoadjuvant chemotherapy (PET2), respectively. PET parameters (maximum standardized uptake value [SUVmax], metabolic tumor volume [MTV], and total lesion glycolysis [TLG]) and their % changes were calculated, and histological responses were evaluated after surgery. ROC curve analyses and the Cox proportional hazards model were used to analyze whether imaging and clinicopathologic parameters could predict event (metastasis or local recurrence)-free survival. **Results:** A total of 36 patients (49%) exhibited a poor histologic response and 17 patients (23%) had experienced events (metastasis in 16 and local recurrence in 1). Both on PET2 and PET3, the % change of SUVmax most accurately predicted events by ROC curve analysis (area under the curve = 0.667 for PET1 and 0.685 for PET2, respectively). By multivariate analysis including the % changes of SUVmax on PET2, PET2, histologic response, age, sex and AJCC stage (A or B), only the % change of SUVmax on PET3 > -54% independently shortened event-free survival (relative risk, 6.39; 95% confidence interval, 1.45-28.10). Patients with the % change of SUVmax on PET3 > -54% had worse 3-y (72% vs. 93%) and 5-y (67% vs. 93%) metastasis-free survival rates than the others (P = 0.005). **Conclusion:** The % changes of SUVmax both on PET2 and PET3 could predict the outcome of patients with osteosarcoma of the extremities. The % changes of SUVmax on PET3 better predicted the outcome than histologic response.

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Comparison of ^{99m}Tc-MDP bone scintigraphy and ^{18}F-FDG PET/CT to predict histologic response to neoadjuvant chemotherapy in patients with osteosarcoma. Sangmoo Lim, Byeonghyeon Byeon, Kyochul Lee; Korea Institute of Radiological and Medical Sciences, Seoul, Republic of Korea

Objectives: We compared the usefulness of ^{99m}Tc-MDP bone scintigraphy and ^{18}F-FDG PET/CT in predicting histologic response in patients with osteosarcoma receiving neoadjuvant chemotherapy. **Methods:** We retrospectively enrolled 62 patients with high-grade osteosarcoma treated with two cycles of neoadjuvant chemotherapy (NAC) and surgery. All patients underwent ^{99m}Tc-MDP bone scintigraphy and ^{18}F-FDG PET/CT before and after NAC. ^{99m}Tc-MDP uptake of primary tumor was measured quantitatively as the maximum tumor-to-nontumor ratio (T/NT) and ^{18}F-FDG uptake was measured as the maximum SUV (SUV) before and after NAC. The percent changes of T/NT (%T/NT) and SUV (%SUV) after NAC were calculated respectively, and the correlations between these parameters were evaluated. After surgery, the effects of NAC were graded histopathologically (good vs. poor) and the optimum cut-off values of %T/NT and %SUV for predicting histologic response were assessed by ROC curve analysis respectively. **Results:** %T/NT and %SUV positively correlated with each other (r = 0.549, p < 0.001). Based on ROC curve analysis, both %T/NT (AUC = 0.768, p < 0.001) and %SUV (AUC = 0.829, p < 0.001) predicted good histologic response. However, there was no significant difference between the AUCs of %T/NT and %SUV (p = 0.373). The sensitivity and specificity for predicting good histologic response were 83.3% and 75.0%, for the criterion of %T/NT < -10% and 80.0% and 81.2%, for the criterion of %SUV < -45%. **Conclusions:** Both ^{99m}Tc-MDP

bone scintigraphy and ^{18}F-FDG PET/CT are useful for predicting histologic response after NAC in osteosarcoma.

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Dose optimization of ^{177}Lu-labeled phosphoramidate-based PSMA inhibitor with an albumin-binding motif (CTT1403) and therapeutic efficacy comparison to ^{177}Lu-PSMA-617. Xiaoxi Ling[1], Cindy J. Choy[2], Joseph D. Latoche[1], Jonathan J. Geruntho[1], Beatrice Langton-Webster[2], Clifford E. Berkman[1], Carolyn J. Anderson[1]; [1]University of Pittsburgh, Pittsburgh, PA, [2]Cancer Targeted Technology, Woodinville, WA

Prostate-specific membrane antigen (PSMA) is a potential biomarker for prostate cancer treatment and imaging. It has restricted expression on prostate cancer cells and its expression level is often associated with tumor progression stage and metastatic disease. Previously, we developed a new PSMA targeting small molecules that also has a small albumin-binding structure (CTT1403). This molecule is designed to bind to albumin to extend circulation half-life of the entire molecule, ameliorating the rapid renal clearance pharmacokinetic profile that characterizes most PSMA targeting agents. As a result, higher tumor uptake over time and ultimately, superior therapeutic efficacy, were observed with 29 MBq CTT1403 (over 50% animals survived over 200 days). Here, we present a dose optimization study of CTT1403 by comparing the tumor progression and overall survival of PSMA+ PC3-PIP human xenograft mice that were given 1, 2 and 3 doses of 14.5 MBq CTT1403 compared to control agents. Tumor growth inhibition was observed in animals that received one dose of CTT1403. Some mice receiving 2 to 3 doses of CTT1403 exhibited complete tumor remissions. The median survival of animals receiving 1 dose of CTT1403 (51.5 days) is slightly longer than control groups (43.5 – 45 days), but is not significant. The 2 and 3 dose groups have a median survival significantly longer (110.5 and 121 days, respectively; $P = 0.0005$) than controls. Lastly, we compared our therapeutic efficacy results against ^{177}Lu-PSMA-617, which is currently in Phase II clinical trials in the US, using the same mouse model. A brief period of tumor growth inhibition was observed in animals that received 29 MBq ^{177}Lu-PSMA-617 treatment, with a median survival time of 56 days (versus 42 days for saline control, $P = 0.005$). In contrast, CTT1403 showed superior therapeutic efficacy with more than 50% of animals that received 29 MBq CTT1403 having tumor remissions and surviving over 200 days ($P = 0.0002$ versus ^{177}Lu-PSMA-617 group). These data demonstrate that CTT1403 may be an alternative to ^{177}Lu-PSMA-617, with tumor growth inhibition occurring at lower doses.

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64Cu-labeled DGEA-RGD heterodimer for microPET imaging of prostate cancer. Shuanglong Liu, Ryan Park, Peter S. Conti; University of Southern California, Los Angeles, CA

Objective: Prostate cancer is one of the leading causes of cancer-related deaths in the United States and Europe. Both DGEA and RGD peptides have been suitably radiolabeled for prostate cancer imaging, by targeting $\alpha2\beta1$ and $\alpha v\beta3$ integrin respectively. We hypothesize that a peptide ligand recognizing both integrins will demonstrate some synergistic advantages because of its dual-receptor-targeting ability. **Methods:** A DGEA-RGD heterodimer was synthesized from DEGA and RGD peptides through Sarcophagine (Sar) chelator, which was subsequently labeled with 64Cu to make 64Cu-Sar-DGEA-RGD. The resulting probe was evaluated in PC3 tumor bearing nude mice by microPET imaging. The receptor-binding characteristics and tumor-targeting efficacy of 64Cu-Sar-DGEA-RGD were tested in vitro and in vivo. **Results:** 64Cu-Sar-DGEA-RGD was obtained in almost quantitative yield after mixing 64Cu and Sar-DGEA-RGD in ammonium acetate buffer at 37 °C for 10 min. MicroPET analysis shows the PC3 tumor uptake was 6.21 ± 0.48, 4.51 ± 1.65, and 3.82 ± 1.77 %ID/g at 0.5, 1, and 2 h post injection, respectively. Due to the synergistic effect, 64Cu-Sar-DGEA-RGD had significantly higher tumor uptake compared with monomeric RGD and monomeric DGEA peptide tracer analogs at all time points examined. For example, 64Cu labeled DGEA and RGD monomer gave 2.08 ± 0.74 %ID/g and 2.40 ± 0.17 %ID/g PC3 tumor uptake in mice at 0.5 h after injection. The PC3 tumor uptake of 64Cu-Sar-DGEA-RGD was inhibited by both either DGEA or RGD peptide. The combined peptides inhibited to the greatest extent of 64Cu-Sar-DGEA-RGD tumor uptake. Dual integrin $\alpha2\beta1$ and $\alpha v\beta3$ recognition showed significantly improved tumor-targeting efficacy and pharmacokinetics compared with monomer RGD and DGEA analogs. **Conclusion:** The heterodimeric molecule is a promising agent for PET imaging of prostate cancer by targeting two receptor entities. Plus the easy labeling character, 64Cu-Sar-DGEA-RGD might lead to a clinical application for improved diagnostic sensitivity and therapeutic efficiency.

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Radiation dosimetry of 64Cu-BaBaSar-RGD2 determined from whole-body PET/CT in non-human primates. Shuanglong Liu, Ivetta Vorobyova, Ryan Park, Peter S. Conti; University of Southern California, Los Angeles, CA

Introduction: 64Cu-BaBaSar-RGD2 is a positron emission radiotracer taken up by integrin $\alpha v\beta3$, which is overexpressed in many malignancies. The aim of this study was to evaluate the biodistribution of 64Cu-BaBaSar-RGD2 in non-human primates with positron emission tomography and to estimate the absorbed doses in major organs for humans. **Materials and methods:** Whole-body PET imaging was done in a Siemens Biograph scanner in male macaque monkeys. After an i.v. injection of 13.1–19.7 MBq/kg of 64Cu-BaBaSar-RGD2, a whole body scan was collected for a total duration of 180 min. Attenuation and scatter corrections were applied to reconstruction of the whole-body emission scan. After image reconstruction, three-dimensional volumes of interest (VOI) were hand-drawn on the PET transaxial or coronal slices of the frame where the organ was most conspicuous. Time-activity curves for each VOI were obtained, and residence time of each organ was calculated by integration of the time-activity curves. Human absorbed doses were estimated using the standard human model in OLINDA/EXM software. **Results:** Injection of 64Cu-BaBaSar-RGD2 was well tolerated in the macaque monkey, with no serious tracer-related adverse events observed. 64Cu-BaBaSar-RGD2 was cleared rapidly from the blood pool, with a 12.1-min biological half-time. Increased 64Cu-BaBaSar-RGD2 uptake was observed in the kidneys, and bladder, with mean percentage injected dose (ID%) values at 1 h after injection approximately 35.50 ± 6.47 and 36.89 ± 5.48, respectively. The calculated effective dose was 15.30 ± 2.21 μSv/MBq, and the kidneys had the highest absorbed dose at 108.43 ± 16.41 μGy/MBq using the non-voiding model. For an injected activity of 925 MBq 64Cu for humans, the effective dose would be 14.2 ± 2.1 mSv. **Discussion:** Measured absorbed doses and effective doses of 64Cu-BaBaSar-RGD2 are comparable to other reported RGD-derived radiopharmaceuticals labeled with 64Cu and 18F. Therefore, 64Cu-BaBaSar-RGD2 can be safely injected into humans for studying integrin $\alpha v\beta3$ expression non-invasively.

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Development of ^{89}Zr-atezolizumab for PET imaging of PD-L1 levels in the tumor microenvironment. Mark S. Longtine, Richard L. Wahl; Washington University, St. Louis, MO

Immune checkpoint blockade (ICB) therapies are proving effective for treatment for multiple types of cancer. ICB releases the patient's immune cells from inhibition, promoting tumor-cell killing. ICB targets include PD-1 and PD-L1. In 2016, atezolizumab, a humanized anti-PD-L1 antibody, was approved for treatment of patients with locally advanced or metastatic urothelial carcinoma who have disease progression following platinum-containing chemotherapy or are not eligible for cisplatin-containing chemotherapy and, more recently, for the treatment of patients with non-small-cell lung cancer (NSCLC) who have disease progression during or following platinum-containing chemotherapy. Notably, only a subset of patients respond positively to ICB (e.g., ~10-25% of bladder cancer patients and ~15% of lung cancer patients treated with atezolizumab). Current data indicate higher TME PD-L1 levels may correlate with the effectiveness of PD-L1 ICB therapy. However, immunohistochemical approaches using tumor biopsies to quantify PD-L1 levels in the TME are limited by assays that use different antibodies and scoring metrics, by small sample size, by intra- and inter-tumoral heterogeneity of PD-L1 expression, and by potentially dynamic expression of PD-L1. Thus, a non-invasive method to quantify PD-L1 levels in the TME could aid in the selection of

patients to receive anti-PD-L1 therapy. We hypothesize that anti-PD-L1 PET/CT imaging may provide such a method. Here, we report the generation and pre-clinical characterization of atezolizumab labeled with the PET radioisotope, ^{89}Zr. PD-L1 conjugated with isothiocyanatobenzyl-desferrioxamine (Dfo) was chelated with ^{89}Zr. Specific activity of >370 MBq/mg and radiochemical purity of >98% were achieved, as assayed by iTLC. FPLC revealed no significant aggregation of Dfo-conjugated atezolizumab or of ^{89}Zr-atezolizumab. ^{89}Zr-atezolizumab retained excellent immunoreactivity, with an immunoreactive fraction of ~80% as assayed by binding to PD-L1 expressing cells and >90% as assayed by FPLC after binding to purified PD-L1. Cell binding assays indicated a Kd of ^{89}Zr-atezolizumab of ~1 nM, comparable to the Kd of native atezolizumab as assayed by flow cytometry. Preliminary biodistribution data in immune compromised mice show a more rapid blood clearance of low protein mass injections of ^{89}Zr atezolizumab than of ^{89}Zr-labeled control human IgG, suggesting mass effects may be important in the animal model. Further biodistribution analyses and quantitative microPET/CT imaging of mice bearing PD-L1-expressing tumor xenografts after injection of ^{89}Zr-atezolizumab are ongoing.

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Quantitative assessment of antibody distribution in a first-in-human clinical trial of pancreatic cancers. Guolan Lu, Willemieke Tummers, Brock Martin, Nynke van den Berg, Nutte Teraphongphom, Robert Ertsey, Steven Hong, Christina Kong, Teri Longacre, George Fisher, Eben Rosenthal; Stanford University, Stanford, CA

Pancreatic ductal adenocarcinoma (PDAC) is highly lethal and resistant to systemic therapy. Although the epidermal growth factor receptor (EGFR) is overexpressed in greater than 70% of pancreatic adenocarcinomas, the anti-EGFR monoclonal antibody cetuximab has failed to show therapeutic efficacy in a phase III clinical trial in unselected pancreatic cancer patients. Besides the genomic heterogeneity, the failure of the clinical trial is often ascribed to ineffective drug delivery. Preclinical studies have demonstrated that the dense desmoplastic stroma creates a pathophysiological barrier for drug delivery and thus limits the therapeutic efficacy. Yet, the influence of the stroma on the intratumoral distribution of antibody in human patients remains to be elucidated. We propose to leverage near-infrared fluorescence imaging to directly visualize the intratumoral antibody distribution and identify the stromal components associated with the distribution in PDAC patients. To this end, our lab has conducted a first-in-human clinical trial utilizing cetuximab-IRDye800 to guide surgical resection in pancreatic cancer patients. About two to five days before surgery, patients received intravenous administration of 100 mg loading dose of unlabeled cetuximab, followed by infusion of cetuximab-IRDye800 at 50 mg or 100 mg. The surgical specimen was formalin fixed and paraffin embedded (FFPE) and then sectioned into serial sections for fluorescence scan, hematoxylin and eosin (H&E) stain and immunohistochemistry (IHC) of multiple molecular markers of tumors and its microenvironment. Fluorescence images of tissue specimen and serial staining of IHC images were co-registered with H&E images to map the tumor boundary onto the corresponding images. The mean fluorescence intensity within the tumor region was calculated to represent the levels of antibody uptake. IHC images were analyzed to generate quantitative expression scores for molecular markers such as EGFR, collagen, hyaluronan, etc. Molecular markers were correlated with the antibody distribution to identify the most predictive factors for antibody distribution in pancreatic cancers. This study demonstrated the feasibility of using NIR fluorescence to directly visualize and quantify the intratumoral antibody delivery in pancreatic cancer patients. Identification of stromal markers predictive of antibody distribution could allow for selection of patients who could potentially be treated with appropriate agents to overcome the pathophysiological barriers, thus enhancing drug delivery and improving therapeutic outcomes.

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Real-time imaging of senescence in tumors with DNA damage. Xiaowei Ma, Ying Wang, Jun Liu, Philip Deenik, Lina Cui; University of New Mexico, Albuquerque, NM

Senescence, a stage when cells arrest the proliferation, plays a key role in tumor suppression, tumorigenesis and aging. Cells lacking senescence characteristics are cancer-prone, while DNA damaging agents such as chemotherapeutics can induce cellular senescence. In order to monitor the responses to chemo- or radiotherapy, senescence is an important parameter for the evaluation of drug efficacy in a tumor. Multiple agents are being developed for detection of senescent cells, but none is currently available for the detection of senescent cells in vivo in real time. One hallmark of cellular senescence is the overexpression of lysosomal beta-galactosidase (beta-gal), and indeed senescence-associated beta-gal (SABG) has been the most widely used biomarker for senescent cells. Many probes are available for the detection of beta-gal, but they are limited for the use in histology or in vitro experiments. Near-infrared (NIR) fluorescence is favored for in vivo studies due to the decreased tissue autofluorescence, high penetration depth, and low light scattering. NIR probes have also been developed for beta-gal detection in engineered cells with knocked-in lacZ (gene for beta-gal expression), but the small Stoke's shift limits the detection sensitivity and accuracy. We have developed a NIR molecular probe that is designed for real-time imaging of senescence. This poster includes the chemical design, optical properties and imaging studies of molecular probes in drug-induced senescent human cancer cells and tumor models.

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The disintegrin vicrostatin (VCN) is an effective PET imaging agent to monitor ovarian cancer growth and progression. Stephen Swenson, Radu Minea, Kai Chen, Hossein Jadvar, Francis S. Markland, Jr.; Keck School of Medicine, University of Southern California, Los Angeles, CA

Although prevention and early detection are the ultimate goals for ovarian cancer (OC), the fact cannot be ignored that in 2017, 22,440 women will be diagnosed with ovarian cancer in the U.S. 60% of these women will be confronted with distant-stage disease and a five-year survival rate of 29% (American Cancer Society, 2017). For these patients, improved methods for following progression as well as treatment of residual disease following surgery would reduce mortality and improve their quality of life significantly. After surgery and first-line chemotherapy, 50% to 75% of responders will relapse within approximately 18 months and require further systemic therapy; rapid detection of the relapse would greatly enhance the therapeutic options. Exfoliated ovarian tumor cells are carried via peritoneal fluid to secondary sites in the abdominal cavity, where they attach, invade the submesothelial connective tissue and proliferate to create peritoneal micrometastasis. These cancer cells in effusions are not amenable to surgical removal and are difficult to detect; failure of their detection and eradication is one of the main causes for unsuccessful treatment and recurrence. Vicrostatin (VCN) is a rationally designed recombinant disintegrin peptide based on the sequence of contortrostatin (CN), a naturally occurring disintegrin isolated from the venom of the southern copperhead. We have shown that VCN can be expressed with high yields (~200 mg/L) in an E. coli strain and purified as a stable and active polypeptide. VCN interacts with a class of mammalian cell surface receptors, the integrins, through an Arg-Gly-Asp (RGD) motif at the tip of an 11-member amino acid loop and by use of amino acids at the COOH-terminus of the disintegrin that fold in close proximity to the RGD motif. VCN retains the same high binding affinity (Kd in low nM range) to integrins as its predecessor CN. The ability of VCN to bind, with specificity, to a variety of solid tumors has been previously demonstrated in orthotopic models of breast, ovarian and brain tumors and in subcutaneous and bone metastasis models of prostate cancer. We have now shown that VCN can be effectively modified at its amino-terminus by attachment of a copper chelate cage that allows it to be an effective PET imaging agent, based on interaction with OC integrins, for evaluation and assessment of ovarian cancer. This PET imaging agent has the potential to be developed and clinically translated as a molecular imaging probe for OC.

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Systems level visualization of activated T cell dynamics enables early classification of response to local cancer immunotherapy. Aaron T. Mayer, Israt S. Alam, Idit Sagiv-Barfi, Kezheng Wang, Ophir Vermesh, Debra K. Czerwinski, Emily M. Johnson, Michelle L. James, Ronald Levy, Sanjiv S. Gambhir; Stanford University, Stanford, CA

Clinical success of cancer immunotherapies has renewed interest in imaging the behavior of immune cells. Unlike traditional treatment

strategies, immunotherapies modulate the body's immune system to seek out and destroy malignant disease. Due to the spatiotemporally varying signatures of immune response, it has been difficult to monitor and predict outcomes to cancer immunotherapy. ImmunoPET imaging, defined herein as positron emission tomography utilizing radiolabeled antibodies, has the potential to enable noninvasive, sensitive and longitudinal interrogation of immune cell subset and state. Cell states including activation, anergy, and exhaustion may be more prognostic of disease outcome than the presence of tumor infiltrating immune cells alone. In particular, T cell activation is thought to be critical to treatment success across many classes of cancer immunotherapy. In this work, we present the first radionuclide imaging of OX40, a novel and specific biomarker of activated antigen specific T cells. Activation dependent and T cell restricted expression of OX40 was validated in vitro via flow cytometric analysis. Cell uptake studies with radiolabeled 64Cu-DOTA-AbOX40 demonstrated ~11 fold ($p < .0001$) higher uptake in dyna-bead activated T cells compared to resting. The tracer showed negligible non-specific uptake in OX40 blocked or OX40-/- T cells and low background levels across a panel of 5 cancer cell lines tested. In vivo, ImmunoPET imaging coupled with immunological and statistical techniques revealed new insights into response following in situ tumor vaccination with CpG, an adjuvant immunotherapy currently in clinical trials. Balb-C mice bearing dual A20 lymphoma tumors were administered low dose CPG directly in the left tumor (n=7-10), while vehicle control mice received PBS (n=7-10). Early after therapy, imaging revealed increased OX40 radiotracer uptake in the CPG treated tumor (TT) ($p<0.05$). ViSNE, a visualization technique for high-dimensional cytometry data, classified OX40+ single cells in a cluster associated with a non-regulatory, activated CD4 T cell phenotype. CPG treatment led to local expansion of this unique OX40 cell population (63%; $p<0.05$). Other immune markers, such as PD-1 and CTLA-4, exhibited no change or correlation with treatment. A simple machine learning strategy based on OX40 imaging biomarkers enabled accurate classification of therapeutic response, while anatomical and blood based measurements failed to do so (82.6% accuracy, 85% sensitivity and 82% specificity, n=46). Remarkably, a generalized linear regression model indicated early PET signal (mean %ID/g) in the local tumor environment to be highly predictive of response outcomes at late timepoints ($r2=0.746$). 64Cu-DOTA-AbOX40 ImmunoPET provides a readily translatable approach for monitoring activated T cells with high sensitivity and specificity. In this instance, integration of molecular imaging and computational immunology enabled systems-level interrogation of vaccine response.

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PET tracers targeting glutamine metabolism to enhance precision cancer medicine. Michael Nickels, Adam Rosenberg, H. Charles Manning; Vanderbilt University Medical Center, Nashville, TN

L-Glutamine (Gln) is essential for cell growth and proliferation. In addition to glucose, cancer cells utilize Gln as a carbon source for ATP production, biosynthesis and as a defense against reactive oxygen species. The utilization of both [18F]Gln and [11C]Gln has been previously reported as biomarkers for tissues with an elevated demand for Gln and novel therapies are emerging that block Gln uptake in cancer cells, which suggests the critical importance of these types of non-invasive PET imaging biomarkers. Unfortunately, the methodologies reported for the preparation of these compounds were found to be lacking in several crucial aspects that are important for the transition of a drug product from preclinical to clinical use. These reports utilized broad approaches to purification and characterization that were found to be lacking in several aspects, including purity, yield and characterization. In addition to the purity concerns, all methodologies for the preparation of [11C]Gln have been reported using non-commercialized, custom built, reaction platforms. In an effort to allow for fast transfer of technology from production site to production site, development of both the [11C]Gln and [18F]Gln must be transitioned onto widely available automated commercial platforms. Herein, we report the development and utilization of methodology for the automated production of [11C]Gln that meets criteria for human use and the optimization of [18F] Gln production and characterization. The radiosynthesis of [11C]Gln was carried out using a modified form of a published procedure (Amino Acids. 2015, 47, 525) on either a GE FX2N or an iPhase Flexlab system. Briefly, [11C]HCN was reacted with a commercially available precursor to provide an intermediate that was then purified by HPLC, partially hydrolyzed and deprotected under acidic conditions. The product was filtered and formulated for injection. [18F]Gln synthesis was optimized from previously reported procedures (J. Nuc. Med. 2011, 52, 1947) to produce greater overall yields. Quality control was performed by derivatizing the final compound to provide an optically active final drug product capable of quantification by standard HPLC techniques. Automated production of [11C]Gln provides over 100 mCi with >99% radiochemical purity, <5% of D-Gln present, no detectable impurities, and a pH in the range of 5.5-6.0. Production of [18F]Gln is accomplished with yields routinely over 50 mCi and quantification of the final injectable showing the first observed specific activities. In summary, automated radiosyntheses were developed using commercially available reaction modules in a rapid time frame. Additional purification techniques allow for removal of undesired chemical impurities and modified QC techniques allow for additional understanding of the final product composition. Efforts are currently underway to develop protocols for the determination of specific activity of [11C]Gln and for modified purification approaches for [18F]Gln.

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PET imaging of tumor PD-L1 status using ^{89}Zr-DFO-6E11. Lotte K. Kristensen, Camilla Christensen, Carsten H. Nielsen, Andreas Kjaer; Rigshospitalet and University of Copenhagen, Copenhagen, Denmark

Objectives: Despite remarkable clinical response and long-term survival in some patients, not all patients benefit from immune checkpoint therapy with PD-1/PD-L1 blockage. Assessment of tumor PD-L1 expression by immune-histochemistry is therefore increasingly applied to identify patients most likely to benefit from PD-1/PD-L1 blockage. However, tissue based methods are invasive and prone to sampling error. The objective of the current work was therefore to develop a PD-L1 specific radiotracer for non-invasive PET imaging of PD-L1 expression as a tool for drug development. **Methods:** Anti-PD-L1 (clone 6E11, Genentech) was randomly labeled on lysine residues with the p-SCN-Bn-Deferoxamine (DFO-Bz-NCS) chelator and subsequently radiolabeled with ^{89}Zr (^{89}Zr-DFO-6E11). The immunoreactivity and in vitro stability of ^{89}Zr-DFO-6E11 was assessed in buffer and plasma. Longitudinal PET/CT imaging was performed at various time points in HCC827 tumor (lung adenocarcinoma) bearing animals with or without co-injection of 10, 30, 100 or 500 μg unlabeled 6E11. Additional PET/CT imaging studies were carried out in syngeneic tumor models with varying degrees of PD-L1 expression. **Results:** ^{89}Zr-DFO-6E11 was successfully labeled with satisfying radiochemical yield and with a radiochemical purity >99%. The HCC827 tumors were identified by ^{89}Zr-DFO-6E11 PET imaging, and co-injection with 6E11 increased the relative tumor uptake. In contrast co-injection with 6E11 reduced the relative uptake in the spleen. Results from PET/CT imaging performed in a panel of syngeneic models are pending. **Conclusion:** Tumor PD-L1 imaging by ^{89}Zr-DFO-6E11 PET/CT is an attractive approach for non-invasive whole body visualization of PD-L1 expression. Tumors were readily visualized by ^{89}Zr-DFO-6E11 PET imaging. However the mass amount of injected 6E11 had a profound impact on the biodistribution in the tumor and PD-L1 rich organs.

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Development of a minibody that binds PD-L1 in high affinity for immunoPET. Shubahnchi Nigam, Robert S. Edinger, Carolyn J. Anderson, W. Barry Edwards; University of Pittsburgh, Pittsburgh, PA

A primary challenge of using intact antibodies for molecular imaging is the long circulating half-life which results in imaging days post administration. Genetically engineered antibody fragments overcome these challenges with more rapid serum clearance while maintaining high target tissue uptake. The aim of this study is to construct and validate a novel high affinity minibody (Mb) for PDL-1 imaging. **Methods:** The variable heavy and light chains of intact anti-PDL1 antibodies were derived from Atezolizumab and ligated upstream of the CH3 domain of the heavy chain to generate the Mb. The Mb was expressed in Expi293 cells and isolated by affinity

chromatography five days post-transfection. Immunoblot, surface plasmon resonance (SPR), and flow cytometry (PD-L1 positive B16F10) were employed to validate the Mb. **Results:** Immunoblots from non-reducing SDS-PAGE gels showed both dimer at 80 kDa and monomer at 40 kDa which is consistent with the known molecular weights of dimer and monomer of the Mb. SPR demonstrated nM affinity of the Mb to both mouse and human PD-L1 ectodomain which is consistent with the binding properties of the Mb. Flow cytometric analysis showed dose-dependent binding of the Mb and saturation of the B16F10 cells. In conclusion, we have designed and validated a high affinity anti-PD-L1-Mb that will be used for non-invasively monitoring PD-L1 levels by immunoPET. Currently, we are evaluating the efficacy of the Mb in murine melanoma tumor models.

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PET imaging using an apoptosis probe, [Cu-64]-NODAGA-Duramycin, for therapy assessment in solid tumors. Lea Nyiranshuti[1], Joseph D. Latoche[1], Koon Pak[2], Brian Gray[2], Carolyn J. Anderson[1]; [1]University of Pittsburgh, Pittsburgh, PA, [2]Molecular Targeting Technologies, Inc., West Chester, PA

Objectives: The early assessment of anticancer agents is essential for monitoring therapy response, disease progress, and improvement of treatment outcomes in clinical oncology. The mechanism of action of many chemotherapy agents includes the induction of cell death through a well-known process, apoptosis. Therefore, it would be desirable to develop a noninvasive imaging method to detect and monitor apoptosis to provide early information on the effectiveness of chemotherapy agents in cancer patients. **Methods:** A low molecular weight peptide, duramycin, that binds an apoptosis biomarker, phosphatidylethanolamine (PE), was conjugated with the chelator NODAGA and radiolabeled with Cu-64. A study to determine the response to therapy using a combination treatment of gemcitabine and chloroquine (to inhibit autophagy) in Panc02 pancreatic xenograft tumor bearing albino C57BL/6 mice was investigated. In a pilot study, tumor-bearing mice were treated with a combination of chloroquine (50 mg/kg) and gemcitabine (40 mg/kg) (n=2) or no treatment (n=2) as a control. In this study, chloroquine was injected daily while gemcitabine was injected on days 0, 2, and 4. All mice were injected with 200 μCi of Cu-64 labeled NODAGA-duramycin and imaged at 4 and 18 h post-injection (p.i) on a small animal PET/CT scanner (Inveon) at day 5 post-treatment. After 18 h imaging, all mice were sacrificed and organs were harvested for a biodistribution study. In a follow-up study, tumor-bearing mice were treated IP with chloroquine alone (n=4) (injected daily), gemcitabine alone (n=4) (injected on days 0, 3, 6 and 9), both chloroquine and gemcitabine (n=4) or no treatment (n=4). The PET/CT images at 4 h p.i were obtained at day 0 (baseline before treatment), and day 5 during treatment and day 14. One animal from each group was sacrificed at days 0, 5, and 14, and tumors were harvested for histology studies. **Results:** The preliminary pilot PET/CT imaging and biodistribution study showed a significant tumor uptake for the mice treated with gemcitabine and chloroquine, with SUVmean of 0.412±0.013 and 0.345±0.020 at 4 and 18 h p.i respectively. The SUVmean of the control group was relatively low (0.222±0.223 at 4 h p.i and 0.205±0.190 at 18 h p.i). Also, the biodistribution study showed higher tumor uptake in the treated mice at 18 h p.i, 6.05±1.63 %ID/g versus 3.52±1.34 %ID/g for the control group. Data analysis is underway for the second study. **Conclusion:** Preliminary results suggest that [Cu-64]-NODAGA-duramycin PET imaging could potentially be used for early assessment of therapy response of chemotherapy agents. **Research support:** This research was supported by Department of Energy, DE-SC0008833. UPMC Hillman Cancer Center shared resources (In Vivo Imaging Facility) were supported in part by NCI P30CA047904.

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Imaging immunotherapy resistance in melanoma in vitro and in vivo employing magnetic resonance. Shivanand Pudakalakatti, Ashvin Jaiswal, Prasanta Dutta, Michael Curran, Pratip Bhattacharya; The University of Texas MD Anderson Cancer Center, Houston, TX

Introduction: Cancer immunotherapy mainly works on blocking the immune checkpoint proteins: either Cytotoxic T- Lymphocyte-1 (CTLA4) or Programmed death -1 (PD1) or both. The technique has witnessed successful application in melanoma. However, not all melanoma patients respond to immunotherapy. The biological molecular mechanisms which drive resistance to immunotherapy are elusive. To understand this crucial knowledge gap, immunotherapy resistant melanoma mouse strains are developed, and underlying biological molecular mechanisms to resistance are unraveled employing Nuclear Magnetic Resonance Spectroscopy (NMR) and Magnetic Resonance Imaging (MRI). The immunotherapy resistant melanoma strains were developed by an in vivo serial passage approach. The cell lines derived from parental and resistant strains were used for in vitro NMR studies. **Methods:** Nuclear Magnetic Resonance (NMR) spectroscopy is employed as an analytical tool to understand the metabolic responses of immunotherapy resistant and responding melanoma cell lines and tissues in vitro and ex vivo respectively. All data were acquired on a Bruker NMR spectrometer operating at 500 MHz ^{1}H resonance frequency. Identification of metabolite peaks was done through Chenomx and the Human Metabolomic Database (HMDB). The ^{13}C magnetic resonance spectra of hyperpolarized 1-^{13}C pyruvate were acquired at 7T Bruker MRI scanner on immunocompetent mice models with melanoma tumor implanted in the flank. The dissolution DNP (HyperSense, Oxford Instruments) operating at 3T was employed to hyperpolarize. **Results:** NMR results revealed upregulated concentration of lactate, acetate, alanine and glycine in resistant cell lines compared to responding cell lines. Whereas adenosine mono phosphate and phosphocholine are downregulated in resistant cell lines compared to responding, the ex vivo tissue analysis of resistant and responding mice tumors confirmed the upregulation in concentration of lactate in the resistant mice. Metabolic imaging by hyperpolarized ^{13}C pyruvate using magnetic resonance (MR) revealed higher pyruvate to lactate conversion in immunotherapy resistant mice compared to responding ones in vivo. **Discussion:** Upregulation in the concentration of lactate, acetate, alanine and glycine and downregulation of adenosine mono phosphate (AMP) and phosphocholine in resistant cell lines are compared to responding cell lines. This demonstrates adaptations in metabolic pathways of glycolysis, fatty acid synthesis and purine synthesis. **Conclusion:** Altered metabolism in glycolysis, purine metabolism and fatty acid metabolism validated by NMR spectroscopy in vitro and hyperpolarized MR spectroscopy in vivo are important metabolic pathways to be targeted for effective immunotherapy. NMR and hyperpolarized MR are promising tools that may be employed to distinguish patients responding and resistant to immunotherapy in melanoma in the near future.

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NMR spectroscopy based blood test to diagnose brain cancer at early stages. Shivanand Pudakalakatti[1], Alessandra Audia[1], Anirudh Mukhopadhyay[2], Krishna Bhat[1], Pratip Bhattacharya[1]; [1]The University of Texas MD Anderson Cancer Center, Houston, TX, [2]Rice University, Houston, TX

Introduction: Early detection of brain cancer will help saving lives. The currently available diagnostic techniques are not robust and are expensive. Therefore, it is necessary to develop cost effective, minimally invasive, and highly sensitive analytical tools to identify brain tumors at an early stage. In this study we are investigating metabolism based biomarkers in platelets derived from low grade glioma II, glioblastoma, and healthy patients identified by nuclear magnetic resonance (NMR) spectroscopy. Recent studies by other groups have shown that platelets exchange information from diseased or affected parts of the body. The in vitro studies of platelets incubation with GBM cancer cells has confirmed the transfer of RNAs from the GBM cells to platelets. Also, the study in a set of glioblastoma (GBM) patients has shown the carriage of RNA by the platelets which are specific to GBM. Based on these studies we hypothesized that platelets derived from low grade glioma, high grade glioma and aggressive glioblastoma will have different metabolic profiles. **Methods:** The metabolites were extracted using 2:1 methanol-water solvent extraction by mechanical vertex and freeze-thaw. The data were acquired on a Bruker NMR spectrometer operating at 500 MHz proton resonance frequency equipped with a cryogenically cooled triple resonance TXI probe. Identification of metabolite peaks was done through Chenomx and the Human Metabolomic Database (HMDB). **Results:** Analysis of ^{1}H-NMR metabolic profiles of low-grade glioma patient platelet samples (n = 10) and glioblastoma patient platelets (n = 10) revealed that glucose, citrate, and succinate are significantly lower in concentration compared to control platelets ($n = 4$, $p < 0.01$). **Discussion:** We hypothesized that platelets derived from brain cancer patients exhibit altered metabolism compared to platelets from healthy volunteers. Glucose is altered

significantly in glioma and GBM patients manifesting reprogrammed metabolic glycolysis pathway. The altered citrate and succinate shows not only glycolysis but also the tricarboxylic acid cycle (TCA) pathway supporting cell proliferation either by providing energy requirements or providing building block moieties. However, it is still needed to be understood how and why platelet's metabolic reprogramming occurs. **Conclusion:** The results suggest that citrate and glucose can be used as a potential biomarker for brain cancer prognosis. This method is promising because it is analogous to a simple blood test which is both cost-effective and is minimally invasive to the patients. The results need to be compared with other diagnosis techniques as Magnetic Resonance Imaging (MRI), Computerized Axial Tomography (CAT), Positron Emission Tomography (PET), and biopsy (histology) for further validation. Finally, more robust studies should be developed in order to understand the mechanism behind the regulation of key metabolic pathways in the platelets in the brain cancer system.

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Evaluation of new PET tracers for in vivo imaging of PD-L1 expression in non-human primate. Daniel J. Rubins, Paul McQuade, Michael Klimas, Marie Holahan, Mona Purcell, Liza Gantert, Hyking Haley, Shu-An Lin, Dinko González Trotter; Merck, West Point, PA

Introduction: In vivo imaging of PD-L1 could potentially monitor changing PD-L1 expression and heterogeneity of PD-L1 expression within and across tumors in subjects following treatment. Previously, we evaluated the PD-L1-targeting Affibody molecule $Z_{PD\text{-}L1\_1}$ as a potential PET tracer in a mouse tumor model of human PD-L1 expression (González Trotter, et al., 2017). A similar study has since been performed with the affinity-matured Affibody molecule $Z_{PD\text{-}L1\_4}$, showing improved tumor targeting in the same mouse model. In this study, we evaluated $Z_{PD\text{-}L1\_4}$ in Rhesus monkeys to evaluate biodistribution in the presence of an endogenous antigen sink and to estimate clinical radiation dose. **Materials and methods:** [^{18}F]AlF-NOTA-$Z_{PD\text{-}L1\_4}$ and [^{68}Ga]NOTA-$Z_{PD\text{-}L1\_4}$ were synthesized, and whole body PET was performed in Rhesus monkeys (N = 3 per tracer) for 180 minutes following IV administration of tracer. Venous samples for blood tracer levels were obtained throughout PET data collection. Absorbed radiation doses were calculated with OLINDA, using human adult models with no scaling. **Results:** Both tracers cleared primarily through the kidneys, and showed modest liver accumulation: [^{18}F]AlF-NOTA-$Z_{PD\text{-}L1\_4}$: Kidney 94 ± 8 SUV, Liver 1.26 ± 0.13 SUV; [^{68}Ga]NOTA-$Z_{PD\text{-}L1\_4}$: Kidney 100 ± 7 SUV, Liver 1.11 ± 0.06 SUV. Lymph nodes, previously shown by IHC to express PD-L1, were clearly visible in PET images, and spleen showed moderate accumulation: [^{18}F]AlF-NOTA-$Z_{PD\text{-}L1\_4}$: Axillary LN 2.36-7.41 SUV_{max}, Spleen 2.24-4.77 SUV; [^{68}Ga]NOTA-$Z_{PD\text{-}L1\_4}$: Axillary LN 1.64-2.36 SUV_{max}, Spleen 1.55-2.42 SUV. Both tracers showed rapid clearance from blood. Estimated radiation dose limits would allow ~3 clinical PET scans per year, with somewhat limited doses: [^{18}F]AlF-NOTA-$Z_{PD\text{-}L1\_4}$: Critical Organ: Kidney 0.62 ± 0.07 mSv/MBq, USA Dose limits: 243 MBq/yr, 81 MBq/dose, Effective Dose: 22.7 ± 6.5 μSv/MBq, EU Dose limit: 441 MBq/yr; [^{68}Ga]NOTA-$Z_{PD\text{-}L1\_4}$: Critical Organ: Kidney 0.87 ± 0.04 mSv/MBq; USA Dose limits 173 MBq/yr, 58 MBq/dose, Effective Dose: 35.1 ± 3.5 μSv/MBq, EU Dose limit: 285 MBq/yr. **Conclusions:** Both tracers demonstrated accumulation in PD-L1-expressing tissues. Modest signal in liver will be helpful for identifying liver metastases. High kidney accumulation caused somewhat restrictive predicted dose limits, although historically dose limits predicted by Rhesus monkeys studies have been more restrictive than those determined in the clinic. Overall, both are promising clinical PD-L1 PET tracer candidates, warranting further evaluation. **Reference:** González Trotter, D., . . . Evelhoch, J. L. (2017). In vivo Imaging of the Programmed Death Ligand 1 by ^{18}F Positron Emission Tomography. *Journal of Nuclear Medicine*, Epub ahead of print.

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Preclinical evaluation of new PET tracers for in vivo imaging of PD-L1 expression. Daniel J. Rubins, Paul McQuade, Michael Klimas, Xiangjun Meng, Hyking Haley, Shu-An Lin, Krista L. Getty, Dinko González Trotter; Merck, West Point, PA

Introduction: PD-L1 expression in some tumor types correlates with therapeutic efficacy of PD-1 inhibitors. In vivo imaging of PD-L1 could potentially monitor changing PD-L1 expression and heterogeneity of PD-L1 expression within and across tumors in a subject. Previously, we evaluated the PD-L1-targeting Affibody molecule $Z_{PD\text{-}L1\_1}$ as a potential PET tracer in a mouse tumor model of human PD-L1 expression (González Trotter, et al., 2017). In this study, we performed a similar evaluation with the affinity-matured Affibody molecule $Z_{PD\text{-}L1\_4}$, to determine if the greater affinity for PD-L1 of $Z_{PD\text{-}L1\_4}$ would result in improved in vivo targeting of PD-L1. **Materials and methods:** The binding affinity of $Z_{PD\text{-}L1\_4}$ for human PD-L1 was measured using Surface Plasmon Resonance (SPR). For imaging, $Z_{PD\text{-}L1\_4}$ was conjugated with NOTA, and radiolabeled with either [^{18}F]AlF or ^{68}Ga. Both PET tracers were evaluated in SCID Beige mice with LOX (hPD-L1+) and SUDHL6 (hPD-L1-) tumors. PET data were acquired for 90 minutes following I.V. administration of the tracer. Immediately after imaging, mice were euthanized for ex vivo biodistribution measurements. **Results:** $Z_{PD\text{-}L1\_4}$ demonstrated very high affinity for human PD-L1 (apparent K_D = 0.07 nM). [^{18}F]AlF-NOTA-$Z_{PD\text{-}L1\_4}$ and [^{68}Ga]NOTA-$Z_{PD\text{-}L1\_4}$ were both successfully synthesized: [^{18}F]AlF-NOTA-$Z_{PD\text{-}L1\_4}$: SA 330-460 Ci/mmol @EOS; 100% RCP and [^{68}Ga]NOTA-$Z_{PD\text{-}L1\_4}$: SA 240-250 Ci/mmol @EOS; 100% RCP. PET imaging showed similar pharmacokinetics and clearance for both tracers. LOX tumors were clearly visible in PET images. Renal clearance was the primary route of elimination for both tracers. Ex vivo biodistribution measurements showed that both tracers had >25 fold higher accumulation in LOX tumors than SUDHL6: [^{18}F]AlF-NOTA-$Z_{PD\text{-}L1\_4}$: LOX: 8.7 ± 0.7 %ID/g (N = 4) SUDHL6: 0.2 ± 0.01 %ID/g (N = 6) and [^{68}Ga]NOTA-$Z_{PD\text{-}L1\_4}$: LOX: 15.8 ± 1.0 %ID/g (N = 6) SUDHL6: 0.6 ± 0.1 %ID/g (N = 6). For comparison, for [^{18}F]AlF-NOTA-$Z_{PD\text{-}L1\_1}$, LOX tumor uptake was < 3 %ID/g. Blood and plasma accumulation measurements were > 3 fold higher for [^{68}Ga]NOTA-$Z_{PD\text{-}L1\_4}$ than [^{18}F]AlF-NOTA-$Z_{PD\text{-}L1\_4}$, and very high kidney accumulation was measured for both tracers. **Conclusions:** $Z_{PD\text{-}L1\_4}$ had markedly higher apparent affinity for human PD-L1 than the previously evaluated $Z_{PD\text{-}L1\_1}$, and demonstrated improved targeting of PD-L1 in a mouse model. The development of both ^{18}F- and ^{68}Ga-labeled PET tracers may expand access to clinical sites, as many sites can only produce one or the other. These results demonstrate that both PD-L1 Affibody PET tracers are promising clinical candidates, warranting further evaluation. **Reference:** González Trotter, D., Meng, X., McQuade, P., Rubins, D. J., Klimas, M., Zeng, Z., . . . Evelhoch, J. L. (2017). In vivo Imaging of the Programmed Death Ligand 1 by ^{18}F Positron Emission Tomography. *Journal of Nuclear Medicine*, Epub ahead of print.

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Differential diagnosis in pancreatic lesions with Octreoscan. Fernanda Salomao Costa, Maria Marta Maggiotto Sabra, Isabella Caterina Palazzo, Gabriela Nara Sadek, Douglas Moeller, Alan Chambi Cotrado, Wilter Ker, Nilton Lavatori, Jader Cun Azevedo, Claudio Tinoco Mesquita; Hospital Procardiaco, Rio de Janeiro, Brazil

Introduction: The most aggressive pancreatic malignant tumor is adenocarcinoma, the fourth cause of death related to cancer. It's differential diagnosis must consider the clinical condition, radiologic and pathophysiologic aspects, confirming with biopsy. **Clinical case:** Male patient, 84 years old, hypertension, and no other comorbities. Chronic osmotic diarrhea, nausea and vomiting, causing intense uncomfortable situation and difficulty in social life. It was tried to treat with restricted diet and different medications, with no success. This clinical condition, with no satisfactory evolution, motivated a tomographic study of the abdomen (13/11/2016): pancreatic tumor localized in body; liver with homogenous texture. MR (13/12/2016): confirmation of anatomic diagnosis, with no other aspects. Guided biopsy via upper endoscopy (14/01/2017): necrosis area with negative result for neoplasic cells. A new biopsy was needed (24/01/2017): positive to neoplasic cells, but insufficient material for imunohistochemic tests—no definition of the tumor. Considering the oligosymthomatic evolution and the good overall state of the patient, it was thought to be a tumor with indolent behavior—neuroendocrine tumor? The patient was led to a scintigraphy with somatostatine analogue: hypercaptation lesions in the liver and in pancreatic body, with counts higher than the hepatic healthy parenchyma, in contrast to PET-CT with 18-F-FDG, which presented concomitant hypercaptation although lower intensity. Chromogranine level was 340ug/dL. **Discussion:** Differential diagnoses of solid pancreatic mass are principally: exocrine primary cancer, neuroendocrine tumor, lymphoma and metastasis. NETs are typically hyper vascular, with increase in vascular precocity phase and

washout in portal precocity venous phase in the CT contrasted images. Typically well-differentiated, leading to classic carcinoid syndrome, after tumoral serotonin secretion. The new biopsy quantified ki67, which is valorous information, once the lesions show more affinity to octreotide than the 18-F-FDG, characteristic of well-differentiated tumor (more affinity to somatostatine analogue than glucoses). With these descriptions and low ki67 (ideally lower than 2), this patient is a candidate to 177-Lu treatment, with good answer. **Follow up:** Our patient started 177-Lu therapy, today he is at the second round, in a total of four, referring important improvement of clinical symptoms. There are no renal consequences although a light medullar depression (RBC = 4,47 to 3,94millions/m3; WBC = 6,8 to 4,1tousend/mm),3 which made us choose for a bigger space between the rounds. This answer could be related to the patient's age; the medical decision of spacing the rounds does not interfere on the therapeutic results.

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Metabolic evolution of patient-derived glioblastoma xenografts through in vivo hyperpolarized ^{13}C magnetic resonance spectroscopic imaging and ex vivo nuclear magnetic resonance spectroscopy. Travis Salzillo, Joy Gumin, Jaehyuk Lee, Islam Hassan, Niki Zacharias, Rivka Colen, Frederick Lang, Pratip Bhattacharya; The University of Texas MD Anderson Cancer Center, Houston, TX

Introduction: Glioblastomas originate from a variety of cells such as astrocytes and neuronal stem cells which, along with their advanced stage, make these tumors diverse in mutations. Thus, targeted therapies can rarely block all mechanisms of proliferation and survival, leading to median survival times of merely 15 months. This research aims to characterize the evolution of tumor metabolism that either leads to or results from these somatic mutations. We hypothesize that tumor metabolism is correlated with tumor aggressiveness, and that metabolic imaging such as hyperpolarized MRI can be employed in the early detection of lesions, serve as a prognostic predictor of survival, and track the efficacy of treatment. **Methods:** Glioblastoma sphere-forming cells (GSC) were cultured from patient biopsies and injected intracranially in the caudate nucleus of the brains of nude mice. The anatomic development of these GSC-derived tumors was followed with periodic MRI using T1-weighted, T2-weighted, and fluid-attenuated pulse sequences. At the 20, 40, 60, 80, and 100% of median survival time points, we performed dynamic metabolic MRI experiments. Hyperpolarized [1-^{13}C] pyruvate was injected through the tail vein, and its metabolic conversion into lactate in the tumor was non-invasively measured using slice-selective pulse-acquired spectroscopy every two seconds for three minutes. This conversion was quantified with the metric nLac which is the time-integrated ratio of hyperpolarized lactate-to-pyruvate+lactate signal. Following the hyperpolarization experiments, mice were euthanized and their tumors harvested for NMR spectroscopy experiments to measure steady-state metabolite pool sizes at the aforementioned time points. H & E staining was also performed to visualize ex vivo tumor samples. Two-way student T-tests and two-way ANOVA with multiple-comparison corrections were performed to identify significant differences of nLac and ex vivo metabolite concentrations between time points. **Results:** Tumor growth was closely followed with conventional MRI scans. From the in vivo experiments, we found that nLac increases with both tumor size and maturity, suggesting elevating levels of aerobic glycolysis. From the ex vivo NMR experiments, 25 metabolites were identified and their concentrations quantified between time points. Several metabolites either positively or negatively correlated with tumor development such as lactate, glutamate, glutamine, creatine, and formate. **Conclusion:** This work demonstrates the utility of hyperpolarized MRI to not only detect the existence of tumors, but also track their metabolic development from tumorigenesis to full maturation, non-invasively. Additionally, longitudinal measurements of global metabolism with NMR spectroscopy monitors the metabolic transformations that occur during tumor growth. This data can be combined with enzyme and sequencing results to verify specific metabolic pathways being affected.

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Comparison of positron-emission tomography reporter gene imaging systems in adoptive T cell therapy of cancer. Noriko Sato[1], John S. Davies[1], Gadi V. Cohen[1], Phuongnga T. Ton[1], Stephen S. Adler[2], Falguni Basuli[3], Xiang Zhang[3], Rolf E. Swenson[3], Christian S. Hinrichs[1], Peter L. Choyke[1]; [1]National Cancer Institute, Bethesda, MD, [2]Leidos Biomedical Research, Bethesda, MD, [3]National Heart, Lung, and Blood Institute, Bethesda, MD

With the development and improvements of genetically engineered T cells, adoptive T cell therapies of cancer have dramatically increased in recent years. Currently, monitoring of such therapy relies on biopsy or blood analyses. Biomedical imaging of these T cells would provide investigators with a tool to assess T cell modifications that enhance targeting, confirm proliferation, and understand the fate of T cells after the target is eradicated. In order to quantitatively and longitudinally monitor T cell therapy over the long-term, a reporter gene strategy is needed. We compared two positron-emission tomography (PET)-reporter gene imaging systems, sodium-iodide symporter (NIS) and somatostatin receptor 2 (SSTR2) systems, in a T cell cancer therapy model in mice. Human T cells were transduced with a T cell receptor (TCR) gene against E7 antigen of human papillomavirus (HPV) and either human solute carrier family 5 member 5 or SSTR2 gene using retroviral vectors. Transduced T cells were sorted for E7-TCR$^+$NIS$^+$ cells and E7-TCR$^+$SSRT2$^+$ cells and underwent a rapid expansion protocol. In vitro studies indicated that both NIS and SSTR2 expressing cells incubated with relevant tracer, F-18 tetrafluoroborate (F-18 TFB) or Ga-68 DOTATATE, respectively, upregulate CD25, CD69 and 4-1BB upon TCR stimulation at comparable levels to non-labeled cells. Two million E7-TCR$^+$NIS$^+$ or E7-TCR$^+$SSRT2$^+$ T cells were transferred to NOD/SCID/IL-2 gamma null mice bearing subcutaneous human HPV$^+$ cancer (SS4050) when the tumors were approximately 4 mm in diameter. Systemic distribution of T cells expressing NIS or SSTR2 was visualized with microPET 1h after administration of the tracer (3.7 MBq/mouse) on multiple days after the T cell transfer. Both reporter systems successfully visualized T cells in the tumor and, as T cells proliferate, the tumors began to regress. Higher tumor-to-background signal ratios were obtained with the Ga-68 DOTATATE/SSTR2 system (8.6±1.6) than with the F-18 TFB/NIS system (4.3±1.0) at the peak of T cell accumulation observed between 7-14 days after T cell transfer. In addition, the strong physiological expression of NIS, such as in the thyroid, stomach, and testes, seemed potentially problematic for analysis of systemic T cell migration. Collectively, both NIS and SSTR2 systems enable longitudinal PET imaging of adoptive T cell therapy. However, the SSTR2 system has advantages over the NIS system due to the high tumor-to-background signal ratios observed.

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SUMMIT: a functional proteomics platform for lead compound generation and target identification. Marc Seaman[1], Dustin Bauknight[2], Kimberly Kelly[1]; [1]Blue Rudge Biosciences, Earlysville, VA, [2]University of Virginia, Charlottesville, VA

Molecular imaging is impactful in many stages of cancer therapy development—guiding the preclinical development of targeted therapeutics and biomarkers and monitoring response to therapy. The process of developing a single compound into a molecular imaging agent is time consuming and costly. However, we have created a platform called SUMMIT to ease these burdens. SUMMIT permits the selection of lead peptides in a reduced timeframe (days versus weeks) while ensuring exquisite selectivity. High throughput screening of combinatorial chemical libraries, particularly phage display, is a powerful approach for identifying targeted molecules. Phage display offers a number of important advantages such as rapid and economical biological expansion, vast peptide diversity and a rapid screening process. However, current phage screening protocols are hampered by false positive rates caused by non-specific phage binding and unequal rates of amplification as well as by loss of potential candidates early in the process due to low starting phage concentrations. Furthermore, it is necessary to hand pick and Sanger sequence individual plaques at the end of the traditional screening process, a tedious process that decreases the data output to a fraction of the total end phage pool. These limitations do not permit a robust analysis of the selectivity of identified peptides across the universe of potential off-targets. Therefore, we sought to harness the power of phage display screening and take advantage of next generation sequencing (NGS) to develop a platform that allows discovery of peptides that can selectively distinguish cells in vitro and in vivo. NGS overcomes some of the drawbacks associated with phage screening by greatly increasing the depth of

characterization of post-screening libraries. Additionally, all sequencing data from past phage screens can be archived and compared to data from the current screen to quantitatively and more robustly assess selectivity. Thus, have we developed a comprehensive in silico analysis platform (SUMMIT) that can predict selective peptide sequences and circumvent many of the weaknesses of traditional phage display. Further, if SUMMIT is used on cells or in vivo where the target is unknown we can use functional proteomic methods to identify novel targets, resulting in the generation of specific lead compounds for imaging and/or therapy and identification of novel targets all in one process. As validation of our newly created SUMMIT platform, we successfully identified a consensus peptide motif HPQ in a well characterized in vitro screen on streptavidin using less rounds than are used in traditional phage display. Further, in an in vitro screen on purified CD4, we identified peptide motifs that mimic the CD4 binding pocket of MHC Class II, the binding partner of CD4. Our SUMMIT platform provides a time and cost efficient tool to identify better lead peptides and novel targets, positively impacting molecular imaging and ultimately, cancer patient care.

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Multi-modal imaging of biological responses to nano immunotherapy and checkpoint blockade in a murine model of breast cancer. Rita E. Serda, Kimberly Denman, Colin Wilson, Reed Selwyn; University of New Mexico, Albuquerque, NM

Nanoparticles for immunotherapy represent an emergent field with evidence for enhanced activation of immune cells over free vaccine agents. In addition to the advantages of nanoparticles for immunotherapy, recent clinical trials support paradigm-changing successes in treatment outcomes using checkpoint inhibitors (CPI) for alleviating immune suppression. While immune checkpoint blocking antibodies have shown significant benefits for patients with a wide range of malignancies, some patients experience immune-related adverse events (irAEs). Here we studied the therapeutic benefits and risks of combination nano immunotherapy and CPI treatment using three variants of the 4T1 murine model of breast adenocarcinoma. Mice with tumors established using 4T1 cells transduced with lentivirus to express firefly luciferase and tdTomato red under the control of the human ubiquitin C promoter became moribund when treated with anti-PD-1 antibody, exhibiting rapid tumor growth and excessive weight loss. Multi-modal imaging of biological responses in mice treated with single agent or combination therapy included bioluminescence, magnetic resonance imaging, 18F-fluorodeoxyglucose positron emission tomography, and tissue electron microscopy.

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Various applications of CUBIC 3D imaging for cancer research. Kei Takahashi, Shimpei I. Kubota, Jun Nishida, Shogo Ehata, Etsuo A. Susaki, Hiroki R. Ueda, Kohei Miyazono; The University of Tokyo, Tokyo, Japan

There have been obstacles to monitor micro-metastasis of cancer cells in vivo, because of the lack of high-resolution imaging. These days, tissue-clearing-based 3D imaging is available with recently developed cocktails, including CUBIC (clear, unobstructed brain/body imaging cocktails and computational analysis), which enables us to analyze the whole body/organ at single-cell resolution. Here, we applied this CUBIC-based cancer (CUBIC-Cancer) analysis to monitoring of micro-metastasis of cancer cells, evaluation of the effects of anti-tumor drugs in whole organ, and elucidation of the roles of EMT in cancer metastasis. Firstly, we applied our new method to various types of mouse tumor models including experimental lung or brain metastasis models. CUBIC-Cancer analysis enabled us to distinguish the patterns of metastasis easily with 3D images and to monitor the metastasis at single-cell resolution in various organs. Then, anti-tumor drug effects were also evaluated with CUBIC-Cancer analysis with mouse breast cancer 4T1 cells. Our new method made it possible to quantify the number and the volume of metastasis foci precisely after the treatment of anti-tumor drugs. Moreover, we succeeded in the detection of the few cancer cells left after continuous anti-tumor drug treatment. Furthermore, we applied this CUBIC-Cancer analysis to study biology of cancer metastasis. Although epithelial-mesenchymal transition (EMT) is known for its importance especially in the intravasation process, the involvement of EMT in extravasation is still controversial. Therefore, we applied our new system for figuring out the role of EMT in the extravasation process using an experimental lung metastasis model. Human lung adenocarcinoma A549 cells were stimulated with or without TGF-b1 for 3 days in vitro and inoculated into nude mice intravenously. CUBIC-Cancer analysis of whole lung revealed that the number of TGF-b-treated A549 cells significantly increased, compared to non-treated A549 cells, 24 hours but not 1 hour after inoculation. In addition, the number of metastatic foci remained from 24 hours to 14 days after inoculation, suggesting that EMT played an important role not only in extravasation but also in survival of cancer cells at metastatic site. Thus, CUBIC-Cancer analysis has various applications including precise evaluation of the effects of anti-tumor drugs in vivo and investigation of the mechanisms of cancer metastasis.

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Sensitivity and specificity study of panitumumab-IRDye800 as targeted agent for image guided surgery in patients with head and neck cancer. Nutte (Tarn) Teraphonphom, Steven Hong, Rebecca Gao, Nynke Van den Berg, Robert Ertsey, Nick Obehelman, Adam Gomez, Brook Martin, Christina Kong, Eben Rosenthal; Stanford University, Stanford, CA

Background: Real-time fluorescence imaging offers the potential to enhance tumor resection by precisely determining where the tumor margin is located. In this study, we combined panitumumab, an FDA approved antibody that targets the epidermal growth factor receptor (EGFR), to the near infrared (NIR) dye IRDye800 (LI-COR Biosciences) to evaluate the agent for sensitivity and specificity as intraoperative fluorescence imaging in primary head and neck squamous cell carcinoma (HNSCC) patients. **Methods:** Cohort A (n=5) received 1/12 of the therapeutic dose, cohort B (n=7) received 1/6 of the therapeutic dose, and cohort C (n=6) received a flat dose of 50 mg of panitumumab-IRDye800, 2-5-days prior to surgery. The resected tumor samples were assessed ex vivo through fluorescence imaging on a Pearl (LI-COR Biosciences) imaging system. Standard histological assessment was performed and unstained paraffin-embedded histologic slides were analyzed using a fluorescent scanner (Odyssey CLx [LI-COR Biosciences]). Fluorescence intensities were correlated with hematoxylin and eosin (H&E) staining in which the pathologist demarcated the tumor area to determine the sensitivity and specificity of the approach. **Results:** Ex vivo imaging findings also correlated well with intraoperative findings. We found average tumor to background ratio (TBR) of primary tumor of, 5.40±0.6 for cohort A, 5.44±0.69 for cohort B and 9.29±1.24 for cohort C. The analysis results in a sensitivity of 91.8% and a specificity of 74.3% for cohort A, sensitivity of 86.13% and a specificity of 78.14% for cohort B, and sensitivity of 95.45% and a specificity of 80.28% for cohort C. There is no statistical difference between the three cohorts. **Conclusion:** Panitumumab-IRDye800 can provide very high specificity and sensitivity to aid in real-time detection and surgical resection of HNSCC.

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Detecting prostate cancer in voided urine: A genomic, optical imaging assay. Sushil K. Tripathi, Edouard Trabulsi, Charalambos Solomides, Leonard Gomella, Matthew L. Thakur; Thomas Jefferson University, Philadelphia, PA

Background and hypothesis: The risk of prostate cancer (PCa), the most common urologic malignancy in men over 60 yrs of age, is 17%. Due, primarily, to its non-specificity, the PSA measurement is no longer considered reliable. Due to the heterogeneity of PCa, the recently developed liquid biopsy assays require multiplex gene sequencing and render the assays expensive and frequently unreliable.We hypothesized that PCa can be imaged optically, by a simple, reliable and relatively inexpensive assay by targeting genomic VPAC receptors expressed on PCa cells shed in voided urine. **Methods:** VPAC1 receptors were targeted with a receptor specific biomolecule, TP4303 developed in our laboratory. An aliquot of voided urine, collected as a standard of care from patients presenting to a Urologic clinic (N=241, >21 yrs of age), was cytospun, cells fixed, treated with TP4303, washed and allowed to react with DAPI (4.6 Diamidino-2-phenylindole, Dihydrochloride). The cells were then observed under a microscope. Cells with TP4303 orange fluorescence around the blue (DAPI) nucleus were malignant as validated by gene sequencing and NKX3 immunohistochemistry (IHC). Because of lack

of VPAC receptors, the normal epithelial cells had no fluorescence around them. All specimens were labeled only with presenting diagnosis with no personal identifiers or other clinical data. **Results:** The assay detected VPAC1 positive cells in 98.6% of patients with PCa (N=173) and none of the patients (N=12), with benign prostatic hyperplasia (BPH). Of the 56 "normal" subjects, 62.5% (N=35) were negative for VPAC1 positive cells and 17.8% (N=10) were uninterpretable due to excessive crystals in the urine. In differential gene expression study of four patients with known PCa and three normal volunteers, 5 PCa genes were upregulated (>1.5 fold, P<0.05) and 19 were down regulated (1.5 fold P<0.05). In IHC there was complete concordance with the optical assay in two PCa and one BPH patients. **Summary:** The data are highly encouraging and warrant further evaluation of the assay to serve as a simple, reliable, non-invasive and inexpensive tool to detect PCa. **Acknowledgment:** MLT thanks his colleagues and gratefully acknowledges the support of NIH/NCI (R01 CA157372).

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Cancer targeting using exosomal lipids toward detection enhancement of microlesion. Yuki Toda[1], Hikaru Kawakami[1], Saeka Ukai[1], Shin-ya Morita[2], Kazuyuki Takata[1], Eishi Ashihara[1]; [1]Kyoto Pharmaceutical University, Kyoto, Japan, [2]Shiga University of Medical Science Hospital, Otsu, Japan

Cancer-targeting must be a crucial technology to develop diagnosis and therapy of cancers. Target-specific accumulation of imaging probes makes the detection of tumor microlesion more clear. However, almost all current technology for cancer targeting has not had a clinical impact on human health. Understanding how cells specifically transfer the biological information to target cells could help us construct a novel targeting strategy. Exosomes are the extracellularly released nanovesicles that present in all of body fluid. MicroRNAs encapsulated in exosomes are stably conveyed to distant cells and support various biological phenomena. We hypothesized that exosomes will be given the address for cell-specific molecular delivery, according to their constituent tuning. In this study, we explored the exosomes internalized into cancer cells effectively and clarified their uptake mechanism. Ultrapure exosomes (about 100 nm vesicles expressing CD63 in *d*=1.16 mg/L fraction) were collected from cell culture media using density-gradient ultracentrifugation. Exosomal lipids were extracted by the Bligh&Dyer method and analyzed each amount of components by enzymatic fluorometric assay [Morita S.Y. and Terada T. *Sci. Rep*., 5:11737, 2015]. Fluorescently-labelled exosomes or reconstructed liposomes were treated to cancer/non-cancerous cells to evaluate internalizing efficiency using image analysis of a laser scanning microscopy. We identified glioblastoma-derived exosomes (Exo-U251) that were internalized more effectively by some types of cancer cells (glioblastoma: U251; breast cancer: MDA-MB-231; fibrosarcoma: HT-1080) than by non-cancer cells [Toda Y., et al. *Biochem. Biophs. Res. Commun*., 456:768-773, 2015]. The exosomes that derived from another cell line did not show the effective internalization into U251 cells, indicating that the tropism is owing to the specific recognition between U251 cells and Exo-U251. This tropism was still seen in the lack of protein ligands on the Exo-U251 surface. Interestingly, Exo-U251 lipid-reconstructed liposomes (Exolip-U251) partly mimicked the tropism of the original exosomes. The enzymatic fluorometric assays showed the uniqueness of the exosomal lipid components according to the cells from which they are derived. Finally, the agents conjugated with Exolip-U251 were effectively delivered inside the cells. We propose that the synthetic drug cargos applied from these unique lipids are more easily quality-controlled in the manufacturing process than protein-decorated ones. Further study will be performed to clarify how the exosomal lipids affect the uptake efficiency on U251 cells. The destination of exosomes might be partly regulated by their own lipid components, and mimicking this manner will lead to a promising targeting technology.

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MRI quantification of SPIO-labeled immune cell recruitment to tumors in murine cervical and breast cancer models. Marie-Laurence Tremblay[1], Brianna Kelly[2], Zoe O'Brien-Moran[2], James Rioux[3], Andrea Nuschke[1], Christa Davis[1], Kimberly Brewer[1]; [1]IWK Health Center, BIOTIC, Halifax, Canada, [2]Dalhousie University, Halifax, Canada, [3]NSHA, BIOTIC, Halifax, Canada

Immunotherapies are becoming a pillar of cancer therapy, but despite initial successes, many challenges remain, particularly related to individual variability. Molecular imaging is an attractive option for monitoring immunotherapy responses by enabling longitudinal characterization of individual tumor microenvironments and providing opportunities to investigate biological relationships between tumors and immune systems. This work uses pre-clinical magnetic resonance imaging (MRI) and positron emission tomography (PET) for immunotherapy monitoring, characterization, and combination. Superparamagnetic iron oxide (SPIO)-labeled cells can be detected and quantified in vivo using R_2* mapping via a multi-echo single point imaging sequence (TurboSPI). The goal is to assess immune cell recruitment to tumors and lymph nodes (LN) in response to immunotherapy to assess links between immune response and therapeutic efficacy at the individual level. **Methods:** C57/BL6 mice were implanted with $5x10^5$ C3 cervical cancer cells and Balb/c mice were implanted with $5x10^3$ 4T1 breast cancer cells. C57BL/6 mice were either untreated or treated with anti-PD1, a peptide-based vaccine DepoVax, or a combination of both. Balb/c mice were either untreated or treated with anti-TIM3. CD8+ cytotoxic T cells (CTLs; C3 and 4T1), dendritic cells (DCs; C3), and myeloid derived suppressor cells (MDSCs; 4T1) were isolated from diseased-matched donor mice and expanded in culture before labeling with SPIO for adoptive cell transfer into mice receiving scans. Simultaneous PET/MRI data was acquired on a 3T pre-clinical scanner 1 day following adoptive cell transfer days 21 and 28 post-implant for C3 mice or days 18 and 25 for 4T1 mice. MR anatomical data was collected using a balanced steady state free precession sequence, and the SPIO recruitment to draining LN and tumors was quantified via R_2* maps generated from TurboSPI. Tumor metabolism was assessed with 18F-fluorodeoxyglucose (FDG) uptake using simultaneous PET/MRI. **Results:** Cellular recruitment of all cells types were observed in both the C3 and 4T1 model. According to R_2* maps, an average of ~3-10% of injected CTLs (8 million) were recruited to the tumor and ~0.1% to the lymph nodes (LN). CTL recruitment to both models was not correlated to tumor volume but in C3 mice, the CTL recruitment rate was significantly different for the combination treatment compared to control group, and the intra-group variability decreased in response to treatment. DCs (1 million injected) migrated primarily to the LNs while MDSCs (3 million injected) were recruited primarily to the tumor. C3 tumors were found to be FDG avid, displaying areas of necrotic cores while 4T1 tumors displayed varying amounts of FDG uptake, indicating a highly heterogeneous metabolic environment. Immune cell tracking and quantification by MRI using TurboSPI is a promising new tool for evaluating immunotherapy efficacy and provides insight into their mechanism of action.

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Dimeric and monomeric glucose-transporters (GLUT-1) targeting conjugates for imaging and alpha-emitter therapy of metastatic melanoma. Izabela Tworowska, Nilesh Wagh, Ebrahim Delpassand; RadioMedix Inc., Houston, TX

Objectives: Targeted α-emitter therapy (TAT) allows for highly localized delivery of the cytotoxic radiation resulting in irreversible damage of DNA. This current project focuses on validation of dimeric and monmeric GLUT targeting agents for TAT of the highly aggressive cancers. We have previously proved that radiolabeled-monomeric glucosamine conjugates selectively target GLUT transporters in mice bearing metastatic melanoma xenografts. The purposes of this study were: (1) evaluation of GLUT-specificity of radiolabeled dimeric bioconjugate, RMX-GC2; (2) design a new scaffold for multi-targeting of GLUT and other receptors/transporters in cancer cells. **Methods:** The monomeric and dimeric RMX-GC were synthesized using coupling peptide chemistry. The Pb212/Cu64-labeling of RMX-GC were carried out under mild conditions in 0.4M NH4OAc buffer (pH 5.5). The GLUT1 binding properties of RMX-GC were determined by cellular uptake in melanoma cancer cell lines, B16-F10, A375, and MW375 cells. GLUT-specificity was confirmed by co-incubation of the agents with GLUT-specific ligands in cancer cell lines. The biodistribution studies of Pb212-RMX-GC and Pb212-RMX-GC2 (10uCi) in B16(10) xenographs of C57/B6 mice were done at 1h, 4h, and 24h time-points. **Results:** Synthesis of Cu64-labeled conjugates (5ug/1.1mCi) was completed under mild conditions (15min/37oC; RCY >99.9%). Pb212-labeling of conjugates (15ug/1mCi) proceeded at room temperature (10min; RCY >96%). In vitro uptake of monomeric Cu64-RMX-GC was 3x-fold lower (<5.5% ID/mg) than the RMX-GC2 in tested cancer cell lines. Cu64/Pb212- RMX-GC2

demonstrated high retention in cancer cell lines with 2x-fold higher accumulation in B16(F10) compared to A375. These results correlated well with known-elevated expression of GLUT transporters in B16(F10) cells relative to A375 cells. The co-incubation of the 64Cu-labeled RMX-GC2 with GLUT specific ligands (cytochalasin B) reduced its uptake by 91% in A375 cells and 87.5% in B16(F10) cells. The biodistribution studies showed >2%ID/g accumulation of Pb212-RMX-GC in B16(F10) tumor at 1h and decreased to 1%ID/g at the 24h time point. There was limited/no uptake of agent by bone, spleen, liver and pancreas. The retention of agent in kidney was 13%ID/g at 1h and decreased to 1.6% ID/g at the 24h time point. **Conclusion:** Our feasibility studies confirmed favorable tumor targeting properties of dimeric RMX-GC2 compared to monomeric analog. As expected, the kidney will be the dose limiting organ for TAT therapy. However, the dose of agent retained in kidneys can be decreased by co-injection of positively charged amino acids (if retention is based on the charged of molecule) and we will evaluate the effect of co-infusion of Lysine-Arginine or other alternative approaches in future studies. Further work is required to optimize the specific activity of the Pb212-labeled dose since all these studies were performed using non-GMP grade agent synthesized in our laboratory.

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Can we use near-infrared fluorescence imaging of panitumumab-IRDye800 to predict intraoperative lymph node status in patients with head and neck cancer? Nynke S. van den Berg, Nutte Teraphongphom, Steven Hong, Brock Martin, Nicholas Oberhelman, Michael Kaplan, Vasu Divi, Christina Kong, Dimitri Colevas, Eben Rosenthal; Stanford University, Stanford, CA

Background: The presence of lymph node (LN) metastasis is considered the most important negative prognostic factor for survival in patients with squamous cell carcinoma of the head and neck (HNSCC). Interestingly, besides preoperative imaging methods (e.g., 18F-FDG PET), there is no intraoperative imaging methods available to predict the LN's status. We here present the first results of our ongoing study using the near-infrared fluorescently labeled epidermal growth factor receptor (EGFR) antibody panitumumab (panitumumab-IRDye800) for intraoperative visualization of metastatic LNs. **Methods:** We are currently running a first-in-human dose-escalation clinical trial of panitumumab-IRDye800 in patients with HNSCC (21/29 patients included). Only adult patients with biopsy-confirmed primary or recurrent HNSCC scheduled to undergo standard-of-care surgery were eligible. Cohort 1 (n=3) received an intravenous microdose of 0.06 mg/kg; cohort 2 (n=5) received 0.5/kg; and cohort 3 (n=7) received 1mg/kg. Imaging of the LN specimens was performed using a dedicated closed-field fluorescence imaging device. Fluorescence signal intensities in the LNs were compared to the histo-pathological status of the LNs. Subsequently, sensitivity and specificity were calculated. **Results:** At the moment data is available from 15/21 patients. Of the 563 LNs removed during surgery, 20 were found tumor-positive at pathology. Fluorescence imaging of panitumumab-IRdye800 revealed 456 true-negative nodes (not fluorescent, not tumor-positive), 19 true-positive nodes (fluorescent, tumor-positive), 88 false-positive nodes (fluorescent, not tumor-positive) and 1 false-negative node (not fluorescent, tumor-positive) resulting in a sensitivity of 95.0%, a specificity of 83.8%. **Conclusion:** Preliminary results from our ongoing study suggest panitumumab-IRDye800 has the potential to be used as an intraoperative method to, in real-time, identify metastatic LNs with high sensitivity and specificity.

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Magnetization transfer MRI performed during neoadjuvant therapy of breast cancer correlates with declines in tumor size. Jack Virostko[1], Anna G. Sorace[1], Chengyue Wu[1], Angela M. Jarrett[1], Stephanie L. Eldrige[1], Jeffrey Luci[1], Debra Patt[2], Boone Goodgame[3], Sarah Avery[4], Thomas E. Yankeelov[1]; [1]University of Texas at Austin, Austin, TX, [2]Texas Oncology, Austin, TX, [3]Seton Hospital, Austin, TX, [4]Austin Radiological Association, Austin, TX

Due to the complex nature of the tumor microenvironment and variability in tumor response to targeted therapies, an array of imaging measurements is needed to fully characterize tumor treatment response. Tumor development and progression are influenced by the surrounding extracellular matrix (ECM), but the macromolecules that comprise the bulk of the ECM are difficult to image directly using magnetic resonance imaging (MRI). However, magnetization transfer MRI (MT-MRI) can indirectly probe the macromolecular pool in biological tissue by probing the interaction of macromolecules with free water, without the use of any exogenous contrast agent. We have employed MT-MRI in a quantitative breast imaging protocol in healthy volunteers and breast cancer patients. MT-MRI measurements were performed using two gradient echo sequences each identical save for the inclusion of a 1500 Hz off resonance MT saturation pulse included on one acquisition for a total scan time of 53 seconds. Magnetization transfer was quantified using the magnetization transfer ratio (MTR), which is calculated as the difference in signal intensity with and without the MT saturation pulse divided by the signal intensity without MT saturation. In healthy volunteers, we found that the MTR of healthy fibroglandular tissue was repeatable in two scans of the same subject, with an average difference of 11.6% (n = 10). Furthermore, MTR measurements were reproducible across three imaging sites, with an average difference of 12.7% between scanners (n = 3). Longitudinal MTR measurements were performed at 4 time points in women undergoing neoadjuvant therapy (NAT) for breast cancer: 1) prior to the start of NAT, 2) after 1 cycle of NAT, 3) after 3-4 cycles of NAT, and 4) after 4-5 cycles of NAT. Averaging across the population (n = 10), the MTR value of the tumor increased serially, with an average increase in MTR of 14.3% from the measurement prior to the start of NAT to the final measurement after 4 cycles of NAT. However, there was a high amount of variability between patients which correlated with the magnitude of decline in tumor volume. The MTR measurement performed after 4 cycles of NAT correlated with declines in tumor size, with smaller increases in MTR corresponding to greater declines in tumor volume (R^2 = 0.6; p = 0.009; n = 10). Further work is underway to correlate MTR measurements with pathology to determine if MTR can predict patients who will achieve pathological complete response. This quantitative breast protocol is currently deployed in the community setting at two imaging clinics.

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Imaging hypoxia-driven regulation of GLUT1, GLUT2 and GLUT5 in breast cancer. Ingrit Hamann, Daniel Krys, Darryl Glubrecht, Vincent Bouvet, Alison Marshall, Larissa Vos, John R. Mackey, Melinda Wuest, Frank Wuest; University of Alberta, Edmonton, AB, Canada

Use of [^{18}F]FDG-PET in clinical breast cancer (BC) imaging is limited mainly due to insufficient expression levels of GLUT1 in up to 50% of all patients. Alternatively, GLUT5 (dominant fructose transporter) and GLUT2 (glucose and fructose transporter) provide possible alternative targets for BC imaging. Transcription factor $HIF1_{alpha}$, activated during elevated growth and metabolism in cancer cells, represents the master regulator for downstream targets such as GLUT1. Here we examined mRNA expression of GLUT1, 2, 5 and $HIF1_{alpha}$ in BC cells and patient-derived samples (CBCF Tumor Bank), the effects of hypoxia on their protein levels and functional changes of glucose and fructose transport using PET radiotracers [^{18}F]FDG and 6-[^{18}F]FDF versus hypoxia PET tracer [^{18}F]FAZA in human estrogen-receptor positive (ER+) and triple-negative BC (TNBC) cell lines and tumor models. Cell uptake of [^{18}F]FAZA correlated well with increased HIF1α protein levels under hypoxia. Both [^{18}F]FDG uptake and GLUT1 protein levels were highest in MB231 cells, while MCF10A and MB231 cells showed higher GLUT5 protein levels and about 2 times more uptake of 6-[^{18}F]FDF compared to MCF7 cells. High GLUT2 levels were detected in all cell lines. Uptake of 6-[^{18}F]FDF into MB231 cells was blocked with fructose, glucose and cytochalasin B, supporting the functional involvement of GLUT2 transport. MB231 tumors showed positive staining for HIF1α, while MCF7 tumors remained HIF1α negative. Uptake of [^{18}F]FAZA into MCF7 and MB231 tumors was comparable with slightly higher uptake in MB231 tumors. In MB231 tumors, [^{18}F]FDG showed continuous increase in uptake, whereas a lower plateau uptake level was reached in MCF-7 tumors. In accordance with PET imaging data, stronger GLUT1 staining was observed in MB231 versus MCF7 tumor tissue. 6-[^{18}F]FDF revealed similar but overall lower uptake in both tumor types. GLUT5 staining intensity was overall lower in both tumors with stronger GLUT5 staining in MB231 tumors. Interestingly, GLUT2 staining was also strongly positive for both BC tumors. Using in vitro and in vivo blocking experiments fructose-mediated transport with 6-[^{18}F]FDF was found to use both GLUT2

and GLUT5. Expression profiles of HIF1α-induced genes are associated with recurrence of BC in patients. We confirmed that GLUT1 serves as a downstream target of HIF1α, as detected on the molecular and functional level. In addition, we found that GLUT5 seems to be regulated by HIF1α as well, pointing to the complexity of BC exhibiting a unique molecular "fingerprint." The present results also strongly support a functional involvement of GLUT2 in fructose metabolism, possibly by compensating for the weaker expression and function of GLUT5 in BC. Hypoxia induces increase in HIF1α leading to an increased GLUT5 protein expression but not GLUT2 concluding that fructose metabolism may play a stronger role in TNBC based on higher HIF1α protein expression in these types of tumors.

INVITED SPEAKER ABSTRACTS

1

Real-time molecular imaging of colorectal cancer with hyperpolarized silicon nano and microparticles. Nicholas Whiting[1,2], Jingzhe Hu[1,2], Shivanand Pudakalakatti[1], Caitlin McCowan[1,2], Jennifer Davis[1], Niki Zacharias Millward[1], David G. Menter[1], Pamela Constantinou[2], Daniel Carson[2], Pratip Bhattacharya[1]; [1]The University of Texas MD Anderson Cancer Center, Houston, TX, [2]Rice University, Houston, TX

Introduction: Colorectal cancer is the second-leading cause of cancer-related mortality in the United States (1), despite the prevalence of existing screening measures, such as the colonoscopy. In addition to the risk of intestinal perforation, traditional colonoscopies are also poorly suited to detect nonpolypoid colorectal neoplasms (i.e., 'flat lesions') (2). Furthermore, the recent increase in colorectal cancer prevalence amongst traditionally younger populations (<50 yrs) (3) incentivizes the need for advanced screening methods that will allow early detection, observation of treatment efficacy, and monitoring of disease recurrence for colorectal cancer. Silicon nano- and micro-particles are potentially suitable molecular imaging agents, as they are non-toxic, easily functionalized for targeting, and can accommodate drug payloads (4–6). ^{29}Si MRI can be used for *in vivo* tracking of silicon particles (7) that have been hyperpolarized via dynamic nuclear polarization (DNP), a method that temporarily increases the MRI signal by 4-5 orders of magnitude through enhanced nuclear spin alignment (8). The resulting enhanced ^{29}Si MR signal is preserved within the core of the particle (9), and is retained for significantly longer than other hyperpolarized contrast agents *in vivo*; typical T_1 values range from 20-40 minutes (10). We have previously demonstrated that silicon particles conjugated with tumor-targeting antibodies have equivalent hyperpolarization characteristics as bare particles, and HP ^{29}Si MR signal could be detected in subcutaneous colorectal tumors for at least 20 minutes post-injection. Here we demonstrate that *in vivo* ^{29}Si MRI can be used to detect silicon particle targeting in orthotopic Mucin-expressing colorectal tumors in mice. **Methods:** Silicon particles (2 μm) were surface functionalized with a *214D4* antibody that targets the large glycosylated ectodomain of human MUC1—a transmembrane Mucin protein that is overexpressed in some forms of colorectal cancer. The particles were hyperpolarized using a laboratory-constructed ^{29}Si solid-state DNP device (10); imaging studies were performed on a 7T small animal MRI. Following hyperpolarization, the targeted particles (in ~300 μL PBS) were rectally administered to transgenic mice that spontaneously produced human MUC1-expressing colorectal tumors in the lower intestinal tract, followed by a 10-15 minute wait prior to imaging. Co-registered [^{1}H:^{29}Si] MRI was performed using a dual-tuned ^{29}Si/^{1}H Litz coil: *in vivo* ^{29}Si imaging used a coronal RARE sequence ($\alpha = 90°$; TR/TE: 60 ms/1.8 ms; 6.4 cm FOV; 2 mm resolution), while ^{1}H imaging utilized a coronal RARE scan ($\alpha = 90°$), TR/TE: 1927 ms/9.5 ms with a RARE factor of 8; 6.4 cm FOV (0.25 mm resolution) and 4 averages. Experiments (n=3) were repeated with relevant chemical (non-targeting particles) and biological (non-MUC1-expressing tumors) controls. Following imaging, the mice were sacrificed and tissues were collected for histological analysis. **Results:** Silicon particles conjugated with the antibody were shown to target MUC1-expressing colorectal tumor cells *in vitro* both before and after hyperpolarization, demonstrating that the targeting ability of the antibody was not affected by the harsh conditions of DNP. The presence of tumors in mouse models was confirmed prior to MRI scans using a veterinary endoscope. Imaging studies of the targeted particles in MUC1-expressing mice demonstrate bright ^{29}Si MR signal at the tumor sites, while the controls provided weaker, sporadic signal that was not associated with the presence of tumors. These results were correlated with tissue histology. **Discussion:** Demonstration that the targeting veracity of the antibody is not negatively affected by the harsh conditions of DNP is a critical step in developing hyperpolarized silicon particles as targeted imaging agents, not just for colorectal cancer but also as a platform technology for interrogating a variety of disease systems. The *in vivo* studies presented here are the first demonstration of MRI detection of targeted imaging by hyperpolarized silicon particles, and potentially open the door to a variety of disease applications. The experiments were repeated (n=3) and compared to both chemical and biological controls (n=3 each), and histological analysis; tissue immunohistochemistry confirmed particle binding only under conditions of *214D4* particle conjugation and MUC1-expression. Furthermore, the long-lasting hyperpolarized ^{29}Si signal was still well-visible for at least 10 minutes after injection. Future studies will translate these advances to nano-scale particles, which should demonstrate improved mobility and allow molecular targeting of other cancer systems. **Conclusion:** We demonstrate targeted molecular imaging of MUC1-expressing colorectal cancer in orthotopic mouse models using targeted silicon particles. When fully developed, these particles are engineered to be a platform system, where different targeting agents and therapeutic drugs can be attached for advanced molecular imaging and therapeutic interventions in the clinic. **References:** (1) SEER Cancer Statistics Review (2016). (2) Soetikno, *et al*., JAMA (2008). (3) Siegel, *et al*., JNCI (2017). (4) Park, *et al*. Nat. Mat. (2009). (5) Tasciotti, *et al*. Nat. Nano. (2008). (6) Santos, Porous Silicon for Biomedical Applications, Elsevier (2014). (7) Cassidy, *et al*. Nat. Nano. (2013). (8) Dementyev, *et al*. Phys. Rev. Lett. (2008). (9) Aptekar, *et al*. ACS Nano (2009). (10) Whiting, *et al*. J. Med. Imag. (2016).

2

Shedding light on tumor oxygenation with photoacoustic imaging. Sarah E. Bohndiek; University of Cambridge, Cambridge, United Kingdom

Photoacoustic imaging is an emerging clinical imaging modality that combines the high contrast of optical imaging with the high spatial resolution of ultrasound. Here, I will first give a general overview of the strengths and weaknesses of photoacoustic imaging. I will then describe our recent experience applying this new modality for quantitative imaging of tumor hemoglobin concentration and oxygenation, as well as tumor vascular function, in small animal models of breast and prostate cancer, as well as exploratory clinical trials in the breast. Finally, I will provide an outlook on the potential of photoacoustic imaging in light of the current state-of-the-art.

3

Cancer nanotheranostics. Xiaoyuan Chen; National Institute of Biomedical Imaging and Bioengineering (NIBIB), National Institutes of Health (NIH), Bethesda, MD

Theranostics (Rx/Dx) aims to develop molecular diagnostic tests and targeted therapeutics with the goals of individualizing treatment by targeting therapy to an individual's specific disease subtype and genetic profile. It can be diagnosis followed by therapy to stratify patients who will likely respond to a given treatment; it can also be therapy followed by diagnosis to monitor early response to treatment and predict treatment efficacy; it is also possible that diagnostics and therapeutics are co-developed (nanotheranostics). This talk will give examples of optotheranostics, magnetotheranostics and immunotheranostics. The translational potential of Nanotheranostics will also be briefly discussed.

4

Imaging tumor associated macrophages with MRI. Heike E. Daldrup-Link; Stanford University School of Medicine, Stanford, CA

Many malignant tumors, including breast cancer, lung cancer, colon cancer, pancreatic cancer, lymphomas, sarcomas and neuroblastomas (among many others), are associated with an anti-inflammatory tumor microenvironment,

which is characterized by infiltration of leukocytes where increases in some leukocyte subsets parallels disease progression and worse clinical outcomes. Tumor-associated macrophages (TAMs) play a key role in this context. New therapeutic drugs that target TAM are currently being developed and are starting to enter the clinic. Thus, it becomes increasingly important to identify patients whose tumors are heavily infiltrated by TAM. To serve this goal, an imaging test would be advantageous over invasive biopsy because it would be non-invasive, cover the whole tumor and could repeatedly interrogate treatment effects on the complex cross talk between innate and adaptive immune responses in vivo, in patients. To the best of our knowledge, no such imaging test exists to date. We use ferumoxytol nanoparticles for TAM imaging. Ferumoxytol is the only FDA-approved iron oxide nanoparticle compound, which can be offered to patients for non-invasive TAM imaging through "off label" use as a contrast agent. Ferumoxytol is a colloid-based ultrasmall superparamagnetic iron oxide nanoparticle (USPIO) compound with a hydrodynamic diameter of 28–32 nm, an r1 relaxivity of 38 mM–1s–1 and an r2 relaxivity of 83 mM–1s–1 at 20 MHz and 39°C. Our team showed that intravenously injected ferumoxytol nanoparticles cause an initial perfusion effect of the tumor tissue, followed by retention in the tumor via the "enhanced permeability and retention (EPR) effect" and subsequent phagocytosis by TAM, which results in a marked negative (dark) signal effect on delayed T2-weighted MR images. This can be used to noninvasively track the degree of TAM infiltration in a tumor. Our clinical MR imaging studies after intravenous ferumoxytol injection demonstrate unique enhancement properties of glioblastomas, lymphomas and sarcomas in the presence of TAM-mediated inflammation, providing a novel, immediately clinically applicable imaging test to non-invasively track innate immune responses from macrophages in patients. This new TAM imaging test is immediately clinically available, could permeate our understanding of immune cell responses to cancer therapies, enable us to overcome the bottleneck of monitoring anti-tumor immune responses in vivo, improve patient stratification to TAM-targeted therapies and aid in assigning non-responders to alternative treatment options.

5

Liquid biopsy for early detection of cancer—Opportunities, promise, challenges and solutions. Kapil Dhingra; KAPital Consulting LLC, Sparta, NJ

Early detection of cancer remains one of the most important unmet medical needs in public health today. Current methods largely rely on periodic history and physical examination, visualization through endoscopic and radiologic means, and, for certain organ sites, biochemical tests. Each of these methods have their own limitations relating to one or more of the following issues, sensitivity, specificity, invasive/cumbersome methodology. Furthermore, many of these tests cannot be repeated frequently due to cost, logistics, or potential side-effects. Additionally, most of the current screening methods are uniquely individualized for each organ, thereby limiting their generalizability for early detection of cancer. As the genetic basis of cancer has become better understood over the last thirty years, molecular approaches have begun to be applied to early diagnosis of cancer. Initial efforts largely focused on inherited cancer mutations in order to identify individuals most at risk of developing cancer. As the catalogue of somatic cancer mutations continues to build, these are increasingly being looked for in various body tissues as well in order to improve diagnosis and prognostic assessment of cancer. However, similar to previous approaches of imaging and direct visualization, these molecular analyses have generally been applied to discrete organs/lesions. It is important to distinguish the challenge of early detection of cancer from that of early detection of recurrence of cancer. Even though in many instances the same technological tools may be applicable to both the situations, the performance characteristics required of the diagnostic tests and by inference the acceptable risk-benefit are quite different. The liquid biopsy option—CTCs: Liquid biopsies have the potential to circumvent many of the limitations of tissue-based diagnostics by allowing access to cancer-derived molecules without prior knowledge of the location of origin of a tumor or even the organ of origin. Initial liquid biopsy techniques largely relied on detection of shed tumor cells in the circulation (CTCs). In certain tumor types, relatively large number of CTCs can be detected consistently in patients with advanced metastatic disease. In certain specific situations, e.g., patients with metastatic prostate cancer, they may be useful to predict therapeutic response (1). However, in most tumor types, it has proven difficult to harvest these cells consistently. In the early detection setting, where even fewer tumor cells are likely to be shed, it is extremely unlikely that CTC detection can be performed consistently and reliably enough to be systematically useful for early detection of cancer. cfDNA: In recent years, it became apparent that a small amount of circulating nucleic acids, cell-free DNA (cfDNA), could be detected in all individuals. The origin of these is generally believed to be from dying cells. In patients with cancer, cfDNA is derived from both normal as well as dying neoplastic cells (circulating tumor DNA or ctDNA). Highly sensitive techniques for isolation, quantification, and characterization of cfDNA have been developed and are being routinely employed by a number of academic institutions and commercial vendors to identify actionable oncogenic mutations in order to drive therapeutic decision making in patients with cancer (2). The first such tests have also received recent FDA approval as companion diagnostics for novel EGFR (epidermal growth factor receptor)-targeted drug. A number of groups have independently shown that ctDNA quantitation not only provides actionable therapeutic information but may also be an early indicator of therapeutic response as well as a harbinger of early progression. While the clinical relevance of early treatment response prediction or early progression detection may seem intuitively obvious, actual clinical trials need to be done in order to prove that modifying treatment based on such early molecular markers leads to an improved outcome for the patient! While the use of ctDNA analyses is increasing rapidly, there are significant limitations that are common to all of the ctDNA platforms. Because ctDNA is derived from dying cells, it represents the average genotype of the dead cells and not necessarily the viable tumor cells which ultimately determine the outcome for a patient. Also, while ctDNA is an excellent source material for 'fixed' genetic lesion, e.g., mutations, translocations etc., it obviously misses enormous information relevant to the real-time behavior of the tumor, i.e., alterations related to transcription, including alternative splicing, as well as alterations related to post-transcriptional processing. Additionally, by its very nature, the amount of ctDNA in the circulation is generally a reflection of the tumor burden in a given patient. Therefore, ctDNA techniques for detection of actionable mutations are generally quite sensitive in the setting of advanced metastatic disease but have poor sensitivity in the setting of early stage disease. Therefore, it is very unlikely that this type of ctDNA analysis will prove useful as a general strategy for early detection of cancer. Some groups have attempted to address this by analyzing an entirely different class of markers on the genome that may be more broadly applicable. Specifically, analysis of methylated genes is being tried as an early detection tool. Other groups are developing assays to detect specific micro-RNAs for the same purpose. Future studies should clarify whether they can provide adequate sensitivity, specificity and broad applicability to become early detection tools for cancer. Exosomal biomarkers: A somewhat newer modality whose potential in oncology is being rapidly recognized is the analysis of biomarkers in exosomes. Exosomes are shed by most cells in the body but in especially large numbers by cancer cells and neuronal cells. Historically, exosomes were considered 'garbage bags' of the cells that were used by the cell to get rid of unwanted metabolites and waste products. Over the last two decades, it has become increasingly apparent that exosomes are an important cargo traffic mechanism that the body uses for a variety of important biological functions, both in health and disease. Specifically, in the context of oncology, they are now known to play an important role in tumor growth, immune system regulation, and, preparation of the pre-metastatic niche at distant organ sites. They are important tools for intercellular communication. It is not unreasonable to describe them as nature's 'FedEx' packages in that they have surface proteins that represent the Sender's and the Recipient's address and have a cargo inside destined to be delivered to the recipient at a near or distant site. The cargo of exosomes contains proteins and enzymes, including growth factors, oncoproteins, immune-regulating proteins, and proteases. Importantly, it also contains a snapshot of nearly the entire transcriptome of the cells, including up to full length mRNAs. This is in sharp contrast to the prevalent notion as recently as a few years ago when exosomes were not believed to contain any mRNA. RNA is highly stable in exosomes enabling successful interrogation of the transcriptome from archival samples after several years. Thus, exosomes, unlike ctDNA, can provide a real-time insight to the prevailing biology, normal and pathological, of a living organism. The first clinically useful application to be commercialized using exosomes as the source material is ExoDx Prostate Intelliscore (EPI). This is a three gene mRNA signature derived from urinary exosomes in individuals with suspected prostate

cancer. The signature has been optimized to discriminate the presence of high grade carcinoma vs. no/low grade carcinoma (this is a more relevant question in prostate cancer than simply discriminating between cancer vs. no cancer). In the validation study (3), urine samples were obtained from men who presented for an initial biopsy following a screening PSA in the range of 2-10 ng/ml. The study included 1,564 men. The three gene signature demonstrated a sensitivity of 87% and a specificity of 46% with a negative predictive value of 90%. Thus, this test could avoid 37% of the planned biopsies if used routinely. This is far superior to the performance of the current most widely used test, i.e., PSA, based on which nearly all of these men would have been biopsied. This is the first application of a novel molecular liquid biopsy to an important public health problem. It stands to reason that exosomes would provide an appropriate liquid biopsy resource to detect oncogenic RNA alterations such as fusion transcripts (e.g., ALK fusions) as well as alternatively spliced transcripts (e.g., EGFRvIII) which cannot be detected by the ctDNA platforms. The feasibility of this has already been demonstrated. Even for DNA mutations, combined exosomal RNA + ctDNA analysis has been shown to have significantly greater sensitivity than analysis of ctDNA alone, especially when the copy burden of the mutations is low. Additionally, in the context of non-small cell lung cancer (NSCLC), in patients with advanced cancer confined to the thoracic cavity only (a situation relatively close to that of patients with primary NSCLC), inclusion of exosomal RNA provides far greater sensitivity than ctDNA for detection of EGFR T790 mutation. Interestingly, for patients with extensive extrathoracic metastatic disease, the sensitivity of ctDNA and combined ctDNA + exosomal RNA for detection of T790 mutation is similarly high. The overall sensitivity of combined ctDNA + exosomal RNA in this study was 87% with a specificity of 98%, negative predictive value of 94% and positive predictive value of 95%. Looking to the future—Multi-platform biomarker integration: Notwithstanding the impressive progress in liquid biopsy over the last few years, further advancements are likely to be needed before these can be widely tested for early detection of cancer more generally. Given the low volume of cancer burden in this setting, further improvements in sensitivity are likely to be required. Fortunately, exosome based techniques lend themselves readily to enrichment/deselection strategies by incorporating an additional step to isolate exosomes derived from specific organ systems/tissue types. In addition, the known driver oncogenic mutations are not present in all tumor types. It is likely that any early detection algorithm needs to incorporate other forms of signatures, including epigenetic alterations, field carcinogenic defects, transcriptomal signatures, and multiparametric proteomic profiles. In the intermediate term, it is unlikely that any of the imaging or liquid biopsy technologies in isolation can be adequate for early detection of all or even most cancers. Optimal early detection strategies will likely include an integrated approach involving clinical and molecular epidemiologic risk factors, liquid biopsy, advanced textural image analysis, and precision nuclear imaging. This will require development of well thought out algorithms for application of the various technologies and the emergent data in order to guide the physician and the patient to an optimal early detection paradigm while minimizing unnecessary invasive interventions and patient anxiety resulting from incidentally discovered, clinically inconsequential findings. **Bibliography:** 1. Heller G, Mccormack RT, Kheoh T, et al. Circulating tumor cell (CTC) number as a response endpoint in metastatic castration resistant (mCRPC) compared with PSA across five randomized phase 3 trials. J Clin Oncol 2017, 35:15_suppl, 5007-5007. 2. Phallen J, Sausen M, Adleff V, et al. Direct detection of early-stage cancers using circulating tumor DNA. Science Translational Medicine 2017, 9 (403):eaan2415. 3. McKiernan J, Donovan MJ, O'Neill V, et al. A Novel Urine Exosome Gene Expression Assay to Predict High-grade Prostate Cancer at Initial Biopsy. JAMA Oncol. 2016; 2:882-889.

6

Magnetic resonance fingerprinting: Development to clinical translation. Vikas Gulani; Case Western Reserve University, University Hospitals of Cleveland, Cleveland, OH

Magnetic Resonance Fingerprinting (MRF) is a technology that was introduced in 2013, for rapid, simultaneous mapping of multiple tissue properties of interest with MRI. The first portion of the talk will be focused on the rationale behind the MRF approach, how it is implemented, and some directions of further technical development. The remainder of the talk will then shift towards clinical translation, with discussion of the steps needed for such translation, and the clinical results obtained thus far.

7

Tumor-stromal interaction dynamics in osteolytic bone metastasis. Yibin Kang; Princeton University, Princeton, NJ

During cancer metastasis, disseminated tumor cells often hijack existing physiological cellular interactions to facilitate their seeding, survival and outgrowth in distant organs. Bone metastasis is a frequent occurrence in breast cancer, affecting more than 70% of late stage cancer patients with severe complications such as fracture, bone pain, and hypercalcemia. The pathogenesis of osteolyitc bone metastasis depends on cross-communications between tumor cells and various stromal cells residing in the bone microenvironment. We used advanced imaging techniques and molecular biology approaches to prove the signaling interactions between metastatic tumor cells and various stromal cells in bone, in order to identify potential new therapeutic targets for bone metastasis. We identified Jagged1 as a TGFβ target gene in tumor cells that engaged bone stromal cells through the activation of Notch signaling to provide a positive feedback to promote tumor growth and to activate osteoclast differentiation. Using genetically modified mouse models, we revealed a surprising role of Jagged1 in promoting chemoresistance of bone metastasis. Chemotherapy of bone metastasis induced elevated expression of Jagged1 in osteoblasts, which provide a pro-survival niche for tumor cells in the bone. Eselectin is an adhesion molecule that normally functions to recruit leukocytes during infection or vascular damage. E-selectin is also thought to be a major component of the hematopoetic progenitor cell (HPC) niche in the bone. We show that E-selectin functions as an essential component of the endothelial niche for bone metastasis, wherein glycosylated E-selectin ligands expressed by metastatic breast cancer interact with endothelial E-selectin to promote the survival and proliferation of metastatic tumor cells. These findings support the notion that development of organ-specific metastasis depends on the interactions between tumor cells and various stromal niche components in a given organ. Importantly, therapeutic targeting of Jagged1 and E-selectin significantly reduce bone metastasis and sensitize them to chemotherapy, suggesting possible avenues to dramatically improve the treatment of metastatic bone disease.

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Hyperpolarized magnetic resonance for imaging cancer metabolism. Kayvan R. Keshari; Memorial Sloan Kettering Cancer Center, New York, NY

Oncogenic transformation has been shown to have a dramatic impact on the metabolic state of the cell. Recent reports have demonstrated that specific alterations in oncogenes and signaling pathways result in increases in pathway flux as well as diversion of substrates. Hyperpolarized magnetic resonance (HP MR) addresses a fundamental limitation of MRI for interrogating metabolic substrates, sensitivity. Using this approach, endogenous metabolic substrates can be prepared prior to infusion into a living system with dramatically increased signal and followed to their metabolic products. In this talk, I will discuss the development of novel endogenous agents to explore tumor metabolism and the imaging of metabolism in patients using HP MRI.

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Tumor organoids for drug discovery and personalized medicine. Qiong Zhou, Linfeng Li, Adedoyin D. Abraham, Daniel V. LaBarbera; The University of Colorado Anschutz Medical Campus, Aurora, CO

The past decade has seen a revolution in developing 3D tissue models of organ function, anatomy, and disease. These models are referred to as organoid, organotypic, or spheroid and these terms are used interchangeably throughout the literature. Organoids are defined by their ability to mimic in vivo organ function and/or disease, they can be engineered with multiple cell types and microenvironment components, and organoids have the

distinct ability to self-assemble. Like organoids, tumor organoids mimic in vivo tumor biology and recapitulate key interactions between extracellular matrix (ECM) molecules and tumor cell receptors that initiate signaling events regulating and promoting cancer. This presentation will discuss our recent work with colon tumor organoid models of epithelial-mesenchymal transition (EMT). These models feature an innovative dual fluorescent biomarker reporter of EMT (Vimentin Promoter-GFP-E-cadherin Promoter-RFP) that can effectively track the forward and reverse EMT transition in live tumor organoids. We have engineered stable dual reporter SW620 cells as single uniform tumor organoid per well in 96- and 384-well plates, and have conducted 3D high-content screening (HCS) drug discovery of thousands of compounds. Additionally, we will discuss hit confirmation and secondary assays used to validate compounds that modulate or reverse EMT. Finally, we will discuss our most recent advances to develop patient derived tumor organoid (PDTO) models suitable for high-content analysis and screening towards achieving the goal of personalized medicine in cancer.

10

Novel ligands and target identification: Using the correct in vivo models. S.K. Sharma[1], A. Chow[1], S. Monette[1], D. Vivier[1], J. Pourat[1], K.J. Edwards[1], T.R. Dilling[1], D. Abdel-Atti[1], B.M. Zeglis[2], J.T. Poirier[1], J.S. Lewis[1]; [1]Memorial Sloan Kettering Cancer Center, New York, NY, [2]Hunter College and Graduate Center of the City University of New York, New York, NY

A critical benchmark in the development of new agents aimed at novel targets is choosing the correct in vitro and in vivo cancer models to perform evaluation. A prime example of this is when studying antibody-based therapeutics. This is particularly relevant when attempting to demonstrate efficacy in preclinical mouse models of human disease, many of which rely on immunodeficient mice. Relatively little is known about how the biology of various immunodeficient strains impacts the in vivo fate of these drugs. As an exemplar, we used immunoPET radiotracers prepared from humanized and murine monoclonal antibodies against four therapeutic oncologic targets to interrogate their biodistribution in four different strains of immunodeficient mice bearing lung, prostate, and ovarian cancer xenografts. We found that the immunodeficiency status of the mouse host as well as both the biological origin and glycosylation of the antibody contribute significantly to anomalous biodistribution of therapeutic monoclonal antibodies in an Fc receptor dependent manner. These findings may have important implications for the preclinical evaluation of Fc-containing therapeutics and highlight a clear need for biodistribution studies in the early stages of antibody drug development.

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Imaging strategies for CAR T-cell immunotherapy of solid tumors. Nia Emami-Shahri[1], Ana C.P. Pereira Puri[1], Julie Foster[2], Jane Sosabowski[2], Sophie Papa[1]*, John Maher[1]*; [1]King's College London, London, England, [2]Barts Cancer Institute, London, England. *Contributed equally to this work.

The unprecedented efficacy of chimeric antigen receptor (CAR) T-cell immunotherapy of CD19+ B-cell malignancy has paved the way for its establishment as a new therapeutic pillar of hematologic oncology. Nonetheless, formidable obstacles hinder the attainment of comparable success in patients with solid tumors. To accelerate progress and rapidly characterize emerging toxicities, systems that permit the repeated and non-invasive assessment of CAR T-cell bio-distribution would be invaluable. In this presentation, we describe our experience with two clinically applicable strategies to achieve this. Following passive labelling with Indium 111, CAR T-cells can be tracked for up to 96 hours in cancer-bearing hosts using SPECT-CT imaging, an approach that highlights the distinctive pattern of migration of these cells following intravenous or regional delivery routes. To extend imaging capability beyond this timeframe, we have co-expressed the human sodium iodide symporter (hNIS) in CAR-engineered T-cells. This symporter promotes the selective uptake of a number of clinically useful PET or SPECT tracer isotopes, enabling the long-term and repeated quantitative monitoring of the distribution of these cells in vivo. Together, these systems provide a clinically compliant toolkit for serial imaging of CAR T-cells, facilitating the improved understanding and further clinical development of this emerging therapeutic modality.

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Directed evolution of PET radiotracers for oncology imaging. Steven W. Millward; UT MD Anderson Cancer Center, Houston TX

Molecular imaging has enormous potential to qualitatively change the way cancer is understood, diagnosed, and treated. The advancement of this field depends greatly on the development of new radiotracers that combine the affinity and specificity of monoclonal antibodies with the rapid pharmacokinetics of small molecules. Peptides and peptidomimetics represent a region in chemical space that can accommodate these disparate characteristics. Unfortunately, linear peptides comprised of the twenty natural proteogenic amino acids often lack the stability and affinity required for molecular imaging applications. Recent work has shown that directed evolution of covalently-cyclized peptide libraries containing N-methyl amino acids results in high-affinity macrocyclic peptides with dramatically enhanced stability toward proteolytic degradation and metabolic modification. This technique, known as Scanning Unnatural Protease Resistant (SUPR) mRNA Display, was used to select SUPR4, a novel macrocyclic ligand against the Her2 receptor that bound with low nanomolar affinity in both ELISA- and cell-based assays. Near-infrared optical imaging with SUPR4-Cy5 showed rapid and Her2-selective tumor uptake mouse xenograft models with minimal background signal in the liver. Radiolabeling of SUPR4 with ^{18}F was carried out using click chemistry and the specific activity of the resulting probe enhanced by >100-fold using a novel alkyne-scavenging resin. SUPR4-[^{18}F] retained Her2-selective tumor uptake *in vivo* and was almost entirely resistant to modification and degradation in systemic circulation. These data suggest that SUPR peptides possess the requisite affinity, stability, and clearance rates for rapid evolution of PET radiotracers for cancer biomarker imaging.

13

Imaging tumor acidosis with acidoCEST MRI. Mark D. Pagel; University of Texas MD Anderson Cancer Center, Houston, TX

Extracellular tumor acidosis is a well-known consequence of upregulated glycolysis in solid tumors, known as the Warburg effect. We have developed a MRI method that can measure extracellular pH (pHe) in tumor models using Chemical Exchange Saturation Transfer (CEST), known as "acidoCEST MRI." We have used acidoCEST MRI to show that aggressive, faster-growing tumors have lower pHe. Also, tumor pHe can significantly increase in response to treatments that generally decrease tumor metabolism, or that inhibit the lactic acid production pathway. Tumor pHe can decrease in response to a mitochondrial poison that redirects glucose metabolism towards glycolysis, and in response to inhibition of gluconeogenesis that reduces the consumption of lactic acid. More recently, we have combined [^{18}F]DG PET with acidoCEST MRI to simultaneously interrogate changes in glucose uptake and lactic acid production in tumor models undergoing treatment, which provides a more comprehensive understanding of changes in glycolytic metabolism in response to treatment. In addition, we have translated acidoCEST MRI to the radiology clinic. Our preliminary results have suggested that human tumors can be more acidic, approaching pHe 6.2, relative to mouse tumor models that typically are no lower than pHe 6.5.

14

What's new in nuc med instrumentation. Todd E. Peterson; Vanderbilt University Medical Center, Nashville, TN

Hybrid (PET/MR and SPECT/MR) and body-part specific scanners are two of the notable recent and ongoing developments in nuclear medicine imaging. Both of these have been significantly facilitated by semiconductor detector technologies. In addition to their magnetic-field compatibility, the implementation of these technologies has enabled improvements in timing,

spatial, and energy resolutions. While the seemingly simple, yet technically challenging idea of extending the axial field of view of the scanner is set to usher in a new level of sensitivity in PET, the detection of Cherenkov radiation offers entirely new possibilities for imaging. Details of these technical developments will be presented and their impact on cancer imaging discussed.

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Real-time PK/PD imaging of immunotherapy in vivo. Mikael J. Pittet; Massachusetts General Hospital, Boston, MA

Immune Checkpoint Blockade (ICB) drugs are designed to activate the immune system against cancer and can be extraordinarily effective in some patients. Yet, how these drugs behave in complex tumor microenvironments and why they work or fail against cancer requires study. To address these questions, we started to image key readouts of immunotherapy function in real-time at single cell resolution. Specifically, we use in vivo molecular imaging to track ICB monoclonal antibody (mAb) therapeutics and resolve their pharmacokinetics (PK) and pharmacodynamics (PD) in real-time and at single cell resolution (real-time PK/PD imaging or RPPI). We propose that the ability to track the dynamics of immunotherapeutics in complex in vivo microenvironments will be important for deciphering drug action mechanisms. Levering this knowledge should help not only to engineer better therapeutics but also to select combination therapeutics rationally.

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[^{18}F]FAZA PET/CT for monitoring modulation of mitochondrial oxidative phosphorylation in vivo. David Piwnica-Worms; The University of Texas MD Anderson Cancer Center, Houston, TX

An emerging and radically different strategy for molecular therapy is the targeting of genomic deletions, which conventionally have not been considered to be therapeutically actionable. However, it has now been demonstrated that genes that are adjacent or in close proximity to lost tumor suppressor genes, so called "passenger" or "collaterally deleted" genes, expose pharmacologically targetable vulnerabilities if these passenger genes have critical functions. For example, gliomas with passenger deletions in the glycolytic gene Enolase 1 (*ENO1*) are exquisitely sensitive to agents that inhibit mitochondrial Oxidative Phosphorylation (OxPhos). Hypersensitivity to OxPhos inhibition derives from impaired glycolysis, because *ENO1*-deleted tumor cells are unable to initiate compensatory upregulation of glycolysis in the face of inhibition of OxPhos, while normal cells do not share this metabolic vulnerability. This provides a therapeutic window. In pre-clinical tumor models, we demonstrate that inhibition of OxPhos can be non-invasively imaged with the PET redox/hypoxia probe, ^{18}F-fluoroazomycin-arabinoside (^{18}F-FAZA), because inhibiting OxPhos reversed tumor hypoxia. Conversely, increasing oxygen consumption via treatment with a mitochondrial uncoupler (2,4-dinitrophenol) increased ^{18}F-FAZA retention in vivo. These data suggest that over-active oxygen consumption (consumptive hypoxia) by tumor cells is a major driver of tumor hypoxia, not deficient delivery, a framework that challenges current paradigms of the etiology of hypoxia in tumors. We further conclude that PET/CT imaging with ^{18}F-FAZA can be used as a noninvasive marker of OxPhos modulation in vivo.

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EGFR-directed fluorescent affibody ABY-029 for phase 0 trial studies. B.W. Pogue[1], K.D. Paulsen[1], D. Roberts[2], S.K. Samkoe[2], P.J. Hoopes[2], J.T. Elliott[1], S. Hull[1], J.K. Gunn[1], E. Henderson[2], J. Paydarfar[2]; [1]Thayer School of Engineering at Dartmouth, Hanover, NH, [2]Geisel School of Medicine at Dartmouth, Lebanon, NH

Molecular guided surgery has potential as a critical tool to enhance extent of tumor resection in a range of cancers, and the evolution towards this surgical paradigm is largely just limited by the speed by which targeted imaging agents are cleared for human use by the FDA. The ABY-029 small molecule bioconjugate was developed through an NIH-funded Academic-Industry partnership, to test use of synthetic protein-dye conjugates through preclinical development and toxicity studies linked to a Phase 0 microdose clinical trial. The design of a human microdose trial in recurrent glioma patients allows for approval with limited pre-clinical single dose toxicity data in a single species, and small production runs of the agent, through peptide synthesis, thereby providing a low cost and yet high production rate of small specific probe molecules. Specifically, ABY-029 is composed of synthetic anti-epidermal growth factor receptor (EGFR; also called ErbB-1) Affibody® molecule (Z03115-Cys) conjugated to IRDye®800CW under GMP conditions. Toxicity analysis in rats demonstrated that signal-dose ABY-029 produced no pathological evidence of toxicity at any of the dose levels tested (up to 1000x an equivalent human microdose level). The objective of the Phase 0 study is to evaluate the binding specificity of ABY-029 in recurrent glioma patients who have tumors with pathology-confirmed EGFR positive status. Preclinical studies in F98 EGFR-positive and EGFRvIII-mutated orthotopic rat tumors and U251 orthotopic xenograft tumors show a strong EGFR specificity and tumor-to-background ratio of between 4:1 and 8:1 depending on the type and location. EGFR-negative tumors showed only very little, perfusion-driven contrast of 2:1 suggesting a dominant molecular-based contrast mechanism. To enable visualization of sub-micromolar concentrations of ABY-029, a conventional operating microscope was fit with a high-powered light source and a near-infrared sensitive fluorescence camera. Preclinical evaluation of this device showed a lack of sensitivity to 1x microdose levels of ABY-029 in a U251 orthotopic glioma, borderline sensitivity to 3x microdose and robust signal in 6x microdose. Therefore, following successful eIND application, the first in-human clinical study is designed to allow escalation of dose from 1x to 3x to 6x, with transition to the next dose permitted only after an absence of detected signal on at least 2 patients. The study was approved by the Dartmouth IRB and registered on ClinicalTrials.gov (NCT02901925), and enrollment began in December 2016. Sarcoma resection and Head & Neck phase 0 trials have also begun to examine this. In conclusion, ABY-029 was selected because of its wide potential to impact surgical oncology, since EGFR is expressed in a broad range of cancers. A complete first-in-human series of surgeries is necessary to fully assess the effectiveness of ABY-029 specifically, and preliminary data to date is promising and the study is progressing as planned.

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Light up my tumor: Giving surgeons hope. E.L. Rosenthal[1], W.S. Tummers[1,2], N.S. van den Berg[1], G.A. Grant[1], H.L. Gordon[1], T.A. Longacre[1], G.A. Fisher[1], A.D. Colevas[1], A.L. Vahrmeijer[2], G.A. Poultsides[1]; [1]Stanford University, Stanford, CA, [2]Leiden University Medical Center, Leiden, The Netherlands

Cancer remains primarily a surgical disease with 75% of solid malignancies treated with surgery at some point during their management. Limited resectable tissue mass and complicated anatomical structures create tension between an extensive resection and preserving normal function. Defining a consistent border between normal and involved tissues at the primary site and identifying tumor containing lymph nodes remain very challenging for surgeons in most disease types. Advances in optical hardware and reagents have provided unique opportunities for real-time imaging during surgical resection or pathological assessment. Optical imaging has been reported using a range of exogenous and endogenous fluorescent agents; we present early clinical trial data in the field of optical imaging for the diagnosis and treatment in solid tumors.

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Radiometal APC chelates for targeted imaging, therapy, and immunomodulation of cancer. Jamey Weichert; University of Wisconsin-Madison, Madison, WI

We have developed a suite of alkylphosphocholine (APC) analogs for multi-modal imaging and targeted radiotherapy (TRT) of a broad spectrum of human cancers. Over-expression of lipid rafts on the outer membranes of cancer cells versus normal cells serves as the basis for APC tumor selectivity. Radioiodinated (I-124, 131) APC analogs demonstrated tumor-selective uptake and prolonged retention in over 90 rodent and human tumor models and have been evaluated in over 100 cancer patients representing a dozen different cancer types in 9 clinical PET imaging and TRT trials.

Moreover, unique tumor selectivity and retention properties were retained upon substitution of the iodine with larger fluorophores suggesting bulk molecular tolerance in the aromatic end of the molecule. APC metal chelates represent a natural structural extension of previous halogenated and optically active analogs. APC chelates permit the use of a wide variety of imaging and radiotherapy avid metals including more effective alpha- and beta-emitters with much lower exposure hazards than I-131. Accordingly, we recently synthesized several APC metal chelates (NM600 series) for PET imaging (Cu-64, Zr-89, Y-86, Mn-52) and targeted radiotherapy (Lu-177 and Y-90). Using paired PET/TRT isosteres (I-124/131 or Y-86/90) PET imaging allows accurate quantification of *in vivo* biodistribution and dosimetry of the TRT construct. New APC metal chelates also exhibit preferable characteristics relative to their halogenated predecessors. Of 11 syngeneic tumor models evaluated to date, 9 exhibited tumor uptake values from 5-10% ID/g. Moreover, in a T-cell lymphoma model (EL4), 80% of the treated (90Y-NM600, 9.3 MBq) mice remained disease free after 90 days. Finally, our collaborative group is focused on using radiation to condition the patient's adaptive immune system against his or her own autochthonous cancer, thus generating an immune-modulated *in situ* vaccine. We recently demonstrated that combining immunocytokines (IC) with external beam radiotherapy (xRT) afforded a striking *in situ* vaccine response in 70% of melanoma-bearing mice wherein the tumor completely regressed and subsequent rechallenge with the same cancer cells resulted in no tumor growth. This finding suggests the existence of a cancer-selective immune memory. However, when this combination (xRT/IC) approach was used to treat the same tumor in the presence of a second, untreated tumor, surprisingly, no response was seen. We hypothesize that "concomitant immune tolerance" (CIT) results, at least in part, from tumor-specific regulatory T-cells harbored in the peripheral untreated tumor. While CIT can theoretically be overcome by delivering xRT to all tumors, it is not feasible to deliver xRT to all metastatic sites which may be invisible. Preliminary preclinical results suggest that immunomodulatory 90Y-NM600 TRT can overcome CIT and restore the *in situ* vaccine response in the presence of metastases in this syngeneic melanoma model thus expanding greatly the utility of TRT agents.

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Cryo-imaging of metastatic cancer and stem cells. David L. Wilson, Yiqiao Liu, Zheng-Rong Lu, Madhu Gargesha; Case Western Reserve University, BioInVision, Inc., Cleveland, OH

We are creating a preclinical, quantitative cancer imaging and therapy analysis platform, which will allow one to study cancer biology and optimize pipelines of technologies (imaging agents, imaging methods, targeted nano-therapeutics, tumor models, etc.), especially for metastatic and invasive cancers. The platform consists of software and multi-spectral cryo-imaging, and optionally in vivo imaging (e.g., MRI and CT). As an example, we used CryoViz™ (BioInVision, Inc.) to analyze imaging agents with CREKA peptide targeting fibrin-fibronectin complexes expressed in the tumor micro-environment. We injected CREKA-Gd, visible in MR, and CREKA-Cy5, visible in red fluorescence, into a GFP-labeled-tumor metastasis mouse model. MRI was followed by cryo-imaging with CryoViz™, which repeatedly sectioned and tiled microscope images of the tissue block face, providing anatomical brightfield and molecular fluorescence, enabling 3D microscopic imaging of the entire mouse with single metastatic cell sensitivity. Selected histology sections were obtained using a tape system. To register MRI volumes to the cryo-brightfield reference, we developed mutual information, non-rigid registration. The result was GFP tumor, color brightfield anatomy, red fluorescence CREKA-Cy5, MRI CREKA-Gd, and optional histology, all within a high resolution, 3D digital visualization and analysis framework. Interactive, multi-scale visualization allowed us to identify a metastasis in GFP, determine the presence of CREKA-Cy5, determine the presence of MR signal, and optionally examine histology for target molecules. Using a 3D extension of the Rose detection model, we determined metastases with sufficient MR signal for detection. Similar methodologies have been found to be quite useful in a variety of stem cell applications, which included cell counts in target organs, suggesting the ability for quantitative, spatially-aware, immunotherapy analysis. In summary, we developed a new methodology which allows one to evaluate qualitatively and quantitatively stem cell and metastatic cancer applications. We anticipate potential future applications to include unique analyses of cancer nano-therapeutics, immune-therapy, and more.

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Quantitative imaging to enable practical computational modeling of cancer. Thomas E. Yankeelov[1], David A. Hormuth II[2], Angela M. Jarret[1], Ernesto Lima[1], Matthew T. McKenna[2], J.T. Oden[1]; [1]The University of Texas at Austin, Austin, TX, [2]Vanderbilt University, Nashville, TN

Three fundamental challenges in mechanism-based modeling of cancer are: 1) model construction and selection, 2) estimating model parameters and quantifying observational data, and 3) assessing model prediction and utility. From each challenge, we pose a fundamental question. First, given the enormous number of models covering a huge range of physical and biological events, which models are the "best" for predicting quantities of interest in tumor growth? Second, how can we determine reasonable values for the multitude of model parameters—and their associated errors—that appear in models of tumor growth? Third, how is the uncertainty in the predicted quantities of interest characterized, and how can the model predictions significantly improve patient care? We have established a general framework for addressing each of these challenges. For challenge 1, we develop a large family of multi-scale models that employs a combination of continuum mixture theory and reaction-diffusion equations to describe interacting species, the balance laws of continuum physics, and the principal hallmarks of cancer (1). After initializing and constraining the model family with relevant in vitro and in vivo data (challenge 2), we then overlay our Occam Plausibility Algorithm (OPAL) to systematically select the most appropriate model for predicting future tumor and treatment response (challenge 3). OPAL is a novel and powerful Bayesian approach for bringing together model-specific experimental data for parameter calibration, determination of output sensitivities to parameter variances, calculation of model plausibility for model selection, and development of criteria for designing validation experiments (2). We posit that this approach allows us to develop practical, tumor forecasting methods for predicting the response of individual cancer patients to therapy. We seek to accomplish this goal by integrating advanced, quantitative, multi-modality imaging data (MRI, PET, and microscopy) with multi-scale biophysical models that predict eventual response. In this presentation, we will describe our approach to integrating imaging data with model building and then present early applications of the methodology in various in vitro and in vivo studies involving brain (3,4,5) and breast cancers (6,7). Our overarching hypothesis is that patient-specific quantitative imaging data combined with estimates of therapeutic regimens will enable a multi-scale model to accurately predict responder/non-responder status after a single cycle of therapy on an individual patient basis (8). **References:** (1) E. A. B. F. Lima, J. T. Oden, and R. C. Almeida. A hybrid ten-species phase-field model of tumor growth. Mathematical Models and Methods in Applied Sciences. 2014;24:2569-2599. (2) E. A. B. F. Lima, J. T. Oden, D. A. Hormuth II, T. E. Yankeelov, R. C. Almeida, Selection, calibration, and validation of models of tumor growth, Mathematical Models and Methods in Applied Sciences 2016;26:2341–2368. (3) Hormuth DA II, Weis JA, Barnes SL, Miga MI, Rericha EC, Quaranta V, Yankeelov TE. Predicting in vivo glioma growth with the reaction diffusion equation constrained by quantitative magnetic resonance imaging data. Physical Biology. 2015;12:046006. (4) Hormuth DA II, Weis JA, Barnes SL, Miga MI, Rericha EC, Quaranta V, Yankeelov TE. A mechanically coupled reaction-diffusion model that incorporates intra-tumoural heterogeneity to predict in vivo glioma growth. J Royal Society Interface. 2017;14(128). (5) E. A. B. F. Lima, J. T. Oden, Wohlmuth B, Shahmoradi A, Hormuth DA II, Yankeelov TE, Scarabosio L, Horger T. Selection and validation of predictive models of radiation effects on tumor growth based on noninvasive imaging data. Computer Methods in Applied Mechanics and Engineering. 2017, in press. (6) McKenna MT, Weis JA, Barnes SL, Tyson DR, Miga MI, Quaranta V, Yankeelov TE. A Predictive Mathematical Modeling Approach for the Study of Doxorubicin Treatment in Triple Negative Breast Cancer. Sci Reports 2017;7:5725. (7) Weis JA, Miga MI, Arlinghaus LR, Li X, Abramson V, Chakravarthy AB, Pendyala P, Yankeelov TE. Predicting the Response of Breast Cancer to Neoadjuvant Therapy Using a Mechanically Coupled Reaction-Diffusion Model. Cancer Res. 2015;75:4697-707. (8) Yankeelov TE, Quaranta V, Evans KJ, Rericha EC. Toward a science of tumor forecasting for clinical oncology. Cancer Res. 2015;75:918-23.

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Mapping bone marrow niches of disseminated tumor cells. Weijie Zhang, Aaron Muscarella, Wei Dai, Hai Wang, Cuijuan Yu, Patrick G. Mitchell, Fabio Stossi, Michael A. Mancini, Wah Chiu, Xiang H.F. Zhang; Baylor College of Medicine, Houston, TX

We aim to overcome the challenge of eliminating microscopic metastases of breast cancer, so that distant recurrences and related deaths can be significantly reduced. We focused on bone micrometastases, which are precursors of overt bone metastases and possibly other metastases. Our previous studies demonstrate that luminal-like breast cancer cells require the microenvironment niche that exhibits osteogenesis activity. The direct adherens junction-based interaction with osteogenic cells leads to increased cell proliferation of cancer cells, via activation of the mTOR pathway. Here, we presented two further studies on the microenvironment niches of disseminated tumor cells (DTCs) and micrometastases, both of which take advantage of cutting-edge microscopy. In the first study, we uncovered a novel cellular protrusion, which primarily functions to tether DTCs to osteogenic cells and facilitate the establishment of further interactions between the two cell types. In the second study, we attempted to map DTCs relative to the endothelial network in the bone and bone marrow, by adopting technologies utilized to identify hematopoietic stem cells. Our results revealed diverse functions of various microenvironmental constituents in early-stage bone metastasis.