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 11–14, 2015, at the Hard Rock Hotel San Diego. The focus of the conference will be to educate, promote, and support the application and use of molecular imaging in cancer biology. This joint meeting will bring together imaging scientists with biologists in basic, translational, and clinical cancer research to explore how new findings in molecular imaging can improve cancer diagnostics, treatment, and patient outcomes.

Exciting developments in the field of cancer imaging have led to important insights into the origins and biology of cancer, as well as novel techniques for early diagnosis, accurate staging, and monitoring of response to therapy for cancer patients. These new discoveries have great potential as tools for basic and translational research in cancer biology and for clinical use to improve patient outcomes.

The conference will feature several concurrent educational sessions on Wednesday, including "Imaging Genomics and Big Data," "Interrogation of Innovative Therapeutic Strategies," and "Challenges and Innovations of Mouse Models of Cancer and Novel Molecular Imaging Ligands." Wednesday evening will include the welcome reception and a special keynote address on targeting PI3K for cancer therapy by Lewis C. Cantley of the Sandra and Edward Meyer Cancer Center at Weill Cornell Medical College in New York. Thursday and Friday will feature 8 plenary sessions focused on immunotherapy and imaging, theranostics and companion diagnostics, image-guided therapy, cancer imaging and early diagnostics, and more.

New this year will be a Distinguished Lecture at 10:00 AM on Thursday, February 12, 2015. Roger Y. Tsien of the University of California, San Diego, and recipient of the 2008 Nobel Prize in Chemistry will be presenting "Building Molecules to Image and Treat Cancer."

This conference will also offer opportunities for participation from junior scientists, with presentations selected from the most highly rated abstracts. In addition to the didactic lectures, the meeting will include poster presentations. The abstracts in this supplement are listed alphabetically by the last name of the presenting author, with the main abstracts followed by the invited speaker abstracts. Please note that the presenting author at the conference is not always the first author of the abstract. Abstracts selected to receive Young Investigator Travel Awards (AACR-Aflac, Incorporated, Scholar-in-Training Awards) are indicated by a footnote.

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

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Abstract Reviewers

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MAIN ABSTRACTS

1

Differential radiosensitivity of transgenic breast cancer tumors is associated with immune cell infiltration. T.A. Aguilera, M. Rafat, E.E. Graves, A.J. Giaccia; Stanford University, Stanford, CA

Understanding factors that influence radiosensitivity of tumors is important in guiding combination therapies that may enhance the efficacy of cancer treatment. Given the increasing indication for stereotactic radiotherapy with high dose radiation in one or a few fractions, therapeutic combinations may enhance the biologic effect and could improve current cancer treatment approaches. We sought to develop a model to study the genetic, microenvironmental, and immunologic factors of radioresistance after stereotactic radiation. We employed syngeneic orthotopic implantation of PyMT mammary tumors to evaluate radiosensitivity of different clones from the same parental transgenic strain. We irradiated tumors in a single fraction at 12 or 20 Gy and measured tumor size 10 days after treatment. We found that Py8119, a rapidly growing tumor with epithelial to mesenchymal transition characteristics, is radioresistant, with average tumor volumes increasing by 3 and 4-fold of their initial size after 12 and 20 Gy of radiation. Alternatively, similarly rapidly growing epithelial Py117 tumors decreased by 3- and 4-fold after irradiation, indicating radiosensitivity. Untreated mice of each tumor clone had similar growth curves. To evaluate the inherent radiosensitivity of the two tumors in culture, clonogenic survival was performed and revealed no differences between the two tumor cell lines. Tumors were then dissociated and analyzed with flow cytometry to evaluate cellular inflammatory factors of the microenvironment. We found that the total % of CD45+ leukocytes in the radioresistant Py8119 tumors was 4.1% compared to 19.7% in the Py117 radiosensitive untreated tumors. The relative differences were unchanged after radiation, but the Py117 and not the Py8119 tumors had a decreased percentage of infiltrating macrophages (CD11b+ F4/80+) and myeloid derived suppressor cells (MDSCs; CD11b+ Gr1+), which may allow for greater T-cell effector function. These data suggest that radiation of Py117 tumors may be sufficient to stimulate an immune-mediated antitumor response that can deplete and then overcome the immunosuppressive microenvironment mediated by macrophages and MDSCs. This is an excellent model to study the factors that impact the antitumor immune response after radiation. This work could have implications in radiotherapy and immunotherapy combination treatments.

2

Enhanced diagnostic performance of FDG PET/CT using delayed time point imaging and partial volume effect correction of quantitative PET parameters: a lesion-based analysis of suspected lung lesions. A. Salavati, S. Houshmand, B. Khiewvan, G. Cheng, S.R. Akers, T.J. Werner, A. Alavi; Department of Radiology, Hospital of the University of Pennsylvania, Philadelphia, PA

Purpose: Several studies have shown the enhanced diagnostic performance of delayed/multiple time point FDG-PET/CT imaging in differentiating benign from malignant lesions. However, visual scoring or maximum standardized uptake value (SUVmax) were used for this purpose. Additionally, the role of "partial volume effect" (PVE) in the accurate quantification of PET/CT parameters, although heavily emphasized in phantoms and simulations, has been investigated by only a limited number of clinical studies. The aim of this prospective study was to assess the potential value of other FDG-PET/CT quantitative parameters including SUVmean, partial volume corrected SUVmean (pvcSUVmean), metabolic tumor volume (MTV), total lesion glycolysis (TLG=SUVmean*MTV), and pvcTLG (pvcSUVmean*MTV) in delayed/multiple time point FDG-PET/CT for detection of lung cancer and assessment of the potential added value of PVE in this regard. **Method and Materials:** One hundred suspected lung cancer lesions (60 benign and 40 malignant) with histopathologically proven diagnosis were included in this research. Patients underwent FDG-PET/CT at 1, 2, and 3 hours after IV administration of FDG. SUVmax, SUVmean, pvcSUVmean, TLG, pvcTLG and SUVpeak were calculated for each time point. Retention index (RI) of parameters [(P3-P1)/P1] was calculated. The diagnostic performance of all parameters and RIs was compared by pairwise comparison of receiver operating characteristic (ROC) curves. A combined adaptive contrast-oriented thresholding segmentation and PVE correction algorithm was used to measure same parameters at the first time point. A subgroup analysis for lesions with metabolic volume less than the median (MTV=6.6) was also performed. **Results:** Based on these measurements, malignant lesions showed moderate increase from 1st to 2nd hour and from 1st to 3rd hour. However, tumor-negative lesions showed mixed pattern of changes between early and delayed time point imaging but the overall average changes were less than those of malignant lesions for all parameters with statistical significance. The area under the curve (AUC) of ROC curves of all parameters increased at 2nd and 3rd hours of imaging; however, only some parameters at 3rd hour showed statistically significant improvement in comparison with 1st hour while AUCs were 0.91, 0.90, and 0.86 for 3rd hour SUVmean, SUVpeak, and pvcTLG versus 0.85, 0.83, and 0.81 for corresponding 1st hour parameters. In subgroup analysis considering only lymph nodes, 3rd hour pvcSUVmean and SUVmax had the highest diagnostic performance compared to other PET/CT parameters but the differences were not statistically significant. RIs did not provide any significant added value over 3rd hour parameters. FDG uptake parameters showed superior diagnostic performance over volumetric parameters while AUCs were 0.90, 0.87, 0.86, 0.84, 0.82, 0.75, and 0.55 for pvcSUVmean, SUVmax, SUVmean, SUVpeak, pvcTLG, TLG, and MTV, respectively. In pairwise comparison of ROC curves, pvcSUVmean showed statistically significant better diagnostic performance over SUVmean (p-value=0.04), SUVpeak (p-value=0.02) but not over SUVmax (p-value=0.16). Similarly, PVC enhanced the diagnostic performance of TLG. In subgroup analysis of small lesions, pvcSUVmean had the highest diagnostic performance compared to all other PET/CT parameters with statistical significance. **Conclusion:** Based on this study, delayed time point imaging to some extent improved the diagnostic performance of PET/CT in differentiating suspected lung lesions while retention indices did not increase the diagnostic accuracy. Additionally, PVE correction improved the diagnostic performance of PET/CT in differentiating the suspected lung lesions, particularly for small lesions.

3

Biological volume variation due to chemoradiotherapy and modeling. E. Hareram[1], M. Alnaaimi[1], M. Alduaij[1], M. Alkhorayef[2]; [1]Department of Nuclear Medicine, Kuwait Cancer Control Centre, Kuwait, [2]Department of Radiological Sciences, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia

Efforts have been made to study biological tumor volume using positron emission tomography (PET) scans. Thirty patients underwent F-18 FDG PET for nasopharynx, rectum and lung cancers, performed at the beginning, middle, and end of treatment, and also 6-8 weeks after radiotherapy. The F-18 FDG PET specificity for rectum cancer was 70%, while the sensitivity was 90%. The tumor size and N category both had a significant effect on the dose response. The average sensitivity and specificity for SCLC in lung cancer were 83% and 91%, respectively, whereas for CT scan they were 64% and 74%, respectively. Accurate delineation of PET-based GTV is limited due to the poor spatial resolution of PET scanners, which is approximately 4.5 mm in the latest generation of PET/CT scanners. As a result of this, PET can suffer from limited lesion detectability. PET can detect positive lesions, if their size is larger than approximately 1 cm and the tracer uptake in the lesion is at least 4 times the uptake of the surrounding background. Smaller lesions may only be detected if there is a substantially more intense uptake. Fortunately, many aggressive tumors, including most lung cancers, have a very high F-18 FDG uptake and lesions of 5 mm can often be detected. The current model used in radiobiology is a linear quadratic model which describes tumor response by using the factors alpha and beta. Chemotherapy modeling can also be incorporated into the radiobiological model. Fractionated cell survival using the linear quadratic model, the BED equation with repopulation, TCP/NTCP and EUD models can be used to assess the response. Since chemotherapy is delivered with different types of drugs, different formalism can be applied. Chemotherapy acts on proliferative cells, hence inducing high early toxicity, with late effects due to the inhibition of DNA repair. Cancer cells are cell-cycle specific and consequently require different formalisms for uracil and halide reactions, to inhibit enzymatic machinery and cause direct damage to DNA. The models given can be incorporated without considering personalized application, due to the variation of sensitivity from one person to another,

and thus more realistic models can be deduced along with radiotherapy. Delineated volume differences can be characterized by normalizing other volumes from timely spaced scans. Biological volume differences can be predicted by modeling at the start of treatment and expected volumes can be calculated from scans. IMRT planning was carried out on reduced volumes to observe the differences in planning. Results were analyzed and the biological correlation was studied with respect to cell biology and correlated with radiobiology.

4

Real-time fluorescence-guided surgery of prostate cancer xenografts with anti-prostate stem cell antigen diabody. A.S. Behesnilian[1], Z.K. Jiang[2], G. Sonn[1], K.A. Zettlitz[2], A.M. Wu[2], R.E. Reiter[1]; [1]UCLA Department of Urology, Los Angeles, CA, [2]Molecular & Medical Pharmacology, UCLA, Los Angeles, CA

Purpose: The ability to visualize cancer in real time during surgery has the potential to improve surgical outcomes. This is especially true in prostatectomy, where incomplete extirpation of disease leads to cancer recurrence, while aggressive resection leads to serious sexual and urinary side effects. We have previously described the development of a fluorescently labeled antibody fragment against prostate stem cell antigen (PSCA) for the optical imaging of prostate cancer. The goal of this study is to validate the use of our real-time fluorescence-guided surgery platform in mouse models. **Methods:** 22Rv1-PSCA+ human prostate cancer cells were injected into the right thigh muscles of 18 nu/nu male mice and monitored until tumor size reached 5-10mm in diameter. Anti-PSCA antibody fragments (cys-diabody) were labeled with far-red fluorescent dye (Cy5) as previously described and administered 6 hours prior to surgery. Mice were euthanized and underwent a first stage traditional surgery to remove the visible tumor, with attempts to keep close surgical margins to emulate prostatectomy. Following this, mice were randomized to either receive no further surgery (traditional only) or to undergo fluorescence-guided surgery to remove any residual cancer. Fluorescent images were taken at all stages but were not made available to the surgeon, and the surgeon was blinded to final groupings. All resected tissues were submitted for H&E staining and the remaining thigh musculature harvested to determine final margin status by human DNA PCR. **Results:** The prostate cancer was invasive into the thigh musculature, making resection challenging. In the traditional group, one mouse did not have any tumor. Residual fluorescence was seen in 8/8 and 9/9 mice in the traditional and fluorescent groups, respectively. Following fluorescent surgery, all 9 mice were free of fluorescence. Residual tumor <1mm was able to be identified by fluorescence and removed. H&E staining of tissue removed during fluorescence-guided surgery confirmed the presence of prostate cancer, which was missed on the traditional surgery. Human DNA PCR is pending to confirm final margin status. **Conclusion:** Fluorescence-guided surgery using our platform is a viable tool which allowed for complete removal of residual prostate cancer from murine thigh muscle that was otherwise missed on traditional surgery.

5

A new PET amino acid tracer for oncologic imaging: synthesis and biological evaluation in a mouse model of glioblastoma. A. Bouhlel[1], D. Zhou[1], A. Li[1], L. Yuan[2], K. Rich[2], J. McConathy[1]; [1]Washington University in St. Louis, Mallinckrodt Institute of Radiology, St. Louis, MO, [2]Department of Neurosurgery, Washington University in St. Louis, St. Louis, MO

Purpose: Currently used amino acid-based PET tracers for imaging brain tumors are primarily substrates of system L amino acid transporters such as [^{18}F]FDOPA, [^{18}F]FET, and [^{11}C]methionine. Because system L is active at the luminal side of the blood brain barrier (BBB), substrates of system L are able to reach the entire tumor volume of brain tumors even if the BBB is not disrupted. However, system L is not concentrative, and novel tracers are needed that cross the BBB but can provide good tumor uptake as well as higher tumor to brain ratios compared to pure system L substrates. The objective of this project is to develop ^{18}F-labeled amino acid with the optimal balance of transport by system L in order to reach the non-enhancing gliomas and other neutral amino acid systems in order to promote tumor uptake. Thus, the new tracer FAMPe was developed, and this tracer is a longer alky chain analogue of the previously reported amino acids MeFAMP, FAMP, and FAMB. **Materials and Methods:** Through simple and efficient organic and radiosynthetic routes, the two enantiomers of the target compound, (*S*) and (*R*)-2-amino-5-[^{18}F]fluoro-2-methylpentanoic acid ((*S*)- and (*R*)-[^{18}F]FAMPe) were developed. The radiolabeling was successfully performed with the ^{18}F-incorporation conducted in *t*-amylalcohol in 10min, and the tracers were provided in a form suitable for animal studies via a Dionex OnGuard II A cartridge treatment. Both enantiomers were obtained in a moderate radiochemical yield (24-58%) (n = 8 runs) and over 99% radiochemical purity. Biodistribution and small animal PET/CT studies were conducted in male BALB/c mice with DBT gliomas. Amino acid uptake assays were carried out in mouse DBT glioma cells to evaluate the transport mechanism of (*S*)- and (*R*)-[^{18}F]FAMPe. **Results:** The biodistribution studies demonstrated high tumor to brain ratios for both enantiomers. However, (*S*)-[^{18}F]FAMPe showed higher and increasing tumor and brain uptake over time compared to (*R*)-[^{18}F]FAMPe. The small animal PET/CT studies revealed good imaging properties for both enantiomers. The tumor to brain ratios calculated from these data showed that the two new tracers were more similar to (*S*)-[^{18}F]FET, a selective system L substrate, than to (*R*)-[^{18}F]MeFAMP, a selective system A substrate. Indeed, the cell uptake assays confirmed that (*S*)- and (*R*)-[^{18}F]FAMPe enter DBT cells in part through the system L amino acid transporter. The in vitro assays showed also that the uptake of (*S*)-[^{18}F]FAMPe was sensitive to glutamine inhibition and to a combination of neutral amino acids such as alanine, serine and cysteine. These results suggest that (*S*)-[^{18}F]FAMPe may use amino acid transporters that mediate the uptake of glutamine such as system ASC. **Conclusion:** Two enantiomers of a new PET tracer for oncology were developed through efficient organic and radio syntheses. Both enantiomers (*S*)- and (*R*)-[^{18}F]FAMP, obtained in good yields and in a suitable form for animal studies, showed very good imaging properties through the small animal PET/CT. However, (*S*)-[^{18}F]FAMP is a better imaging agent due to its high and increasing tumor and brain uptake compared to (*R*)-[^{18}F]FAMP. (*S*)-[^{18}F]FAMP was also revealed to be a good substrate for system L amino acid transporter but also for other kinds of neutral amino acid transporter and particularly glutamine amino acid transporter such as system ASC. These promising results encouraged us to develop two others tracers, (*S*)-[^{18}F]FAMH and (*S*)-[^{18}F]FAMHep, which are in progress. (*S*)-[^{18}F]FAMH and (*S*)-[^{18}F]FAMHep may show better balance of brain availability and tumor uptake than the amino acids that are selective for system L. Additional studies are ongoing to define better the mechanism of transport of these tracers.

6

Characterization of magnetotactic bacteria as MRI cell labeling and tracking agents. K.D. Brewer[1], A. Chan[2], J.A. Rioux[1], M. Rafat[1], S. Machtaler[1], P. Chakraborty[2], J. Barozzo[2], A. Wakeel[2], J.K. Willmann[1], E. Graves[1], B. Rice[2], C. Bell[2], B. Rutt[1]; [1]Stanford University, Stanford, CA, [2]Bell Biosystems, San Francisco, CA

Superparamagnetic iron oxide (SPIO) contrast agents are widely used to label cells for tracking by MRI. However, these particles are significantly disadvantaged in longitudinal imaging because, as cells divide, the amount of iron per cell decreases and eventually the labeled cells can no longer be detected. MR reporter genes exist that potentially increase iron uptake, but these have met with limited success. Recently, Bell Biosystems Inc. (San Francisco, CA) has developed a bacterial-derived magnetic pseudo-organelle known as the "Magnelle." This construct derives from magnetotactic bacteria, building on previous work that explored bacteria as a potential MR contrast agent. Since magnetotactic bacteria naturally generate magnetosomes (chains of magnetite crystals encapsulated in lipid bilayer) through the coordination of over 100 genes, they may have advantages as MR contrast agents. Since Magnelles were derived from bacteria, they have the intrinsic ability to self-replicate, making them interesting candidates for labeling and longitudinal evaluation of cells. This is crucial for many emerging applications, particularly the evaluation of cell-based therapies. This work presents initial characterization of the MRI relaxivity properties (r_1, r_2) of Magnelles, their cell loading efficiency and cell relaxivity characteristics, as well as in vivo imaging characteristics using a model breast cancer cell line. MR relaxation properties of Magnelles were measured and compared to Molday ION Rhodamine B SPIO particles (BioPAL, Worcester, MA). For in vivo imaging, MDA-MB-231 breast

cancer cells were labeled with Magnelles overnight using manufacturer instructions, then suspended in PBS. Between 10^2 and 10^4 Magnelle-labeled cells were injected intracranially into the left hemisphere of the brain of Nu/Nu mice. An equivalent number of unlabeled cells were also injected intracranially in the right hemisphere as a negative control. MR relaxivity characterization was done at 0.5T, 3T and 7T (using Bruker minispec mq20, GE MR750 and MR950 imagers, respectively) and the in vivo imaging was done at 7T (GE MR901). Average iron content was measured to be 2-3fg/Magnelle. Cellular uptake for 231 cells was between 0.7-1.6pg iron/cell for Magnelles and 2-3pg iron/cell for Molday. Longitudinal relaxivity (r_1) values show that although Magnelles exhibit typical decreasing r_1 with increasing field strength, the absolute r_1 values and their relative field-dependent change are far smaller (10× smaller at 3T, ~2.5× smaller at 7T) than for conventional iron oxide nanoparticle agents such as Molday. The decreased r_1 for Magnelles compared to Molday at all field strengths is probably due to the encapsulation of the magnetite particles, either inside of the magnetosome, or inside the Magnelle itself. Transverse relaxivity (r_2) values for Magnelles were on the same order of magnitude as Molday. Sample r_2 values at 7T and room temperature were ~100$mM^{-1}s^{-1}$ for Magnelles and ~135$mM^{-1}s^{-1}$ for Molday. For 231 cells, the r_2 of Magnelle-labeled cells was ~40$mM^{-1}s^{-1}$ and ~70$mM^{-1}s^{-1}$ for Molday-labeled cells, indicating an approximate decrease in r_2 of just over 50% following cell loading, for both forms of cell labeling agent. Magnelle-labeled cells exhibited very strong MR contrast in vivo allowing detection down to 100 labeled cells. These bacterial-derived pseudo-organelles, "Magnelles," have potential for use as novel self-replicating magnetite-based MR contrast agents. They have r_2 relaxivity values comparable to traditional iron-oxide nanoparticle contrast agents, and demonstrate strong MR contrast when loaded into cells and implanted in tissue. Further exploration and characterization of the relaxivity properties of Magnelles, their magnetosome components, and their behavior in cells could help direct the optimization and application of this novel class of MRI cell-labeling probe.

7

Computational multiplexing imaging: a network approach. E. Capobianco; University of Miami, Miami, FL

Originated in experimental contexts conceived to yield evidences, and then translated into computational domain in which the measurements are to be modeled, computational multiplexing imaging (COMI) is a suggested research direction with two main goals. The first is the integration of the observational power available from imaging techniques with various experimental evidences. The second is an expansion of the system's prediction power by combining information in a unifying computational frame. In particular, the integration by networks implies that the information flowing across connectivity patterns can allow quantification of relationships between many observed variables (related to cell morphology, morphodynamic pattern analysis, etc.) recapitulating molecular activities. The integration of the corresponding data multitude requires that network inference strategies are designed, both correlative and causative, inherently modular and robust. COMI's impact is expected to emerge in diagnostics, prognostics, and therapeutics, embracing also theranostics.

8

Wavelet based feature approach for radiomic texture extraction from glioblastoma phenotypes. A. Chaddad, Jr.[1], P. Zinn[2], R. Colen[1]; [1]The University of Texas MD Anderson Cancer Center, Houston, TX, [2]Baylor College of Medicine, Houston, TX

Purpose: The texture feature extraction based on discrete wavelet transform (DWT) filter is being proposed to estimate the glioblastoma heterogeneity from magnetic resonance (MR) imaging. A novel approach is developed based on radiomics texture feature and applied on the three main volumetric phenotypes of GBM, namely, necrosis, active tumor and edema/invasion. This approach analyzes the texture phenotypes and then these texture features are used for predicting GBM phenotypes based on its different heterogeneity. **Methods and Materials:** T1-WI and FLAIR MRI images from 22 GBM patients were analyzed. Images were registered by using T1-WI and its corresponding FLAIR sequence, and GBM phenotypes were segmented from registered image by using 3D Slicer. 246 texture features were extracted from each of the GBM phenotypes by DWT filter. These features were framed by the wavelet approximations as "low-frequency" and "high-frequency" details of GBM phenotypes. 69 descriptors were found significant with p-value<0.05 to discriminate between GBM phenotypes. Decision tree classifier model was employed on three sample types, namely, necrosis, active tumor and edema. To assess the performance of our classifier using leave-one-out cross-validation, to discriminate between phenotypes, accuracy, sensitivity, specificity and negative predictive value (NPV) were computed. **Result:** DWT based features showed acceptable performance classifier, with accuracy of 76.74%, sensitivity of 70.93%, specificity of 82.56% and NPV of 85.03%. Predictive GBM phenotypes showed high classifier accuracy of 70.93%, 81.39% and 77.90% for active tumor, edema and necrosis, respectively. DWT based features demonstrated high feasibility to enhance the precocious GBM phenotypes and automate the phenotype discrimination. **Conclusion:** This work provides preliminary evidence that MRI-derived DWT texture features are predictive of GBM phenotype. Higher accuracy classifier proved that the DWT feature extraction can be a promising technique to analyze GBM heterogeneity.

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An integrin αvβ3 binding positron emission tomography tracer, ^{18}F-RGD-K5 uptake as a potential prognostic factor in patients with metastatic breast cancer. H.J. Cho[1], J.D. Lee[2], J.Y. Park[2], M. Yun[2], W.J. Kang[2], C.H. Kim[1], J.C. Walsh[3], H. Kolb[3]; [1]Brain Korea 21 PLUS Project for Medical Science, Yonsei University, Seoul, Korea, [2]Department of Nuclear Medicine, Yonsei University College of Medicine, Seoul, Korea, [3]Biomarker Research, Siemens Molecular Imaging Inc., Culver City, CA

Objectives: ^{18}F-RGD-K5 is an αvβ3 integrin binding agent for positron emission tomography (PET). We evaluated whether ^{18}F-RGD-K5 uptake predicts progression-free survival (PFS) and overall survival (OS) in patients with metastatic breast cancer. **Methods:** Eleven patients with metastatic breast cancer were enrolled. Both ^{18}F-RGD-K5 and ^{18}F-FDG PET scans were performed in each patient within 7 days. The maximum standardized uptake value (SUVmax) was measured in each scan. In patients with multiple metastases, only the lesion with the highest SUVmax was included. All patients received chemotherapy alone or combined with hormone therapy and/or immunotherapy. Univariate and multivariate Cox proportional hazards regression was performed to evaluate the association between factors of interest and PFS or OS. **Results:** The mean (± standard deviation (SD)) follow-up time was 26.2 ± 19.1 months (median, 25.5 months). The median PFS was 10.1 months (95% CI, 3.0 to 26.3 months). The mean (± SD) SUVmax on ^{18}F-RGD-K5 PET was 3.94 ± 1.70 (median, 4.03) and the mean (± SD) SUVmax on ^{18}F-FDG PET was 9.21 ± 4.35 (median, 9.70). In univariate analysis, hormone receptor status and SUVmax on ^{18}F-RGD-K5 PET predicted OS. Stepwise Cox regression analysis revealed the SUVmax on ^{18}F-RGD-K5 PET was the strongest predictor. Patients with SUVmax equal to 4 or less on ^{18}F-RGD-K5 PET had a worse prognosis than those with SUVmax greater than 4 (median PFS, 3.4 vs 10.9 months; log-rank, P=.022; median OS, 8.5 vs 45.1 months; log-rank, P=.010). The SUVmax on ^{18}F-RGD-K5 PET was a strong and independent prognostic factor. **Conclusion:** The SUVmax on ^{18}F-RGD-K5 PET is predictive of OS in patients with metastatic breast cancer, though the number of patients was too small to draw a clear conclusion. αvβ3 integrins have important roles in tumor cell migration, invasion, proliferation and survival, and tumor angiogenesis, and their role as either pro- or antitumor regulators is determined by tumor microenvironment. ^{18}F-RGD-K5 uptake may reflect the complicated nature of αvβ3 integrins. Further study in larger populations is required to refine the clinical significance of ^{18}F-RGD-K5 uptake as a prognostic indicator.

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Evaluation of mesenchymal stem cell mediated enzyme/prodrug therapy for radio-resistant glioblastoma cells using BLI/MRI/PET imaging systems. T. Chung, H. Youn, J. Na, Y.I. Kim, H. Kim, K.W. Kang, D.S. Lee, J.-K. Chung; Seoul National University, Seoul, Korea

Background: Molecular imaging methods are powerful tools for an evaluation of therapeutic efficacy of new therapeutic methods. Despite

advances in radiation and chemotherapy after tumor surgery, inherent chemo/radio-resistance of glioblastoma reduces survival of patients, and required development of new therapeutic methods. Mesenchymal stem cell (MSC) mediated enzyme/prodrug therapy can be an option. Combining tumor targeting property of MSCs and non-toxic prodrug therapy which is converted only in the tumor lesion can be suggested to reduce side effect of cytotoxic drugs. In this study, we evaluated the efficacy of combination therapy with MSCs expressing cytosine deaminase (CD) and prodrug 5-FC in mouse glioma model by various molecular imaging modalities. **Methods:** Human glioma cell lines (U373, U87MG) and primary glioblastoma cells from a patient (GBM28) were stably transfected with luciferase reporter genes for tumor cell imaging. Glioma cells were treated with ionizing radiation and 5-FU dose dependently. Microarray and real-time PCR were performed to evaluate the expression levels of radio-resistance and 5-FU metabolism related genes. Therapeutic MSC/CD cells were generated by transfection of CD genes into human bone marrow derived MSCs. *In vitro* and *in vivo* 19F-magnetic resonance spectroscopy (19F-MRS) was performed to validate the conversion of 5-FC to 5-FU by the ability of MSC/CDs. To estimate the *in vitro* bystander effect of MSC/CDs with 5-FC, luciferase expressing glioma cells were co-cultured with MSC/CDs. For monitoring therapeutic effect of MSC/CDs with 5-FC *in vivo*, U87MG/Luc cells were transplanted into the mouse cranium. Four days later, therapeutic MSC/CD cells were transplanted into the mouse cranium. Tumor growth was measured with positron emission tomography (PET), MRI and bioluminescence imaging (BLI). **Results:** U87MG/Luc cells showed 2.5 times more radio-resistance than other glioma cells (U373/Luc, GBM28/Luc). In contrast, U87MG/Luc cells were evaluated 4 times more sensitive to 5-FU than U373/Luc cells. Microarray analysis demonstrated that U87MG cells highly express DNA repair related genes compared to U373 cells. 19F-MRS of MSC/CDs demonstrated effective conversion of 5-FC to 5-FU *in vitro* and *in vivo*. *In vitro* bystander effect of MSC/CD therapy with 5-FC in U87MG cells was 2 times more effective than that in U373 cells. In glioma orthopotic model, we successfully monitored tumor growth by PET/MR/BLI. We also observed 70% of inhibition of tumor growth by MSC/CD with 5-FC therapy. No tumorigenesis or pathological abnormalities were observed in MSC/CD transplantation site. **Conclusions:** We could successfully evaluate the efficacy of a new stem cell mediated enzyme/prodrug therapy by various imaging modalities. Furthermore, we revealed the reason for the different response of therapeutic efficacy on glioma cells through microarray analysis. Therefore, we suggest an effective anticancer therapy using MSC/CD with 5-FC prodrug for radio-resistant and 5-FU sensitive glioma treatment.

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Glycolysis promotes tumor growth by signaling proliferation and survival pathways. I.J. Cohen[1], I. Serganova[2], N. Shah[2], D. Sardar[2], E. Moroz[2], M. Shindo[2], R. Blasberg[2]; [1]GSK Graduate School, New York, NY, [2]Memorial Sloan Kettering Cancer Center, New York, NY

Background: Metabolism is now recognized as a hallmark of cancer. In order to keep up with expansion of the tumor biomass, cancer cells increase their glycolytic metabolism, even in the presence of oxygen (aerobic glycolysis—"Warburg effect"). Glycolysis is used for the production of biomaterials required for the synthesis of nucleotides, lipids and amino acids that are necessary for rapid cell proliferation, as well as for ATP production. It was recently shown that oncogenic pathways not only promote cancer cell proliferation, but also induce a metabolic reprogramming that favors glycolysis. In fact, the PI3K/Akt/mTOR pathway has been shown to upregulate glycolytic genes (GLUT1, HK, LDHA) in order to promote higher rates of glycolysis during rapid tumor cell proliferation, even in the presence of adequate oxygen (1). Disruption of glycolysis leads to drastic changes in cancer cell behavior. LDHA depletion (2) or FBP1 overexpression (3) (both of which result in decreased glycolytic rates) have been shown to change the phenotype of cancer cells and tumors, by reducing cell proliferation and the development of metastases (less aggressive phenotype). While this might be due to the lack of energy and nutrients to fuel tumor growth, it is possible that glycolysis may play a more active role in regulating cancer cell proliferation. In fact, it was recently shown that the addition of excess glucose to the extracellular medium leads to an increase in Akt pathway activity and an increase in the cancer stem cell (CSC) population (4). It was also shown that GAPDH can act as a sensor of glycolytic flux to promote mTOR activation under high rates of glycolysis by regulating RheB (5). These findings suggest a role for glycolysis in the signaling pathways that promote oncogenesis and cancer cell stemness, proliferation, survival and metastases, all of which are associated with aggressive disease. **Experimental Design:** This study uses both murine (4T1) and human (MDA-MB-231) cancer cell lines to study the effects of glycolysis on proliferation/survival and stemness pathways. 4T1 cells carry two retroviral reporter constructs: a constitutively expressed Firefly luciferase/RFP (FLuc-RFP) to monitor tumor growth and metastases, and a hypoxia-inducible exGaussia luciferase-GFP (HRE-exGLuc-GFP) to monitor HIF1 activity in tumors over time. LDHA knockdown or inhibition of glycolysis via 2-deoxyglucose (2DG) was used to alter glycolytic flux. The effect of glycolytic inhibition on proliferation/survival pathways was assessed by Western blotting (phospho-Akt, MAPK, cleaved Caspase-3, b-Catenin). The effect on tumor stem cell population was assessed by Side Population and Tumorsphere assays and by CD44/CD24 FACS staining. **Results:** LDHA depletion leads to an apparent decrease in 4T1 CSC number, as measured by the Tumorsphere assay. A Side Population assay is being developed to quantify the percentage of cancer stem cells within a population and confirm these results. While bone marrow-derived cells show the expected ~1-3% of hematopoietic stem cell population (which can be abolished by verapamil treatment), 4T1 cells show no such CSC population in current culture conditions. The CD44/CD24 assay is also being developed to study CSCs. These assays will be used to assess the effect of increased (glucose addition) or decreased (2DG addition) glycolytic rates in the CSC population. Furthermore, the HRE-exGLuc-GFP reporter gene in 4T1 cells is being used to assess the effects of glycolytic rates on HIF1a signaling in-vitro and in-vivo. Further, the constitutive FLuc-RFP reporter gene will be used to isolate cancer cells from tumors via FACS sorting of RFP+ cells to perform gene expression analysis of control or LDHA knockdown cells, as well as cells under different treatment conditions. **Conclusions:** Thus far, LDHA depletion has been shown to induce a less aggressive cell and tumor phenotype, as well as a decrease in the number of cancer stem cells. Our results, coupled with previous reports showing significant effects on tumor phenotype following the reduction of glycolysis, suggest that high rates of glycolysis are required for rapid growth and expansion of more aggressive tumors. Although this could be in part due to a lack of nutrients and oxygen in the tumor microenvironment, we propose that glycolytic enzymes, glycolytic intermediates and products of glycolysis are important for tumor growth because they directly regulate and signal to oncogenic pathways, in addition to their established function in glycolysis itself. **References:** 1. Ward, P.S., and C.B. Thompson, Metabolic reprogramming: a cancer hallmark even Warburg did not anticipate. Cancer Cell, 2012. 21(3): p. 297-308. 2. Rizwan, A., et al., Relationships between LDH-A, lactate, and metastases in 4T1 breast tumors. Clin Cancer Res, 2013. 19(18): p. 5158-69. 3. Dong, C., et al., Loss of FBP1 by Snail-mediated repression provides metabolic advantages in basal-like breast cancer. Cancer Cell, 2013. 23(3): p. 316-31. 4. Liu, P.P., et al., Metabolic regulation of cancer cell side population by glucose through activation of the Akt pathway. Cell Death Differ, 2014. 21(1): p. 124-135. 5. 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Gold nanoparticles enable improved sensitivity and specificity for X-ray detection of microcalcifications in radiographically dense mammary tissues. L. Cole[1], T. Vargo-Gogola[2], R. Roeder[1]; [1]University of Notre Dame, Notre Dame, IN, [2]Indiana University School of Medicine—South Bend, South Bend, IN

Introduction: Microcalcifications are the most common tissue abnormality detected by mammographic screening for breast cancer. However, sensitivity and specificity for the detection of microcalcifications and diagnosis of breast cancer by mammography are dramatically decreased in women with dense breast tissues. Furthermore, increased breast density is correlated with a four-fold elevated risk of developing breast carcinoma. Improvements are needed for mammographic screening, and in particular for detecting microcalcifications within dense tissue. Therefore, bisphosphonate-functionalized gold nanoparticles (BP-Au NPs) were investigated for

targeting and contrast-enhanced detection of microcalcifications within dense mammary tissues. **Methods:** A transgenic mouse tumor model, MMTV-PyMT (PyMT) was adopted to recapitulate the radiographic density of human breast tissue as mammary glands (MGs) of PyMT mice exhibited significantly greater X-ray attenuation compared to MGs of strain and age-matched wild type (WT) mice. Microcalcifications were created within the MGs of each mouse type by injecting two concentrations of hydroxyapatite (HA) crystals (3.0 and 5.0 mg/mL) into the fat pad of the left number 4 MG (n = 6/HA concentration). Matrigel alone was injected into the right number 4 MG as a contralateral control. Twenty-four hours after creating the microcalcifications, a 100 μL solution of 50 mM BP-Au NPs was injected into both the left and right MGs for a total dose of 2 mg/mouse. Mice were then imaged with computed tomography (CT) prior to BP-Au NP delivery (0 h) and 3, 6, 24, 48 h post-delivery of BP-Au NPs. **Results:** Microcalcifications with 3.0 mg/mL HA were undetectable in PyMT mice prior to delivery of BP-Au NPs. After the delivery of BP-Au NPs, the X-ray attenuation of 3.0 mg/mL HA microcalcifications was significantly increased by 3 h and up to 48 h after delivery compared to before delivery and compared to the Matrigel controls. Thus, BP-Au NPs improved the sensitivity for detection of microcalcifications that were otherwise below the CT detection limit. Microcalcifications with 5.0 mg/mL HA were detectable in PyMT MGs prior to delivery of BP-Au NPs. After delivery of BP-Au NPs, the X-ray attenuation was increased compared to before delivery and to the Matrigel controls. Thus, BP-Au NPs improved the specificity for detecting suspicious abnormalities within radiographically dense mammary tissues. **Conclusions:** The results of this study suggest that BP-Au NPs could provide a more sensitive and specific diagnostic tool for the detection of microcalcifications during mammographic screening for breast cancer in radiographically dense mammary tissues.

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Imaging genomic mapping reveals gender-specific oncogenic determinants of cell death in glioblastoma. R.R. Colen[1], J. Wang[1], D.A. Gutman[2], S. Singh[1], P. Zinn[3]; [1]The University of Texas MD Anderson Cancer Center, Houston, TX, [2]Emory University, Atlanta, GA, [3]Baylor College of Medicine, Houston, TX

Background: Imaging genomics, a newly emerging field, links genomic information with imaging phenotypes. Cell death in glioblastoma is not completely understood; mechanisms such as rapid cellular proliferation-induced necrosis and apoptosis have been proposed. Here, we identify gene and microRNA profiles of cell death as defined by MRI volumetrics and uncover distinct and gender-specific molecular profiles potentially driving oncogenesis and cell death in glioblastoma. **Methods:** Using data obtained from The Cancer Genome Atlas (TCGA) and The Cancer Imaging Archive (TCIA), gene expression, microRNA, and quantitative MR-imaging datasets were generated based on a total of 99 patients. A histopathology dataset was used to recapitulate MRI data. Ingenuity pathway analysis and MirWalk were used for gene ontology and cognate RNA-RNA interaction analysis. **Results:** Female patients demonstrated significantly lower volumes of cell death on MRI compared to males (P=0.03). Gender-specific analysis revealed that female patients with high volumes of cell death on imaging had significantly shorter survival times (P=0.01). This difference was not observed in males (P=0.6). Genomic transcription factor analysis suggested that cell death in female GBM patients is driven by oncogenic MYC, while cell death in male GBM patients is rather induced by TP53 activity. **Conclusions:** To our knowledge, this is the first report in the literature to suggest that GBM cell death may be driven by gender-specific molecular pathways. Gender bias was statistically significant and concordant on MRI, histopathology, and survival phenotype levels. These data suggest a potential gender-specific biology and may lead to targeted gender-genomic GBM therapies.

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Radio genomic mapping in enhancing volume phenotype identifies novel drivers in glioblastoma proliferation. G.A. Thomas[1], A. Kotrotsou[1], M. Leudi[1], M. Hatami[1], S.S. Singh[1], P.O. Zinn[2], R.R. Colen[1]; [1]The University of Texas MD Anderson Cancer Center, Houston, TX, [2]Baylor College of Medicine, Houston, TX

Background: Glioblastoma (GBM) is the most common and most aggressive primary malignant brain tumor in adults. It accounts for 20% of all the intracranial malignancies. Currently the survival of patients diagnosed with GBM remains less than 2 years despite extensive therapy. So time has arrived to look into newer, more focused methods of biomarker discovery that would help to develop targeted therapies and address the issue of tumor heterogeneity. We have been successful in harnessing magnetic resonance imaging (MRI) to identify different imaging phenotypes and assessing the genotypic background responsible for these phenotypes in the newly emerging field of imaging genomics. In this study, we identify the novel genes, pathways and networks relating to the contrast enhancement volume phenotype. **Methods:** We analyzed 106 patients in the TCGA (The Cancer Genome Atlas) on the basis of their contrast enhancement volume phenotype by grouping them based on survival using Kaplan–Meier statistics. Contrast enhancement volumetrics were obtained using 3D Slicer software. Discovery and validation were employed to reduce false discovery. Differentially expressed genes were analyzed using ingenuity pathway analysis. **Results:** Pathways and networks associated with a high contrast enhancing volume phenotype vs a low contrast enhancing volume phenotype were identified. The top proliferative genes were identified as novel molecules involved in driving tumor proliferation in glioblastoma. The upstream regulators previously not described in relation to GBM were found although similar functions have been elaborated in other tumors. Further in vitro studies need to be done to validate the in silico finding. **Conclusion:** Radio genomic discovery of potential molecules describing physiologic phenotypes may open the door to personalized medicine and therapeutics.

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A novel 18F-labeled NGR peptide for microPET imaging of CD13 receptor expression. G. Li[1], Y. Xing[1], J. Wang[2], L.-P. Yap[1], B. Bai[1], K. Chen[1], P.S. Conti[1]; [1]University of Southern California, Los Angeles, CA, [2]The Fourth Military Medical University, Xi'an, Shaanxi, China

Objectives: Noninvasive imaging of CD13 receptor in living subjects is of particular interest for both CD13-targeted cancer diagnosis and treatment. In this study, we radiolabeled an NGR peptide with 18F via bioorthogonal click chemistry. The resulting PET probe (18F-CNGR2) was subject to in vitro and in vivo evaluations. **Methods:** The dimeric NGR peptide (NGR2) was conjugated with an alkyne-containing polyethylene glycol (PEG) unit to provide a precursor, which was then mixed with an 18F-labeled azide moiety (18F-N3) to afford 18F-CNGR2 via catalyst-free click chemistry. The diagnostic value of 18F-CNGR2 was evaluated in CD13-positive HT-1080 and CD13-negative MCF-7 mouse xenografts by static microPET imaging followed by ex vivo quantification of tumor CD13 receptor level. **Results:** The synthesis of precursor was achieved from monomeric NGR peptide in three steps in an overall yield of 70%. The total synthesis time for 18F-CNGR2, including final high-performance liquid chromatography purification, was about 120 min. The decay-corrected radiochemical yield was 25 ± 4% (n = 8). For microPET study, 18F-CNGR2 displayed favorable in vivo performance in terms of good tumor uptake and retention in nude mice with HT-1080 tumor xenografts. 18F-CNGR2 was excreted predominantly through the renal route with most radioactivity in non-targeted tissues cleared at 2 h post-injection. The 18F-CNGR2 uptake in HT-1080 tumor (CD13 positive), MCF-7 tumor (CD13 negative), kidneys, liver, and muscle was 2.98±0.26, 0.45±0.32, 1.35±0.53, 1.02±0.24, and 0.14±0.02%ID/g, respectively. The CD13-specific tumor accumulation of 18F-CNGR2 was accomplished by a blocking study with a non-radiolabeled NGR peptide. Immunohistochemical staining of representative sections of HT-1080 and MCF-7 tumors confirmed the microPET data, indicating that CD13 receptors are indeed overexpressed in HT-1080 tumors, but not in MCF-7 tumors. **Conclusions:** A novel 18F-labeled dimeric NGR peptide has been successfully developed for microPET imaging of CD13 receptor expression. Convenient preparation, high CD13 specificity in mouse tumor xenografts, and favorable excretion profile in mouse models warrant the translational study of 18F-CNGR2.

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[^{68}Ga]MLN6907 first-in-class in vivo imaging of guanylyl cyclase C (GCC) in subcutaneous xenografts. D. Cvet[1], R. Robertson[1], J. Terkelsen[1], M. Borland[1], M. Saylor[1], K. Orcutt[2], R. Coelho[2], M. Schultz[3], W. Woodson[2], K. Zasadny[2], O. Yardibi[1], N. Salem[1], T. Anderson[4], J. Norenberg[4], P. Veiby[1],

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A novel, first-in-class, ^{68}Ga labeled DOTA-para-bn-SCN-Ahx-STp(5-18), 2.2 kDa peptide ([^{68}Ga]MLN6907) with high affinity to the guanylyl cyclase C (GCC) receptor has been developed as a research tool to detect differential GCC receptor densities in patients with primary and metastatic gastrointestinal tumors using positron emission tomography–computed tomography (PET/CT) and has potential to help select patients for treatment with GCC targeted therapies. GCC is sequestered exclusively in the gastrointestinal luminal compartment except under malignant transformation where it is then made accessible to intravenous agents. Conventional patient selection strategies often rely on an analytical IHC or total protein based assessment of a tumor biopsy which can be limited to an archival tissue sample from a single region of a particular lesion. Imaging may offer whole body, real time, multi-region and multi-lesion assessment of target levels. In addition, functional parameters associated with receptor kinetics may also be explored *in vivo*. Previous *in vitro* and *ex vivo* experimentation of [^{68}Ga]MLN6907 has demonstrated highly specific and sensitive uptake in a panel of engineered and primary human tumor cell lines (Cvet et al., AACR 2014). The current work shows the ongoing development of this agent and the first comprehensive assessment of *in vivo* competitive binding assays using PET in a subcutaneous xenograft cell line engineered to overexpress the cell surface target GCC. Female CB-17 SCID mice were inoculated S.C. with a HEK-293 GCC#2 cell line. Xenograft tumors (n=15) were allowed to grow to 200-500mm^3 and then randomized into 5 groups (n=3/group). Groups 1 and 2 received 0.15 and 0.3 µg of 150 and 350 µCi [^{68}Ga]MLN6907, respectively; groups 3-5 received 0.3 µg of [^{68}Ga]MLN6907 (~333 µCi) plus 1, 5, or 20 µg of excess cold peptide, respectively, for *in vivo* competitive binding assessment. Whole body PET scan from 45-90 minutes (9×5 min dynamic scan and summed) post injection of tracer was performed using a 3-mouse hive with a Siemens Focus 220 (Siemens Medical, Knoxville, TN). Following imaging, tumor, liver, spleen, kidneys, and whole blood from all animals (n=3/group; n=15 total) were collected after necropsy and counted on a gamma counter. The data presented will show the whole body (PET) and tissue specific uptake analysis in tumor, kidney, heart, and liver. High specific uptake of [^{68}Ga]MLN6907 was observed in the HEK-293 GCC#2 xenograft tumors. This was demonstrated by the progressively decreased %ID/g in the tumor only as the cold mass was increased. The kidney had the highest %ID/g for all groups. *In vivo* and *ex vivo* analysis showed good agreement supporting future assessment of [^{68}Ga]MLN6907 *in vivo* using PET measurements. [^{68}Ga]MLN6907 will be evaluated as a single, I.V., microdose GCC PET imaging agent in a phase 1 study designed to assess the safety, pharmacokinetics (PK), distribution, and radiation dosimetry in patients with surgically resectable metastatic colorectal carcinoma.

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Immuno-PET imaging of guanylyl cyclase C tumor receptor expression in multiple primary human tumor explant models of colorectal and pancreatic cancer using a zirconium-89 labeled antibody. D. Cvet[1], M. Saylor[1], R. Robertson[1], J. Terkelsen[1], M. Borland[1], K.D. Orcutt[2], O. Yardibi[1], M.S. Berridge[3], N. Salem[1], J. Hesterman[2], J. Hoppin[2], D.P. Bradley[1]; [1]Takeda Pharmaceuticals Company Limited, Cambridge, MA, [2]InviCRO, Boston, MA, [3]3D Imaging, Little Rock, AR

Introduction: Guanylyl cyclase C (GCC) is a cell surface protein expressed by normal intestinal epithelial cells and 95% of metastatic colorectal cancer (mCRC) tumors. MLN0264 is an investigational agent antibody-drug conjugate comprising a fully humanized anti-GCC monoclonal antibody (MLN2045) conjugated to the highly potent cytotoxic monomethyl auristatin E (MMAE) via a protease-sensitive linker (maleimido-caproyl-valine-citrulline [vc]) currently in Ph2 clinical trials (linker/toxin technology licensed from Seattle Genetics). MLN0264 specifically binds to and is internalized by GCC-expressing cells *in vitro* and *in vivo*. Cells expressing GCC were killed by MLN0264 *in vitro*, whereas non-GCC-expressing cells were not. Cytotoxicity was MLN2045-dependent, as a non-targeting antibody-drug conjugate had no effect on the viability of GCC-expressing cells. In GCC-expressing xenograft models with similar levels of GCC expression as human mCRC samples, MLN0264 induced mitotic arrest, leading to tumor regressions and long-term inhibition of tumor growth. Positron emission tomography (PET) imaging with radiolabeled monoclonal antibodies has the ability to visualize both *in vivo* cell surface markers and the overall biodistribution of the labeled antibody. ^{89}Zr has a decay half-life of 3.3 days which matches well to the circulation half-lives of antibodies. To date ^{89}Zr-based PET imaging has been investigated for a wide variety of cancer-related targets which include human epidermal growth factor receptor 2, epidermal growth factor receptor, prostate-specific membrane antigen, splice variant v6 of CD44, vascular endothelial growth factor, carbonic anhydrase IX, and insulin-like growth factor 1 receptor, among others. Considering ^{89}Zr has a decay half-life of 3.3 days, its usage as a PET imaging probe matches well to the circulation half-lives of antibodies. This PET study evaluated GCC protein expression in subcutaneous (S.C.) implants with various GCC-expressing cell lines and primary human explant xenograft models. This is the first disclosure of data describing the tumor uptake kinetics and biodistribution of [^{89}Zr]MLN2045. **Methods:** HEK-293, HEK-293 GCC#2, and T84 cell lines along with PHTX11C (colorectal), PHTX17C (colorectal), and PHTX215PA (pancreatic) primary human tumor explants were grafted S.C. in SCID mice, grown to 200-300mm^3, randomized by tumor volume and selected for imaging. [^{89}Zr]MLN2045 was dosed at 0.5 mg/kg and animals were scanned 2, 24, 48, 72 and 144 hrs after injection. A 30 min static image (followed by a 5-8 minute attenuation correction scan) was acquired using a Siemens Inveon (Siemens Medical, Knoxville, TN). PET data were reconstructed using a 2D ordered-subset expectation maximization (OSEM) method, resulting in whole body images of 128 × 128 × 63 voxels. **Results:** Specific uptake was demonstrated in all GCC-specific tumor lines when compared with the HEK-293 negative control. Increased uptake was also evident in the shoulder and knee joints most likely due to the release of ^{89}Zr from the antibody over time. Free radionuclide can accumulate in the bone and can associate with plasma proteins. **Conclusion:** This study confirms differential selectivity of [^{89}Zr]MLN2045 in GCC-expressing lines over the non-expressing line which can be visualized using PET imaging.

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[^{18}F]FMISO µPET as a potential predictive biomarker of 5-FU therapy resistance in a xenograft mouse model for colorectal cancer. S. De Bruycker[1], C. Vangestel[2], T. Verbrugghen[1], P. Pauwels[2], T. Van den Wyngaert[2], S. Staelens[1], S. Stroobants[2]; [1]University of Antwerp, Antwerp, Belgium, [2]Antwerp University Hospital, Antwerp, Belgium

Introduction: It is well established that tumor hypoxia has a negative impact on the efficacy of chemotherapy (e.g., 5-FU). Predictive biomarkers of tumor hypoxia are critically needed to identify patients with a worse prognosis that may benefit from appropriate alternative therapies. We investigated if baseline [^{18}F]FMISO µPET/CT can predict 5-FU therapy response, as determined with [^{18}F]FDG µPET/CT and Ki67 immunohistochemistry in a xenograft mouse model for colorectal cancer. **Methods and Materials:** Nude mice (n=12) were subcutaneously inoculated with 2×10^6 Colo205 cells. Two weeks later, [^{18}F]FMISO and [^{18}F]FDG µPET/CT scans were performed to determine baseline tumor hypoxia and metabolic activity, respectively. Differential tumor hypoxia was induced by exposing half of the animals to short-term (4 h) hypoxic breathing conditions (10% O_2) prior to the [^{18}F]FMISO scan. Baseline tumor volume in the two cohorts was equal, at 235±70 and 246±52 mm^3, respectively. All [^{18}F]FDG scans were performed under normoxia (ambient air, 21% O_2). Thereafter, animals were treated with 5-FU (150 mg/kg; single dose) under hypoxic or normoxic breathing conditions. Two and six days post-therapy, follow-up [^{18}F]FDG µPET/CT scans were performed to assess therapy response. Ex vivo, Ki67 immunohistochemistry (IHC) was performed on the excised tumors. **Results:** As expected, there was a clear difference in the extent of tumor hypoxia in our two different breathing cohorts, with [^{18}F]FMISO SUV_{mean} being significantly higher in the hypoxic compared to the normoxic cohort (0.68 vs. 0.27 g/cc; p=0.006). A reduction in [^{18}F]FDG SUV_{mean} was observed in both the hypoxic and normoxic breathing cohorts two days post-therapy, but this was only significant in the normoxic cohort (–4.7±7.6 vs. –28.4±5.6 [p=0.03] %, respectively). Relative tumor volumes (RTV), measured on µCT, increased significantly in the animals exposed to hypoxic conditions, whereas no change in RTV was observed in the normoxic breathing cohort (+20.9±7.0 [p=0.04] vs. +5.2±6.6 %, respectively). A simple linear regression including both cohorts established that baseline [^{18}F]FMISO SUV_{mean} could statistically significantly predict the tumor

growth inhibition two days post-therapy (R^2=0.6; p=0.002). However, no significant correlation was found between baseline [^{18}F]FMISO SUV_{mean} and the change in tumoral [^{18}F]FDG signal (R^2=0.2; p=0.2), since the animal-specific metabolic response under therapy was less consistent than the anatomical response. Six days post-therapy, a slight increase in [^{18}F]FDG SUV_{mean} was observed in the hypoxic breathing cohort, whereas under normoxic conditions a reduction in [^{18}F]FDG signal persisted (+8.5±7.4 vs. −23.5±15.2 %, respectively). In both breathing groups, RTV was increased six days post-treatment, but was only significant under hypoxic conditions (+36.0±12.3 [p=0.03] vs. +7.7±15.0 %, respectively). Ki67 expression six days post-therapy was comparable in both breathing cohorts (72±4 vs. 69±1 %, respectively). **Conclusion:** Our preliminary results show that a baseline [^{18}F]FMISO scan might be able to predict 5-FU therapy response in normoxic tumors and resistance in hypoxic tumors. Imaging of a control cohort and additional IHC data at the different time points will be gathered in order to fully comprehend the observed data.

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Spatial and temporal localization of intravasation events during early tumor development. E.I. Deryugina; The Scripps Research Institute, La Jolla, CA

Intravasation, the active entry of malignant cells into newly formed angiogenic blood vessels, is one of the hallmarks of cancer and could be a rate-limiting step in the metastatic cascade. According to established notion, stromal invasion is a prerequisite of cancer dissemination via vascular routes and, therefore, intravasation events should be associated with the invasion front of expanding primary tumors. However, clinical data and emerging experimental evidence, including our own research, indicate that metastasis-seeding intravasation might be initiated very early during cancer progression, namely, before the signs of extensive tumor invasion into the adjacent stroma. Thus, I have proposed that the bulk of intravasation events takes place within the primary tumor interior, involves the intratumoral vasculature, and is initiated prior to and proceeds independently of stromal invasion at the tumor periphery. Herein, this hypothesis was investigated in avian and mouse model systems designed to study coordinately the initiation of tumor angiogenesis and intravasation. These live animal models employ congenic pairs of tumor variants selected in vivo from human fibrosarcoma and prostate and epidermoid carcinomas for the high or low dissemination ability (hi/diss and lo/diss variants). In the chick embryo model, the tumor cells are grafted into the chorioallantoic membrane mesoderm, where extensively vascularized primary tumors are formed within several days. Furthermore, hi/diss tumor variants generate invasive primary tumors with the escaping cancer cells migrating towards and along tumor-adjacent blood vessels. The intramesodermal model, therefore, represents an ideal setting, where the entry of tumor cells into the vasculature could be distinguished between the interior core and invasive periphery of the primary tumor. To spatially and temporally localize the intravasation process, a unique high-resolution image analysis module has been designed to identify and quantify in an unbiased manner primary tumor cells located intravascularly or entering blood vessels and to morphologically segregate these cells from tumor cells positioned extravascularly at the abluminal vessel surface. By this approach, the bulk of actual tumor cell intravasation has been spatially localized to the interior core of hi/diss primary tumors and not to their invasive outgrowths, despite the strong vasculotropic behavior of escaping tumor cells. We have also established that initial intravasation events become detectable as soon as angiogenic intravasation-sustaining vasculature is formed within the core of developing primary tumors, well before the signs of substantial stromal invasion. Since intravasation process involves two major players, i.e., tumor cells with heightened migration abilities and newly formed angiogenic blood vessels, we examined the role of epithelial-mesenchymal transition (EMT) in rendering cancer cells with intravasation capacity and also probed for the role of inflammatory neutrophils and neutrophil MMP-9 in the initiation of intratumoral vasculature with unique intravasation-sustaining characteristics. The major findings from our intramesodermal model have been validated in a mammalian setting in the mouse ear model, where hi/diss tumor cells implanted intradermally into the ear develop invasive and highly vascularized tumors readily accessible for investigation by confocal microscopy and our unique image analysis module. Together, these data demonstrate that metastasizing cancer cells enter the blood circulation very early during tumor development and do so primarily within the tumor interior and independently of stromal invasion. These findings also indicate the necessity for re-focusing studies from the periphery of the primary tumor to its core, where critical processes such as EMT and angiogenesis are induced to facilitate tumor cell intravasation much earlier during cancer progression than conventionally recognized.

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Construction and characterization of a human binary code scFv phage library. X. Wang, H.-Y. Kim, B. Wahlberg, W.B. Edwards; University of Pittsburgh, Pittsburgh, PA

Introduction: A phage display library of antibodies with high diversity and functional antibody expression greatly enhances the chances of discovering antibodies with practical value in phage display techniques. Therefore, we have constructed a scFv phage display library to determine whether it can supply stable, high affinity antibodies against antigens of biomedical interest. **Methods:** The library (BCscFv library) was constructed upon the humanized 4D5 framework with binary code mutations comprising Tyr and Ser residues in all of the complementarity determining regions (CDRs) of the variable heavy chain (VH) and in CDR3 of the variable light chain (VL). To characterize the quality of the BCscFv library, PCR, immunoblotting, and sequencing were performed on antibody clones randomly picked from each sub-library. To determine the functionality of the library, it was tested by screening against a variety of antigens which included antigens of interest for molecular therapies or imaging in cancer (VEGFA, CD44, CD90, and VEGFR1). As an exemplar, anti-VEGFR1 antibodies were examined in detail for affinity, thermal stability, and specificity. **Results:** The diversity of the library, 2.2×10^9, is sufficient for use as an antibody source. PCR analysis of random clones from each sub-library demonstrated that the library consists of the full length sequence of the scFvs (86% of the clones) and immunoblotting demonstrated that the library can produce full length scFvs that are in frame (73% of the clones). After two rounds of panning three rounds of panning against CD44 and CD90 and three rounds of panning against VEGFA and VEGFR1, antibodies for each antigen were isolated with affinity that was great enough for molecular imaging or targeted drug delivery without the need for affinity maturation (K_D <50 nM). Turbidity measurements indicated that the anti-VEGFR1 antibodies possessed an inherent resistance toward thermally induced aggregation which is a good indication of stability. One of the anti-VEGFR1 antibodies did not bind VEGFR2 but stained VEGFR1 in tumor sections indicating selectivity. **Conclusions:** This scFv library based on a binary code can serve as a rich source of soluble, high affinity antibodies for therapeutic purposes.

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Multiple myeloma imaging by C-11-acetate metabolic PET and in vitro metabolite fate mapping by NMR in human cells. F. Fontana[1], K. Shoghi[1], A. D'Avignon[2], K.N. Weilbaecher[1], M. Shokeen[1]; [1]Washington University School of Medicine, St. Louis, MO, [2]Washington University in Saint Louis, St. Louis, MO

Background: Multiple myeloma (MM) is a malignancy of bone marrow (BM) plasma cells (PC), which is characterized by high levels of monoclonal immunoglobulins (Ig), multiple bone osteolytic lesions, and systemic symptoms. While monoclonal Ig are an established serum marker for MM, the severity of skeletal complications of MM warrants efficient imaging modalities to detect and follow the response of bone lesions. ^{18}FDG-PET is the current gold-standard method for imaging of MM, yet its sensitivity can be as low as 61%, particularly in intramedullary lesions. We hypothesized that acetate metabolism could be a target for molecular imaging of MM, which could be rapidly translated to the clinic and highly suitable for preclinical models of disease. **Experimental Methods:** C57Bl/KaLwRij mice were injected with GFP-5TGM1 cells intravenously (for engraftment to bones and spleen) or subcutaneously to obtain plasma cell tumors (PCT). Allogeneic or human myeloma cell lines were injected in Matrigel in the flank of NSG mice to obtain subcutaneous PCT. MM cell engraftment and tumor burden were verified by serum protein electrophoresis (SPEP), histology, and flow cytometry. For small animal imaging, mice were injected

by tail vein with ^{11}C-acetate (20 MBq) for 0-60 min dynamic PET. Small animal PET images were acquired with Focus 220 or Inveon, while the CT images were acquired with the Inveon. Inveon Research Workstation (IRW) was used for image analysis. Regions of interest (ROI) were selected from maximum intensity projection (MIP) PET images using CT anatomical guidelines and the activity associated with ROIs was derived from the IRW. Maximum standard uptake values (SUVs) were calculated using the equation SUV=([nCi/mL]×[animal weight (g)]/[injected dose (nCi)]). Corresponding time activity curves (TACs) were plotted and analyzed with IRW. For bio-distribution, animals were sacrificed 1 h after ^{11}C-acetate injection. Organs of interest were harvested, weighed and counted in the gamma counter. The percentage injected dose per gram of tissue (%ID/g) was determined by decay correction of the radiopharmaceutical for each sample normalized to a standard of known weight, which was representative of the injected dose. In vitro acetate uptake was measured by ^{13}C-edited ^{1}H-NMR analysis of the supernatant at different time points. **Results:** ^{13}C-edited ^{1}H-NMR analysis of the supernatants showed acetate uptake by human and murine myeloma cells in vitro. By using GFP as a genetic reporter, we then found that ^{11}C-acetate PET was comparable or superior to optical imaging in visualizing 5TGM1 subcutaneous plasma cell tumors (PCT). In ex vivo bio-distribution assays, radioactivity correlated with the number of viable engrafted tumor cells ($p<0.01$). In the orthotopic 5TG/KaLwRij model, SUVs for ROI in the tibiae of tumor bearing mice were significantly higher than in control mice ($p<0.05$). As early detection of response to treatment is a yet to be met need in MM research, we asked whether ^{11}C-acetate could be used to image mice before and after pharmacological treatment. Indeed, upon treatment with an anti-myeloma drug (bortezomib day 1-day 4), we were able to measure a significant decrease in acetate uptake ($p<0.01$), which coincided with a reduction of tumor burden measurement ex vivo ($p<0.01$). Translating our findings to non-genetically labeled models, we confirmed ^{11}C-acetate uptake in human myeloma cell xenografts and in one case of spontaneous multiple myeloma arising in an aged KaLwRij mouse. **Conclusions:** Our data show that ^{11}C-acetate PET could provide a reliable and reproducible in vivo technique for preclinical imaging of MM. Furthermore, as ^{11}C-acetate PET is FDA-approved for other conditions, our data provide strong proof-of-principle for the use of the same tracer in clinical trials. In this presentation, the novel in vivo and in vitro findings and future implications will be discussed.

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Automated analysis of drug distribution in intravital imaging. R.J. Giedt, R. Weissleder; Massachussets General Hospital/ Harvard Medical School, Boston, MA

Recent advances in the field of intravital imaging now allow pharmacokinetic and pharmacodynamic studies at the single cell level in live animal models. These studies, often completed via serial measurements of multiple locations in tumors, generate large data sets for thousands of cells. The amount of data generated makes manual analysis impossible but automated methods do not yet exist. In this research we have developed and validated different analysis methods and show how they can be used to measure drug concentrations (e.g., olaparib, a PARP inhibitor) at the single cell level. The method is broadly expandable to other drugs and pharmacokinetic/dynamic analyses. The primary problem involved with creating automated image analysis methods for intravital imaging data sets is finding ways of defining areas of interest (typically single cells) from the background. Due to multiple factors, including heterogenous marker expression, cell movement, and multiple layers of cells among others, developing automated methods of finding objects of interest is a challenging problem in an intravital imaging context. We therefore began this project by quantitatively surveying numerous common thresholding methods on a range of example intravital images to determine which would be most appropriate for identifying fluorescently labeled drugs. Evaluation of thresholding methods was completed on a wide range of thresholding methods and resulted in the identification of three specific methods (Otsu, Huang, Ray) that showed the most promise. From these, we identified Ray's method as the most suitable for incorporation into an algorithm focusing on intravital imaging. Following the implementation of this thresholding method into an overall segmentation procedure, we validated the algorithm on a fluorescently labeled PARP inhibitor in an intravital model by first determining, at the single cell and population levels, cellular drug concentration over time. Using this information we were able to calculate relevant pharmacokinetic information such as single cell variability of drug concentration over time, single cell drug concentration over time, the number of cells receiving a therapeutic dose of drug, and the cytosolic versus nuclear fraction of drug localization. Ultimately, we were able to show that relevant pharmacokinetic information can indeed be extracted from serial intravital imaging in an automated fashion. We believe that the application of this algorithm will be of value to the analysis of intravital microscopy imaging, particularly when imaging drug action in large populations of single cells. More broadly, use of this algorithm in future studies combined with in vivo pharmacokinetic studies utilizing fluorescently labeled drugs should aid in drug development and validation, ultimately resulting in improved treatment options and prognoses.

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A new paradigm for characterization and treatment of bone metastases. B.S. Greenspan; Georgia Regents University, Augusta, GA

The current standard of care for diagnosis and therapy of cancer is unsatisfactory. Results for many cancers (e.g., lung, breast, pancreatic, advanced prostate) are not very good. Side effects of chemotherapy are often severe, unpleasant and force changes in management. We can do better. The proposed new paradigm is based in part on the hallmarks of cancer, as described by Hanahan and Weinberg in their updated review article in 2011. There are 8 hallmarks: 1) self-sufficiency in growth signals (sustaining proliferative signaling); 2) evading growth suppressors; 3) evading apoptosis; 4) enabling replicative immortality; 5) sustained angiogenesis; 6) activating tissue invasion and metastasis; 7) reprogramming of energy metabolism; 8) evading immune destruction. Underlying the above hallmarks, according to Hanahan and Weinberg, are the two enabling characteristics of genomic instability and tumor-promoting inflammation. The current approach addresses screening, diagnosis, staging/re-staging and surveillance. There are NCI clinical trials evaluating the neoplastic processes of metabolism, proliferation, metastasis, angiogenesis, hypoxia, and receptor imaging. However, we need to take into account a number of factors not generally evaluated currently. These include clonal variation, especially within metastases, and the tumor inflammatory microenvironment and intratumoral acidity. Based on the above, I propose the following modern integrated and comprehensive approach for evaluation, characterization and treatment of bone metastases. Detection of bone metastases by the usual array of imaging studies can be expanded to include more positron-emitting labeled radiotracers. We should be developing new prognostic and predictive biomarkers. We should be placing more emphasis on assessment of therapeutic response with a wider array of tracers. Focused screening and/or surveillance would be important. These imaging tools would be critical to improve personalized care for patients based on biologic characteristics of the primary tumor and also metastases. I suggest the following: For characterization of metastatic bone disease, PET/CT will likely continue to be the major imaging modality in the near future. Characterization of bone metastases as well as the primary tumor should include at least all of the following: 1. Evaluation of glucose metabolism by F-18 FDG; 2. Evaluation of bone turnover by F-18 sodium fluoride (NaF); 3. Evaluation of DNA synthesis with a proliferation agent, such as F-18 FLT or F-18 sigma-2 receptor imaging; 4. Evaluation of amino acid metabolism, such as C-11-methionine or C-11-, N-13- or F-18-glutamine; 5. Evaluation of various proteins and cell-surface receptor agents, such as RANKL/RANK, OPG, Wnt signaling pathway, Dkk1, or sFRP-2; 6. Evaluation of angiogenesis, such as Cu-64 RGD; 7. Evaluation of osteoclastic activity (included in part in #5 above); 8. Evaluation of various clones of cells in metastatic deposits. The goal is to fully characterize not only the primary tumor, but also metastatic deposits, including characteristics of each different clone of cells in each metastatic deposit. This should lead to more specific and more comprehensive targeted therapy and therefore more successful therapy. For therapy: 1. Utilize negative feedback loops where possible, as in I-131 therapy for thyroid cancer. 2. Identify enzymatic pathways that are upregulated or could be upregulated, and then shut them down with the use of diagnostic/therapeutic (theranostic) pairs of agents, either chemical antagonists or radionuclides. Examples include tyrosine kinase pathways and androgen receptor blockade in prostate cancer. 3.

Whole body radiation by radiation oncology, for immune stimulation. This idea is well known to radiation oncologists. I suggest starting with 100 rem whole body irradiation initially. 4. Disrupt signaling pathways and chemical mediators appropriate for each primary tumor and its metastatic deposits, including integrins and cadherins, TGF-beta in osteoblastic metastases, and PTHrP. 5. Disrupt the tumor support structure, including recruitment of normal cells that surround metastatic deposits. 6. Disrupt the tumor microenvironment of the metastases, particularly the inflammatory component. 7. Disrupt the acidic intracellular environment, which may allow greater toxic effects on tumor cells by the immune system and may reduce tumor capability of invasion. 8. Use of bisphosphonates. 9. Use of localized external beam irradiation when appropriate. 10. Use of radium-223 dichloride (Ra-223 Cl2), an alpha-emitter. Beta-emitters may also be useful. 11. Use of radioimmunoconjugates as theranostic agents. An example is Zr89-J591/Lu-177-J591 for prostate cancer. I believe that this comprehensive, combinatorial, targeted approach will produce much better outcomes, and do so with significantly less side effects. The goal is to improve survival and also quality of life.

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Evaluation of the alum vaccine depot theory with SPECT/CT. T. Esposito[1], J. Lai[2], K. Tse[1], H. Jiyoung[1], P. Hopkins[2], T. Schneider[1], V. Schmitt[1], K. Saatchi[1], J.P. Dutz[2], U.O. Häfeli[1]; [1]University of British Columbia, Vancouver, BC, Canada, [2]Child and Family Research Institute, Vancouver, BC, Canada

Alum is a broad term that encompasses aluminum-based adjuvants such as aluminum oxyhydroxide (AlO(OH)). Commercially known as Alhydrogel, AlO(OH) consists of 1-20 µm clusters of 10-100 nm nanofibers. This adjuvant promotes strong antibody-mediated immunity and has an extensive safety record; AlO(OH) is found in a variety of human vaccines, such as HAVRIX against hepatitis A. Despite being the only FDA approved adjuvant until 2009, the mechanism of action of alum is poorly understood. AlO(OH) is thought to form a depot of vaccine antigen at the site of injection but this hypotheses has yet to be tested. In this work, we radiolabeled the model antigen ovalbumin with iodide-123 (123I-OVA) and observed at the site of injection the kinetics of its distribution when adsorbed to Alhydrogel 1.3% in relation to free 123I-OVA; monitoring was done using a VECTorPET/SPECT/CT scanner. Transit of 123I-OVA was significantly slowed when adsorbed to alum relative to free antigen following subcutaneous injection. AlO(OH) forms an antigen depot. We have recently developed and characterized a novel tracer for AlO(OH) by doping the adjuvant's crystal structure with gallium-67. Fundamental questions, such as the kinetics and distribution of AlO(OH) in relation to antigen, can now be explored. A better understanding of one of the oldest and most common adjuvants can not only improve vaccine safety, but aid in the development of future vaccines.

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Intravital imaging of monocyte regulation of tumor metastasis in the lung. R.N. Hanna, C. Cekic, E. Herrley, G. Chodaczek, C.C. Hedrick; La Jolla Institute for Allergy and Immunology, La Jolla, CA

Little is known about the physiological function of monocyte subsets in the regulation of tumor metastasis in the lung. In this study we use intravital imaging of transgenic mice to show that a subset of patrolling monocytes (defined as $Ly6C^{-}CX3CR1^{high}Nur77^{high}CD115^{+}MHC2^{-}$) is enriched in the microvasculature of the lung and is important in preventing initial tumor metastasis. Patrolling monocytes were found to establish early interactions with tumor cells and specifically remove tumor material from the lung vasculature. NR4A1 (Nur77)-deficient mice, which have a unique loss of $Ly6C^{-}$ patrolling monocytes in the hematopoietic compartment, have significantly increased metastasis of syngeneic tumor cells in the lung. Transfer of Nur77-proficient patrolling monocytes into Nur77-deficient hosts in a gain-of-function manner significantly reduces tumor lung invasion. These studies indicate an important contribution of Nur77-dependent patrolling monocytes in cancer immune surveillance, which may provide new opportunities for cancer immunotherapy.

*AACR-Aflac, Incorporated, Scholar-in-Training Award winner.

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Imaging the tumor microenvironment of metastasis reveals the sites of transient blood vessel permeability and tumor cell intravasation. A.S. Harney[1], E.N. Arwert[1], D. Entenberg[1], Y. Wang[1], P. Guo[1], B.-Z. Qian[2], B. Smith[3], J.W. Pollard[2], J.G. Jones[1], D.L. Flynn[3], J.S. Condeelis[1]; [1]Albert Einstein College of Medicine, Bronx, NY, [2]University of Edinburgh, Edinburgh, United Kingdom, [3]Deciphera Pharmaceuticals LLC, Lawrence, KS

Macrophages in the tumor microenvironment are critical in promoting tumor progression to metastasis. Hematogenous dissemination of tumor cells from the primary tumor is an essential step in metastasis. Direct contact between a macrophage, tumor and endothelial cell [tumor microenvironment of metastasis (TMEM)] correlates with metastasis in breast cancer patients independently of other clinical prognostic indicators (1). Mechanisms underlying TMEM function and tumor cell intravasation have not been directly visualized. Here we show, using intravital high-resolution two-photon microscopy (2), that tumor cell intravasation occurs only at TMEM. Tumor cell intravasation occurs concurrently with transient, local blood vessel permeability at TMEM in mouse mammary carcinoma and human patient-derived xenograft models. Ablation of the presence or activity of the TMEM-associated macrophages blocks intravasation at TMEM, demonstrating an essential role of perivascular macrophages in TMEM function. A subset of TMEM macrophages are identified as Tie2-expressing macrophages that are characterized by F4/80+/CD11b+/CD68+/MRC1+/$Tie2^{Hi}$/$VEGFA^{Hi}$/CD11c–. VEGFA signaling from $Tie2^{Hi}$ TMEM-associated perivascular macrophages causes the local loss of vascular junctions resulting in transient localized blood vessel permeability and tumor cell intravasation at TMEM. Macrophage-specific ablation of VEGFA results in increased vascular junction stability at TMEM perivascular macrophages, demonstrating that vascular junction dissolution leading to vascular permeability and tumor cell intravasation is a local event at $VEGF^{Hi}$/$Tie2^{Hi}$ TMEM-associated macrophages. The dynamic nature of vascular permeability at active TMEM sites provides insight into the spatial and temporal heterogeneity of vascular leakiness. Inhibition of Tie2 with the first in class small molecular inhibitor Rebastinib impairs TMEM function leading to a reduction in vascular permeability, tumor cell dissemination and metastasis. Further, Rebastinib inhibition of Tie2 blocks tumor cell extravasation and metastatic growth in the lungs. Together, the findings that TMEM macrophages mediate blood vessel permeability and tumor cell intravasation demonstrate an essential role for TMEM microanatomical compartments within the primary mammary tumor as sites of tumor cell dissemination. These data reveal that tumor cell intravasation and vascular permeability are local events at TMEM. These data considerably extend our understanding of the mechanism of tumor cell intravasation in breast cancer and explain the prognostic value of TMEM density in predicting distant metastatic recurrence in breast cancer patients. Advances in molecular imaging technology have allowed for the high-resolution live imaging of the tumor microenvironment in both the primary mammary tumor and secondary sites, including the lungs. With an increased understanding of the molecular mechanism underlying TMEM activity, multimodal imaging is being pursued to develop clinically applicable diagnostics based on the high-resolution imaging developed using animal models. **Acknowledgments:** This research is supported by the Department of Defense Breast Cancer Research Program under award number BC120227 (A.S.H.), NIH CA100324 (J.S.C.), and the Integrated Imaging Program. **References:** 1. T. E. Rohan et al., Tumor microenvironment of metastasis and risk of distant metastasis of breast cancer. J Natl Cancer Inst. 2014;106 (August 1, 2014). 2. D. Entenberg et al., Setup and use of a two-laser multiphoton microscope for multichannel intravital fluorescence imaging. Nat Protoc. 2011;6:1500-1520.

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Imaging macrophages in ovarian cancer. H.M. He[1], A. Chiu[1], C.H. Contag[2], O. Dorigo[1]; [1]Department of OBGYN, Stanford University, Stanford, CA, [2]Departments of Pediatrics, Microbiology & Immunology, and Radiology, Stanford University, Stanford, CA

Ovarian cancer (OC) is the most lethal gynecological cancer for women all over the world. The disease is usually diagnosed at later stages and its

mechanism is largely unknown. Therapeutic strategies targeting various oncogenic pathways have been insufficient. The microenvironment is likely to play an important role in mediating drug response. However, little is known about the microenvironment in OC, which consists of a complexity of cellular components including macrophages. Recent studies correlated high percentage of tumor-associated macrophages (TAMs) with poor patient prognosis. Our previous work demonstrated that the presence of intratumoral CD163 positive macrophages, but not stromal macrophages, is associated with poor survival. To investigate the role of macrophages in OC progression, we established a transgenic mouse model to track macrophages via bioluminescent imaging. We found a dynamic pattern of macrophage activity during OC progression and were able to manipulate the bioluminescent signals by either activating or depleting macrophages. This model also allows us to explore the benefit of pharmacological interventions targeting TAMs.

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Visualizing the fate of immunotherapeutic cells by 19F MRI. B.M. Helfer[1], O.F. Charles III[1], C. Schirda[2], E.T. Ahrens[3]; [1]Celsense, Inc, Pittsburgh, PA, [2]University of Pittsburgh, Pittsburgh, PA, [3]University of California at San Diego, San Diego, CA

Live cell vaccines are increasingly being used in the clinic to modify a patient's immune function. Clinical studies involving human polarized dendritic cells, chimeric antigen receptor T-cells, and NK cells have demonstrated great promise as an immunotherapeutic intervention against cancer. Significant questions concerning the pharmacokinetics of these cells as well as correlations of cell fate to therapeutic potency remain. Here we report on a landmark clinical trial utilizing a 19F magnetic resonance imaging (19F MRI) tracer agent used to visualize the fate of an autologous dendritic cell vaccine used to treat patients with colorectal cancer. This study demonstrates the capability of MRI to detect and quantify labeled cells with a high degree of specificity. With nominal exogenous fluorine naturally present in tissue, labeled cells appear with little background. Pairing this signal with conventional proton MRI from the same imaging session, cells are able to be traced to their anatomical location. Contrary to concerns regarding the sensitivity of this approach, a high resolution image of the labeled cells was acquired in 8 minutes using a 3T scanner, an off-the-shelf surface coil, and standard pulse sequences. Utilizing these methods, the apparent limit of sensitivity was 2.5×10^4 cells per voxel in the region of interest. The MRI tracking capabilities, safety profiles, and clinical sensitivity demonstrate the ability of 19F to be used in additional clinical applications to visualize the fate of cellular therapeutics.

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Contrast agent cocktail for imaging of cancer and inflammation in a mouse model of colorectal cancer. A. Hellebust[1], D. Rosen[2], S. Anandasabapathy[2], R. Richards-Kortum[1]; [1]Rice University, Houston, TX, [2]Baylor College of Medicine, Houston, TX

A minimally invasive, optical-based strategy for monitoring the molecular and architectural changes associated with inflammation and carcinogenesis of the gastrointestinal tract has the potential to improve early cancer detection and therapy. An optical contrast agent cocktail enabling visualization of multiple biomarkers simultaneously would allow clinicians to detect increases in biomarker expression levels corresponding to precancer or to monitor response to therapy with minimal invasiveness. The ability to image multiple markers is especially useful in early cancer detection where patient to patient tumor heterogeneity is high. Evaluating biomarker detection *in vivo* in animal models is a critical step towards the clinical translation of contrast agents for cancer screening and diagnosis. The contrast agent cocktail in this study consisted of three components: fluorescent labeled CD45 targeted antibody, fluorescent labeled tumor necrosis factor α (TNFα) targeted Affibody and proflavine, a vital fluorescent dye which labels cell nuclei. These agents provide examples of different sized contrast agents that could go into a contrast agent cocktail, although other antibodies, peptides and small molecules may be more desirable for other organ sites or specific disease detection. Inflammatory polyps, pre-cancerous lesions and tumors were generated in the distal colon of male BALB/cJ mice through the administration of azoxymethane (AOM) and dextran sodium sulfate (DSS). This model emulates the progression of colitis induced carcinoma and allows for topical contrast agent application *in vivo* as well as the potential for *in vivo* imaging. Contrast agent cocktails were topically applied to the colon lumen *in vivo* to anesthetized mice. After a fifteen minute incubation, mice were euthanized and the colon was harvested and bisected for wide-field and high-resolution fluorescence imaging. The IVIS Small Animal Imager was used for wide-field fluorescence imaging to verify contrast agent application in the distal colon and to identify lesions across the surface of the distal colon. Confocal microscopy was used to visualize contrast agent labeling across the tissue surface and assess permeation of agents of various sizes into tissue. Confocal imaging of the surface allowed high resolution imaging to validate contrast agent localization. Tissue was fixed and processed for standard pathology processing and immunohistochemistry. Mice were imaged at 4, 7, 10 and 13 weeks to ensure the evaluation of inflammatory polyps, pre-cancerous lesions and tumors. Over 20 mice were imaged over the course of the study. Topical administration of the contrast agent cocktail led to consistent labeling of the distal 2-3cm of the mouse colon. The TNFα Affibody provided successful labeling of TNFα positive immune cells during confocal microscopy. The CD45 targeted antibody labeled inflammatory polyps; however, the ability to permeate the epithelium due to its large size (150kDa) limited the staining to the surface of the epithelium. Proflavine staining identified architectural changes in the glandular patterns over time as well as an increased nuclear-to-cytoplasmic ratio in areas of cancer. The simultaneous use of multiple agents in a contrast agent cocktail has the potential to improve early cancer detection and monitor response to therapy over time.

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The influence of carrier concentration on the radiochemical purity, hydroxyapatite affinity and in-vivo bone accumulation of the bone-targeting therapeutic radiopharmaceutical Rhenium-188-HEDP. R. Lange[1], J. de Klerk[1], H. Bloemendal[1], R. Ramakers[2], F. Beekman[2], M. van der Westerlaken[1], H. Hendrikse[3], R. ter Heine[1]; [1]Meander Medical Center, Amersfoort, The Netherlands, [2]MILabs B.V., Utrecht, The Netherlands, [3]VU University Medical Center, Amsterdam, The Netherlands

Introduction: Bone metastases constitute a major complication in the final stage of several cancers, such as prostate cancer, breast cancer and lung cancer. These metastases may lead to serious complications like pain, neurological symptoms, fractures and hypercalcemia resulting in a decreased quality of life and poor survival (1). Palliative pain treatment often consists of opioids or external beam radiotherapy. An effective and safe alternative is treatment with a bone-targeting therapeutic radiopharmaceutical, like the beta emitter ^{188}Rhenium-HEDP (2). Recently, we published the on-site production of GMP grade ^{188}Rhenium-HEDP for application in routine clinical practice and for support of clinical studies (3). There is no standard preparation method for ^{188}Rhenium-HEDP available. It is very important to avoid adverse effects caused by radiochemical impurities that direct beta-radiation to tissues other than bone. It is well known that the presence of carrier (non-radioactive rhenium as ammonium perrhenate) in the reaction mixture during labelling is a prerequisite for adequate bone affinity, but little is known about the optimal carrier concentration. **Methods:** We investigated the influence of carrier concentration in the formulation on the radiochemical purity, in-vitro hydroxyapatite affinity and the in-vivo bone accumulation of ^{188}Rhenium-HEDP in mice. The radiochemical purity was determined by means of thin layer chromatography (3). The hydroxyapatite binding was determined with an assay described previously (4) for ^{188}Rhenium-HEDP with different carrier quantities and was compared to some commercially available bone-seeking radiopharmaceuticals (^{99m}Tc-oxidronate, ^{99m}Tc-medronate and ^{153}Sm-lexidronam). For the in-vivo experiments, SPECT images were acquired by a VECTor/CT preclinical system with subsequent quantification of the healthy-bone-to-soft-tissue ratio of ^{188}Rhenium-HEDP. **Results:** The carrier concentration influenced hydroxyapatite binding in-vitro as well as bone accumulation in-vivo. Carrier amounts below 0.1 µmol and above 20 µmol resulted in low to moderate hydroxyapatite binding. Variation in hydroxyapatite binding with various carrier concentrations seemed to be mainly driven by variation in radiochemical purity and showed a good correlation (R^2 = 0.944). The in-vivo bone accumulation appeared to be more complex: satisfactory radiochemical purity and hydroxyapatite affinity did not necessarily predict acceptable bio-distribution of ^{188}Rhenium-HEDP. **Conclusions:** Variation in carrier concentration during preparation of ^{188}Rhenium-HEDP should be avoided. For development of new bisphosphonate-based radiopharmaceuticals for

clinical use, human administration should not be performed without previous animal bio-distribution experiments. Furthermore, our clinical formulation of ^{188}Rhenium-HEDP, containing 10 μmol of carrier, showed excellent bone accumulation that was comparable to other bisphosphonate-based radiopharmaceuticals, with no apparent uptake in other organs. We are currently investigating the routine clinical use of this promising therapeutic radiopharmaceutical as a single agent as well as in combination with chemotherapy in two clinical trials. **References:** 1. Suva LJ, Washam C, Nicholas RW, Griffin RJ. Bone metastasis: mechanisms and therapeutic opportunities. Nat Rev Endocrinol 2011; 7: 208-218. 2. Lam MG, de Klerk JMH, van Rijk PP, Zonnenberg BA. Bone seeking radiopharmaceuticals for palliation of pain in cancer patients with osseous metastases. Anticancer Agents Med Chem 2007; 7: 381-397. 3. ter Heine R, Lange R, Breukels O, Bloemendal HJ, Rummenie RG, Wakker AM, et al. Bench to bedside development of GMP grade Rhenium-188-HEDP, a radiopharmaceutical for targeted treatment of painful bone metastases. Int J Pharm 2014; 465(1-2): 317-324. 4. Deutsch E, Libson K, Vanderheyden JL, Ketring AR, Maxon HR. The chemistry of rhenium and technetium as related to the use of isotopes of these elements in therapeutic and diagnostic nuclear medicine. Int J Rad Appl Instrum B 1986; 13(4): 465-477.

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A quantitative study of breast cancer glucose and lipid metabolism with nonlinear optical microscopy. J. Hou, A. Paul, E. Gratton, E. Botvinick, E.O. Potma, B. Tromberg; University of California, Irvine, Irvine, CA

We study the correlation of breast cancer cell glucose and lipid metabolism with cancer proliferation and metastasis by nonlinear optical microscopy (NLOM). The cellular glucose metabolism is assessed by imaging the metabolic coenzymes (NADH and FAD+) through two photon excited fluorescence (TPEF) microscopy and fluorescence lifetime microscopy (FLIM). We evaluate the oxidative/glycolytic rate from fluorescence lifetime of NADH and from redox ratio calculated as the intensity ratio of FAD+/(NADH+FAD+). We map out the lipid distribution by coherent anti-Stokes Raman scattering (CARS) microscopy and develop a Matlab program based on machine learning algorithm to compute the lipid percentage from CARS images. Commercial cell lines (MCF10A, T47D, MB231 and PME) and cells extracted from patient biopsies are suspended in Matrigel/collagen mixture and are dynamically monitored for 2-3 weeks. In the 3D environment, we observed that different cell types show different metabolic rates and morphological patterns corresponding to their proliferation and metastatic status. Normal breast cells and non-malignant cells show highest redox ratio and lipid percentage and form polarized structures (acini) with a hollow center. The malignant cells, which form solid spheroids, are the most glycolytic and have less lipid storage in cytoplasm. The metastatic groups, with a moderate glucose metabolic rate and lowest lipid percentage, do not form any growth arrested structure. A shift from glycolytic to oxidative and an increase in lipid content have also been observed during the acini formation. The results of this study have the potential to enhance the understanding of the correlation of cell metabolism and cancer progression and can lead to novel strategies and targets for therapy and prevention.

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Cell to cell communication of exosomal miR-210 released by hypoxic breast cancer cell in tumor microenvironment. K.O. Jung[1], H. Youn[2], M.J. Kim[3], K.W. Kang[4], D.S. Lee[5], J.-K. Chung[6]; [1]Biomedical Sciences, Seoul, Korea, [2]Cancer Imaging Center, Seoul National University Hospital, Seoul, Korea, [3]Department of Nuclear Medicine, Seoul, Korea, [4]Laboratory of Molecular Imaging and Therapy, Cancer Research Institute, Seoul, Korea, [5]WCU Graduate School of Convergence Science and Technology, Seoul, Korea, [6]Tumor Microenvironment Global Core Research Center, Seoul, Korea

Purpose: Breast cancer cells actively release exosomes, which are known to carry specific cellular components such as proteins, mRNA, and miRNA for communicating various cells in the tumor microenviroment. In this study, we visualized exosome-mediated transfer of hypoxia-induced miR-210 in tumor microenvironment cells. **Methods:** Mouse breast cancer cells, 4T1, and mouse endothelial cells, SVEC, were transfected with miR-210 specific reporter (pCMV-luc2/miR-210) which was designed to be turned off luciferase signals by binding of miR-210. Hypoxia was induced by desferoximine (DFO). Mouse blood was collected by cardiac puncture, and exosomes were harvested from mouse blood and conditioned cell culture medium. Exosomes were isolated by ultracentrifugation or ExoQuick and characterized with Western blot and TEM. Real-time PCR was performed to measure the amount of miR-210. Luciferase activity was measured by luciferase activity assay and IVIS imaging. In tumor tissues, immunohistochemistry was performed for detecting HIF-1a, luciferase, Ephrin-A3, PTP1B, and VEGF. **Results:** The amount of miR-210 was increased in hypoxic 4T1 cells (15.70 fold) and in the exosomes from the hypoxic tumor cells (12.73 fold). In bioluminescence imaging, luciferase signals of Exo (+) 4T1 and Exo (+) SVEC cells were decreased compared to that of Exo (−) cells. In xenograft mouse models, luciferase signals decreased (0.53 compared to control tumor) from the tumors treated with DFO, indicating increased miR-210 activity. In addition, the luciferase signals also decreased (0.56) from tumors treated with hypoxic exosomes, suggesting exosome-mediated transfer of miR-210. The expression of Ephrin-A3 and PTP1B, miR-210 target proteins, was also decreased in the hypoxic exosome-treated tumor cells, while the expression of VEGF was increased in those cells. **Conclusions:** Transfer of miR-210 through exosomes was successfully visualized in mouse breast cancer cells and endothelial cells and had an effect on angiogenesis-related genes. This imaging system also can be applicable for monitoring exosomal miRNA transfer in other cells from tumor microenvironment.

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In vivo PET imaging of radiolabeled exosomes from breast cancer cells. K.O. Jung[1], H. Youn[2], M.J. Kim[3], S.W. Oh[4], K.W. Kang[5], D.S. Lee[6], J.-K. Chung[7]; [1]Biomedical Sciences, Seoul, Korea, [2]Cancer Imaging Center, Seoul National University Hospital, Seoul, Korea, [3]Department of Nuclear Medicine, Seoul, Korea, [4]Department of Nuclear Medicine, Seoul National University Boramae Hospital, Seoul, Korea, [5]Laboratory of Molecular Imaging and Therapy, Cancer Research Institute, Seoul, Korea, [6]WCU Graduate School of Convergence Science and Technology, Seoul, Korea, [7]Tumor Microenvironment Global Core Research Center, Seoul, Korea

Purpose: Cancer cells release exosomes to communicate with environment cells, promoting angiogenesis and metastasis. However, physiological distribution of the exosomes was not clarified until now. To monitor the biodistribution of exosomes, we performed PET imaging of radiolabeled exosomes by different injection routes in the mouse. **Methods:** Exosomes were isolated from cultured medium of 4T1, mouse breast cancer cell line, using ExoQuick. Exosomes were characterized by Western blot and TEM. Purified exosomes were labeled with 64Cu or Cy-7 for in vivo imaging. In mice, radio-labeled exosomes were injected by tail vein or footpad injection. PET images were obtained by PETBOX. After sacrificing animals, radio-activities of organs were measured, and immune-histostaining was performed. **Results:** Thin layer chromatography showed 90% labeling purity for 64Cu. PET images for i.v. injected exosomes showed high radioactivities in the lung, liver, and spleen compared to the control 64Cu. Footpad injected exosomes showed more retention time in lymph node than control 64Cu. In immunohistochemistry, brachial and axillary lymph nodes showed more uptake of exosomes than inguinal lymph node. **Conclusions:** Exosomes from tumor cells were successfully labeled and visualized by PET imaging. This is the first PET imaging of radio-labeled exosomes. This imaging system can be useful for monitoring the distribution and tracking of exosomes to understand their localization.

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Near infrared photoimmunotherapy: basis, applications, and beyond. H. Kobayashi; NCI/NIH, Bethesda, MD

Near infrared photo-immunotherapy (NIR-PIT) is a newly developed, molecularly targeted cancer photo-theranostics (imaging diagnosis plus therapy) using conjugates of a near infrared silica-phthalocyanine dye, IR700, to a monoclonal antibody (MAb) thereby targeting specific cell-surface molecules after intravenous administration of antibody-photosensitizer conjugate (APC). When exposed to NIR light, the conjugate induces a highly selective necrotic cell death only in receptor-positive MAb-IR700-bound cancer cells (Nat Med. 2011). Necrosis occurs as early as 1 minute after exposure to NIR light and results in irreversible morphologic changes including cellular swelling, bleb formation, and rupture of vesicles due to

membrane damage based on photo-reactive structural changes of associated proteins with photo-immunoconjugates induced by photo-chemical cleavage of silica-phthalocyanine dye. Meanwhile, immediately adjacent receptor-negative cells are unharmed. Due to the concentration gradient of APC leaking from vessels, NIR-PIT first causes necrosis in perivascular cancer cells resulting in dramatically enhanced vascular permeability with enhanced nano-particle delivery to cancer tissue, an effect termed "super-enhanced permeability and retention (SUPR)" (ACS Nano. 2013). The combination of PIT and SUPR effects can effectively treat a variety of solid cancers including inhomogeneous cancers and cancer stem-like cells by employing different targeting molecules (including but not limited to MAbs) and nano-sized anti-cancer drugs. Preclinical examples of successful NIR-PIT, employing a variety of single- and multi-molecular target NIR-PIT (EGFR, HER2, CD25, PSMA), combined with nano-sized anti-cancer agents (DaunoXome, Ablaxane), will be shown. The combination of NIR-PIT and nano-sized systemic therapies is especially well adapted for real world heterogeneous tumors containing both receptor positive and receptor negative cells. Now NIR-PIT is ready for a first-in-human study against head and neck cancer.

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Multicenter clinical trials using FDG PET to predict chemotherapy response: effects of injection-to-acquisition time variability on required sample size. B.F. Kurland[1], M. Muzi[2], L.M. Peterson[2], R.K. Doot[3], K.A. Wangerin[2], D.A. Mankoff[3], H.M. Linden[2], P.E. Kinahan[2]; [1]University of Pittsburgh, Pittsburgh, PA, [2]University of Washington, Seattle, WA, [3]University of Pennsylvania, Philadelphia, PA

Uptake time (duration between tracer injection and image acquisition) has considerable effect on FDG PET SUV quantitation, and deviations from standardized timing are inevitable in both clinical practice and multicenter clinical trials. When serial scans have dissimilar uptake times, the percentage change in FDG PET SUV may be attenuated or exaggerated. This study examines the influence of uptake time using virtual clinical trials based on kinetic parameters and variability obtained from dynamic FDG PET scans of locally advanced breast cancer patients' tumors. **Methods:** We assessed the impact of uptake time variation in terms of sensitivity and specificity of percent change in FDG PET SUV. Sensitivity was defined as the probability of observing a change of 20% or greater decrease in FDG PET SUV, given a true change of −30%. Specificity was defined as the probability of observing absolute change of <20% if there was no true change. Sensitivity and specificity were based on analysis of 1000 simulated baseline time-activity curves, 1000 simulated repeat scans (no change in the underlying curve), and 1000 simulated scans with 30% decrease from the underlying curve. Variability was added to the model-simulated time-activity curves (TACs) to replicate the level observed in the FDG PET TACs for the breast cancer cohort (overall coefficient of variation of 4% at 45-60 min). Each simulated time-activity curve provided SUVmean values at 5 min time frames, from 45-120 min in 1 min increments covering the range of the observed variation in PET facility uptake time over 5 years. Uptake time to determine SUVmean values for change measures was sampled separately from the baseline, repeat, and 30% decrease curves. Three scenarios were used to sample uptake times. First, SUVmean for 60-65 minutes after injection was selected as an ideal static measure. Second, uptake times were sampled with replacement from a quality control database of FDG body scans at a PET facility with strict oversight of calibration and patient protocols. Third, uptake times were sampled with equal probability from 45-120 minutes after injection, to measure the impact of extremely loose oversight of uptake time. **Results:** Simulated 60-65 minute baseline SUVmean values ranged from 1.1 to 16.9. The uptake time pattern had a marked impact on the distribution of observed percentage change in SUV. Sensitivity was 95% (95% confidence interval 94-96) when all scans began at 60 minutes after injection. The uptake time for 2003 FDG body scans at a well-regulated academic institution's PET facility ranged from 47-114 minutes, with a median of 60 minutes. 23% of scans started outside a window of 55-65 minutes (3% <55 minutes, 20% >65 minutes). The sensitivity was 91% (89-92) when sampling uptake time with replacement from the PET facility recorded times. Finally, sensitivity was 72% (70-75) when uptake time was sampled with equal probability from 45-120 minutes after injection. Specificity for these three scenarios was 98% (97-99), 94% (93-96), and 66% (63-69). **Conclusions:** Minor variations in the uptake time had little impact on sensitivity and specificity of tumor response measurements, while large variations in the uptake time substantially degraded both sensitivity and specificity. While adherence with minor variations is feasible, large variation in uptake time has been observed in practice and can lead to severe underpowering of multicenter trials using PERCIST or other measures of metabolic response.

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Retinography: neurographic molecular imaging with a near infrared optical imaging probe to assess the function and number of retinal ganglion cells in glaucoma. L. Le Roux, X. Qiu, L. Flores II, D. Piwnica-Worms, D. Schellingerhout; The University of Texas MD Anderson Cancer Center, Houston, TX

Objectives: To develop a NIR fluorescent probe for nerve imaging based on the fast retrograde neural transport mechanism to visualize retinal ganglion cells (RGCs) in a glaucoma model. **Methods:** A well characterized model of excitotoxic glaucoma was induced in rat (n=3) eyes by injecting NMDA (N-methyl-D-aspartic acid, 50 nmol/2.5 μL) into the vitreous of one eye. This model is known to induce apoptosis in RGCs. Twenty-four hours after the NMDA injection, the NIR fluorescent neural imaging probe consisting of the non-toxic C-fragment of tetanus toxin (TTc790) was also injected into the vitreous of both the contralateral normal (control) eye and the NMDA-treated eye (8 μg of TTc790/2 μl of PBS). In vivo imaging of the distribution of TTc790 was performed using confocal-scanning laser ophthalmoscopy (cSLO) (Retinal Angiograph II, Heidelberg, Germany). Both eyes and associated neural tissues were harvested at 2-3 hours after TTc790 injection for ex vivo microscopic imaging using an epi-fluorescent microscope with NIR imaging capabilities (AxioZoom16, Zeiss Microscopy, Germany). **Results:** Images were consistent with RCGs showing the most TTc790 uptake. The RGCs are the final projection neurons of the retina, responsible for carrying information from the eye through the optic nerve to the lateral geniculate nucleus. RGC axons are the principal component of the optic nerve. Live animal and ex-vivo neural uptake studies within the rat retina demonstrate a) avid TTc790 uptake in RGCs, b) that both in vivo and ex vivo imaging is feasible and c) that an NMDA-induced model of glaucoma shows greatly reduced uptake and transport of TTc790 that can be detected by live cSLO imaging. Preliminary statistical data analysis of raw fluorescent output from the retina showed 11,460 ± 1,601 AU for normal and 9,255 ± 224 for NMDA-treated eyes ($p<0.05$, two-tailed paired t-test). **Conclusion:** A novel fluorescent NIR probe, TTc790, allows the visualization and quantitation of retinal ganglion nerve cells. We show significant decreases in neuronal uptake and transport of TTc790 in a glaucoma model, a condition in which the hallmark pathology is loss of RCGs. There are clear implications for clinical staging and monitoring of patients at risk of blindness from glaucoma. **Acknowledgment:** Research support: NINDS R01NS070742.

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Eco-therapy: changing the tumor microenvironment treatment of solid tumors. B. Li, L. Zhang; The Second Affiliated Hospital of Shanxi Medical University, Taiyuan, China

Background: The tumor microenvironment has a regulatory role in tumor occurrence, progression and transfer. Tumor cells can constantly adapt to specific environments. Tumor microenvironment can also promote or limit tumor cells by dynamically changing the composition and related functions. To control the tumor through a single drug is impossible to achieve so that we will treat the tumor microenvironment as a new therapeutic target, changing the appropriate microenvironment of tumor cell survival to limit tumor progression and metastasis with the purpose of controlling the tumor. **Methods:** From January 2013 to June 2014, we used eco-therapy to treat 131 cases with advanced lung adenocarcinoma.These patients completed at least one complete cycle of therapy in the Second Affiliated Hospital of Shanxi Medical University, Oncology. We observed its recent efficiency, the score of life quality and one year survival rate. Also, we compared these with data in the relevant literature. Cox regression analyses prognostic factors. **Results:** CR: 4 cases; DR: 99 cases; SD: 28 cases; PD: 0 cases; objective response rate: 78.63%; FACT-L improvement rate: 100%; year survival rate: 67.18%. Indicators were higher than the data in relevant literature. Cox regression analysis showed that the control condition of disease after

treatment has an influence on patients' relief ($p<0.01$) while sex, tumor stage and smoking history do not ($p>0.05$). **Conclusion:** The eco-therapy can significantly improve the recent efficiency of advanced lung adenocarcinoma and obviously increase the one year survival rate. **Discussion:** Eco-therapy is such an organic combination of biological response modifier, autologous immune cells, anti-angiogenesis drug, molecular targeted drug and three oxygen treatment technology, changing tumor's related microenvironment: 1. To reduce the content of collagen fibers in tumor issue and to change its hard physical trait. 2. The normalization of tumor blood vessels, changing the low oxygen, high acid and high interstitial pressure. 3. Enhance the immune effect and reverse immunosuppression status to promote the transformation from microenvironment to immune support. 4. Eco-therapy combining with three oxygen treatment will improve the hypoxia, enhance immune killing effect and scavenge free radicals and toxic substances, thereby establishing normal cell social ecology. Eco-therapy is to reduce the number of tumor cells at the same time to slow down the residual tumor cells and tumor stem cell proliferation, thus significantly improving the clinical effect of treatment of advanced lung adenocarcinoma.

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Imaging cell signaling dynamics in vivo. T.W. Liu, D. Piwnica-Worms; The University of Texas MD Anderson Cancer Center, Houston, TX

Tumor development is mediated by a network of signaling pathways and interactions with the microenvironment. This network changes spatially and temporally over the course of tumor development, resulting in heterogeneous cell populations with differing, and often transient, phenotypes. For example, β-catenin is thought of as the master switch controlling proliferation versus differentiation, where nuclear β-catenin accumulation may induce epithelial-mesenchymal transitions. Only by considering the system as a whole and understanding the interplay between signaling networks and cell phenotypes, can we begin to understand the contribution of the microenvironment during tumor development. Utilizing intravital imaging with dorsal skinfold window chamber animal models and imaging reporters (injectable and genetically encoded), we began to evaluate in real time the interactions between cell phenotypes and signaling cascades of populations and individual cells in heterogeneous living systems. Using a melanoma cell line, B16F10, we demonstrate heterogeneity in the transcriptional activity of nuclear factor κB (NFκB) and β-catenin during tumor development. B16F10 cells were transduced to stably express a click beetle green-green fluorescent protein (CBG-GFP) gene under a ubiquitin promoter and either an NFκB-Firefly luciferase reporter gene (Cignal lenti reporter) or a 7xTcf-Firefly luciferase reporter gene. Following tumor cell injection within dorsal skin window chambers using C57B6 mice, transcriptional activity of the reporters during tumor growth was monitored in vivo using a commercially available small animal bioluminescent and fluorescent imaging system. High resolution imaging was achieved using an automated microscope with high numerical aperture objectives and a deeply cooled one megapixel back illuminated CCD camera. Changes in reporter activity were quantified over time as tumor growth was monitored; the CBG-GFP reporter both monitors tumor growth and serves as an internal control for normalization in this dual-luciferase assay. Finally, this strategy provides an advance of technology by demonstrating high resolution single cell microscopic imaging using bioluminescent reporters in vivo.

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Molecular imaging approaches to study normal tissue protection by multi-functional cerium oxide nanoparticles during radiation therapy. P.R. McDonagh, L. Yang, M. Sun, P. Jose, G. Sundaresan, A. Alam, R. Mikkelsen, J. Zweit; Virginia Commonwealth University, Richmond, VA

Along with chemotherapy and surgery, radiation therapy (RT) is a leading treatment option for cancer. It is an element in the treatment plan of over 50 million cancer survivors worldwide. Even with advances in conformal image guided therapies, RT is still limited by damage to normal tissues. Both short and long term side effects have been associated with RT, including anemia, gastrointestinal distress, skin irritation, tissue fibrosis, and even secondary cancer. These compromise the quality of life for many patients during RT. Our work investigates cerium oxide nanoparticles (CONPs) for their application as radio-protective agents administered during RT to prevent normal tissue toxicities. These nanoparticles have demonstrated an ability to reduce radiation side effects by reducing radicals and preventing radiation induced DNA damage, and doing so preferentially in normal tissue over tumor tissue. The mechanisms of radio-protection by CONPs are not well characterized, and comprehensive *in vivo* studies are currently lacking. The discrepancy in protection of tumor versus normal tissue may be related to differences in the tissue microenvironments. This work has aimed to develop multi-functional CONPs with polymer coatings and radiolabeling which can be used for both biodistribution and mechanistic studies. A polymer coating allows the nanoparticles to become biocompatible as the nanoparticles no longer aggregate and become stable in solution, reducing toxicity with no demonstrated effect on radical scavenging ability. Changing the surface chemistry using several differently charge polymers allows us to manipulate the blood circulation, renal/hepatic clearance, and tumor versus normal tissue uptake of the CONPs. Incorporation of imaging isotopes, including ^{89}Zr, ^{141}Ce, and ^{111}In, into the core of CONPs to create intrinsically radiolabeled CONPs (rCONPs) has allowed the collection of detailed *in vivo* PET/SPECT imaging and *ex vivo* biodistribution data. This has demonstrated the improved distribution and normal tissue retention of polymer coated nanoparticles when compared to uncoated nanoparticles. *Ex vivo* biodistribution results matched SPECT imaging of 141Ce-rCONP, showing accumulation mainly in the lungs, liver, and spleen in a mouse over a one week period with no noted toxicities. Mice bearing colon cancer (HCT116) xenograft tumors showed lower or similar uptake of polymer coated rCONPs in the tumors compared with several normal tissues including heart, lungs, bone marrow, and colon, demonstrating promising biodistribution for protection of at risk tissues. Further studies have demonstrated that pre-treatment of mice with IV injected CONPs before 10Gy irradiation showed radiation protection through reduced apoptosis in normal colon tissue compared to mice only irradiated. Xenograft tumors from these animals showed decreased clonogenic colony formation in mice treated with CONP and 10Gy irradiated, compared to those only irradiated. This demonstrates a possible role of CONPs as radio-sensitizers specific to tumors. FDG PET imaging of spontaneous colon cancer tumors in mice has also shown no difference in effect on tumor size when mice are treated with CONPs and irradiation versus irradiation alone, further indicating the CONPs' radio-protective properties are selective to normal tissue. These studies set the groundwork for future in vivo biomedical imaging examining CONPs' microenvironment dependent properties, which will include MR imaging of tumor pH and photoacoustic imaging of tumor hypoxia. The data from this project should form a picture of how CONPs interact with the intact tumor and normal tissue environments and determine their potential for translation to the clinic as radio-protective agents to increase RT therapeutic index.

*AACR-Aflac, Incorporated, Scholar-in-Training Award winner.

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^{64}Cu-, ^{68}Ga-, and ^{177}Lu-LLP2A conjugates for imaging and treatment of very late antigen-4 (VLA-4). J. Nedrow, W. Beaino, C. Anderson; University of Pittsburgh, Pittsburgh, PA

Background: Melanoma demonstrates therapeutic resistance, aggressive clinical behavior, and predisposition for late metastasis. Very late antigen 4 (VLA4) is specifically overexpressed in metastatic melanoma tumors and provides a potential target for radiotherapy. Radiolabeled LLP2A conjugates can visualize cancer sites through PET imaging (^{68}Ga and ^{64}Cu) or SPECT imaging (^{177}Lu). In addition, ^{64}Cu and ^{177}Lu have tumor cell killing properties for targeted radiotherapy of metastatic melanoma. Here we evaluated modified LLP2A conjugates labeled with ^{64}Cu, ^{68}Ga and ^{177}Lu for PET-CT imaging and investigated the potential of ^{177}Lu-LLP2A for radiotherapy in an aggressive mouse melanoma model. **Methods:** LLP2A was conjugated to NODAGA and CB-TE1A1P for ^{64}Cu labeling, DOTA and NODAGA for ^{68}Ga labeling, and DOTA for ^{177}Lu labeling. The biodistribution of the conjugates was performed in B16F10 tumor bearing mice. PET-CT imaging was performed with the ^{64}Cu and ^{68}Ga tracers. **Results:** ^{68}Ga-NODAGA-PEG$_4$-LLP2A (K_i=0.11 nM, 8.7±1.3 %ID/g) demonstrated similar tumor uptake compared to ^{68}Ga-DOTA-PEG$_4$-LLP2A (K_i=1.56 nM, 9.1±0.9 %ID/g) but with greater renal and liver retention. ^{64}Cu-labeled CB-TE1A1P-PEG$_4$-LLP2A (K_d=0.28 nM, 16.9±2.2 %ID/g) had higher uptake compared to NODAGA-PEG$_4$-LLP2A (K_d=0.23 nM, 13.4±1.7 %ID/g) at 2 h post-injection. Tumor to muscle and blood ratios were significantly higher for ^{64}Cu-CB-TE1A1P-PEG$_4$-LLP2A compared to ^{64}Cu-NODAGA-PEG$_4$-LLP2A. In

addition, liver and kidney uptake was lower for ^{64}Cu-CB-TE1A1P-PEG$_4$-LLP2A. ^{177}Lu-DOTA-PEG$_4$-LLP2A demonstrated the highest uptake at 4 h (28±10.7 %ID/g) compared to the ^{68}Ga and ^{64}Cu tracers with excellent tumor to muscle and blood ratios. **Conclusion:** ^{64}Cu and ^{68}Ga-labeled LLP2A conjugates are excellent PET imaging agents for melanoma, with ^{64}Cu-CB-TE1A1P-PEG$_4$-LLP2A being the superior PET agent. The ^{177}Lu-labeled conjugate demonstrated significant uptake in VLA-4-positive tumors, providing a potential agent for radiotherapy of difficult to treat metastatic melanoma.

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The theranostic somatostatin receptor antagonist pair 68Ga-OPS202/177Lu-OPS201 is superior to the agonist pair 68Ga-DOTA-TATE/177Lu-DOTA-TATE: a preclinical and clinical phase I/II study. G.P. Nicolas[1], R. Mansi[1], J. Kaufmann[2], H. Bouterfa[2], H.R. Maecke[3], D. Wild[1], M. Fani[1]; [1]University Hospital Basel, Basel, Switzerland, [2]OctreoPharm Sciences GmbH, Berlin, Germany, [3]University Hospital Freiburg, Freiburg im Breisgau, Germany

Aim: Radiolabeled somatostatin (sst)-antagonists have shown higher uptake in sst receptor-expressing tumors than sst-agonists. We aimed at evaluating a theranostic pair of the sst2 receptor antagonist Cpa-c(DCys-Aph(Hor)-DAph(Cbm)-Lys-Thr-Cys)-DTyr-NH2 (JR11) and compared the results with sst2-agonist DOTA-TATE for PET imaging and targeted radionuclide therapy of neuroendocrine tumors. **Materials and Methods:** OPS201 (DOTA-JR11) was radiolabeled with 177Lu and 90Y. OPS202 (NODAGA-JR11) was radiolabeled with 68Ga. The theranostic pairs (68Ga-OPS202/177Lu-OPS201 or 90Y-OPS201) were compared head-to-head with the well-established agonists 68Ga-DOTA-TATE/177Lu-DOTA-TATE. Receptor affinity, in vivo biodistribution and pharmacokinetics of all tracers were investigated in HEK-hsst2 xenografts. The influence of the peptide mass was studied in vivo in terms of image contrast and tumor-to-background dose ratios. OPS202 is currently under clinical evaluation in patients with neuroendocrine tumors. **Results:** 177Lu-OPS201 and 90Y-OPS201 have similar affinity for the sst2 (IC50 = 0.73±0.15 and 0.47±0.05 nM, respectively), and showed also similar uptake in vivo in all sst2-expressing normal tissues, like stomach, pancreas and adrenals. However, tumor uptake of 90Y-OPS201 was lower than 177Lu-OPS201 4h p.i. (16.9±2.5 and 23.9±4.3 %IA/g, respectively), while 177Lu-OPS201 showed higher uptake than 177Lu-DOTA-TATE (17.8±4.0 %IA/g, 4h p.i.). The median tumor residence time for 177Lu-OPS201 was 19.2h and for 177Lu-DOTA-TATE 7.4h, resulting in a 2.6 times higher tumor dose for the antagonist than for the agonist. The dose to the kidneys was 1.8-fold higher with 177Lu-OPS201 than with 177Lu-DOTA-TATE. Tumor-to-kidney ratio at 4h was 3.5±0.7, 3.9±0.5 and 4.2±0.6 for 177Lu-DOTA-TATE, 177Lu-OPS201 and 90Y-OPS201, respectively. The therapeutic index defined as tumor-to-kidney dose ratio was increased by 34% in favor of 177Lu-OPS201 compared to 177Lu-DOTA-TATE. Importantly, injection of 10, 200 and 2000 pmol of 177Lu-OPS201 caused no relevant saturation effect in the tumor (23.9, 24.9 and 18.8 %IA/g, respectively, at 4h p.i., $p>0.05$) while the background significantly decreased. Consequently, the tumor-to-background ratios increased, (i.e., tumor-to-liver from 16 to 52 and 84 and tumor-to-bone marrow from 25 to 97 and 159, respectively, 4h p.i.). There was no significant change of the tumor-to-kidney ratio (3.9, 3.3 and 3.3, respectively). The tumor uptake of 177Lu-DOTA-TATE, on the other hand, significantly decreased by 30% and 45%, when 200 or 2000 pmol were injected, respectively ($p<0.05$). Small-animal SPECT/CT images reflected the biodistribution results. Regarding the PET tracers, 68Ga-OPS202 showed 1.7-fold higher tumor uptake compared to 68Ga-DOTA-TATE, at 1h p.i. and higher tumor-to-liver and tumor-to-pancreas ratios (49.5 vs 41.3 and 2.69 vs 1.65, respectively). Initial results of a clinical phase I/II trial using 68Ga-OPS202 PET/CT in comparison to 68Ga labeled agonist confirmed the preclinical results. **Conclusion:** Improved tumor-to-background ratios of 68Ga-OPS202 (liver, pancreas and gastrointestinal tract) in neuroendocrine tumor patients holds promise for increased sensitivity in staging neuroendocrine tumors. The increased tumor uptake and prolonged residence time of 177Lu-OPS201 compared to 177Lu-DOTA-TATE may improve the safety window of targeted radionuclide therapy. The preclinical mass-dependent study provides important information that an optimized antagonist-mass is likely to reduce liver and bone marrow toxicity.

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A genomic study of alteration events caused by ionizing radiation in human embryonic stem cells via next-generation sequencing. V. Nguyen, I.V. Panyutin, R.D. Neumann, I.G. Panyutin; NIH, Bethesda, MD

Ionizing radiation (IR) is widely employed for medical diagnostic and therapeutic purposes. However, the biological effects of IR from clinically relevant diagnostic doses remain unclear. A novel model using human embryonic stem cell (hESC) cultures was established to conduct our study of IR effects. Cells were subjected to CT scans and gamma irradiation at low (0.2 Gy) or high (1 Gy) doses and then maintained in culture until confluence before harvesting for DNA isolation. Since IR is often implicated as a risk for inducing cancer, a primer pool targeting genomic "hotspot" regions that are frequently mutated in human cancer genes was used to generate libraries from low-, high-dose and control samples of four hESC cell lines: H1, H7, H9, and H14. Using a semiconductor-based deep sequencing approach, we were able to consistently sequence these samples with reliable coverage for data analysis. Single nucleotide variants, occurring at very low frequencies (~2%), were identified after the high-dose exposure using two independent analysis software platforms, thus confirming the utility of our method. We did not detect any differences in the number of genetic alterations between the CT or low-dose samples and controls. This suggests that in these highly sensitive hESCs, low-dose IR did not result in a detectable increase in genetic alteration events occurring within the cancer "hotspot" gene regions. Future studies are warranted to confirm the apparent lack of detectable genetic alterations in hESCs by IR at or below 0.2 Gy.

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Irradiation of normal tissues stimulates tumor cell migration. M. Rafat, M. Vilalta, T.A. Aguilera, A.J. Giaccia, E.E. Graves; Stanford University, Stanford, CA

Rates of recurrence remain high for triple-negative breast cancers despite aggressive surgical, radiological, and chemotherapeutic intervention. Recent studies have suggested that recurrence may be facilitated by tumor self-seeding through circulating tumor cells. The role of the tumor microenvironment in recurrence, however, is not fully understood. We have determined previously that radiation enhances the migration of tumor cells in a preclinical breast cancer model. We hypothesize that the irradiated tumor stroma and microenvironment may influence tumor cell migration. In this study, we characterize the effects of normal tissue irradiation on tumor cell migration to evaluate how tumor-stromal interactions modulate recurrence after therapy. This work represents the first step toward elucidating the contribution of stromal tissues in tumor and immune cell recruitment following radiotherapy. Mouse embryonic fibroblasts (MEF) were irradiated at 20 Gy with a cesium source. Supernatant was collected after 2 or 7 d incubation to be used as a chemoattractant in an in vitro transwell assay to investigate the induction of 4T1 murine mammary carcinoma cell invasion. In addition, an orthotopic breast cancer model was used to evaluate the effect of radiation on tumor cell migration to normal tissues. 4T1 cells were stably transduced with viral vectors encoding tdTomato and luciferase for in vivo and ex vivo fluorescence and bioluminescence imaging (BLI). Nude mice were inoculated with 4T1 cells in the mammary fat pad (MFP) and injected in the contralateral MFP with phosphate buffered saline (PBS). The contralateral normal MFP was irradiated to a dose of 20 Gy with a 250 kVp cabinet x-ray machine when tumors reached 100 mm^3. Cell migration was monitored with BLI 10 days after irradiation. Irradiated and control tissues were fixed and embedded in paraffin for immunohistochemistry (IHC) analysis. Tissue sections were stained with F4/80 to determine the extent of macrophage infiltration. Flow cytometry was also performed on dissociated irradiated and control tissues to characterize immune cell populations. Of particular interest were macrophages and myeloid-derived suppressor cells (MDSCs), which were defined as CD11b+F4/80+ and CD11b+GR1+ cell populations, respectively. Tumor cell migration to normal tissues both in vitro and in vivo was enhanced by radiation. 4T1 cells exhibited an increase in invasion ($p<0.05$) in vitro when exposed to irradiated MEF media. Ex vivo BLI analysis demonstrated that normal tissue irradiation attracted tumor cells to the MFP and surrounding tissues, including the peritoneum and muscle ($p<0.001$). IHC staining revealed an increase in macrophage infiltration in irradiated tissue sections. Flow cytometric analysis confirmed

an increase in macrophages as well as MDSCs in irradiated areas. Our study establishes that normal tissue radiation response may play a role in modulating tumor cell migration after radiation. The increase in macrophage and MDSC infiltration after irradiation indicates the contribution of the immune system in tumor cell migration. These results suggest that the tumor stroma may facilitate tumor regrowth and tumor cell invasion following radiotherapy.

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Quantitative PET and MRI to monitor tumor growth and treatment response in hollow fiber bioreactors. S.D. Rani, K.L. Jameson, J. Hao, D.C. Colvin, D.R. Tyson, J. Fellenstein, E.C. Rericha, V. Quaranta, T.E. Yankeelov; Vanderbilt University, Nashville, TN

Objective: A major barrier to quantitatively studying cancer is the lack of a robust, controllable in vitro assay that allows for serial and noninvasive multiscale measurements to interrogate growth behavior before and during therapy. In the current work, the feasibility of performing serial fluorodeoxyglucose positron emission tomography (^{18}F-FDG-PET) and diffusion weighted magnetic resonance imaging (DW-MRI) on three different hollow fiber bioreactors provides preliminary evidence for a robust 3D platform to quantitatively monitor tumor growth. **Methods:** ^{18}F-FDG-PET reports on glucose metabolism, while DW-MRI is a method to estimate cell density. Tumor cell proliferation was longitudinally monitored by ^{18}F-FDG-PET and DW-MRI within three different bioreactor configurations (Fibercell, Fredrick, MD) varying in density and composition of hollow fibers, which exchange media to and from the extracapillary space (ECS) through diffusion. Type I consisted of 20 permeable hollow fibers (1.2 mm outer diameter (OD) and 0.8 mm inner diameter (ID)), type II consisted of >200 fibers (200 μm OD and 180 μm ID), and type III consisted of 25 fibers (1.2 mm OD and 0.8 mm ID). For these studies we used a non-small cell lung cancer cell line (PC9) that is highly sensitive to the small molecule kinase inhibitor erlotinib. Cells were seeded and maintained in the bioreactors for up to 8 weeks. PET imaging started with 60 min dynamic acquisition after ^{18}F-FDG bolus injection into the bioreactor, followed by another 60 min dynamic acquisition during tracer flushing. A two-compartment four parameter ^{18}F-FDG model was employed to estimate the net glucose utilization by the cells in the bioreactor. DW-MRI was acquired on all three bioreactor types to generate apparent diffusion coefficient maps, which were converted to estimates of cell number and, subsequently, cell proliferation over time using our previously published methods (1). **Results:** All three types of bioreactors were capable of maintaining cells in culture for up to eight weeks. Serial ^{18}F-FDG-PET and MRI measurements were acquired during this period and glucose utilization, tumor cell number and proliferation were quantitatively assessed. We observed cell growth in all three types of bioreactors. **Conclusions:** We demonstrate the feasibility of performing ^{18}F-FDG-PET and DW-MRI on hollow fiber bioreactors to monitor tumor cell growth at tissue level densities. Preliminary PET and MRI data suggest that the bioreactor serves as a promising in vitro platform for tumor studies that recapitulates some features of in vivo systems and enables long term multimodal imaging. Ongoing studies are investigating the ability of PET and MRI measures to assess response to a course of oncogene targeted therapy within the bioreactor. **Reference:** Atuegwu et al. Magn Reson Med. 2011;66:1689-96.

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Zaprinast inhibits glutaminase and growth of glutamine-addicted cells. Y. Rao[1], A. Elhammali[2], S.T. Gammon[1], D. Piwnica-Worms[1]; [1]The University of Texas MD Anderson Cancer Center, Houston, TX, [2]Washington University St. Louis, St. Louis, MO

Zaprinast, a PDE5 inhibitor, was identified through a high throughput fluorescent screen as an inhibitor of the production of the oncometabolite 2-hydroxyglutarate (2HG). Through monitoring of metabolites by mass spectrometry, the mechanism of action was not through inhibition of PDE5, but rather, through inhibition of glutaminase (GLS1). Direct inhibition of GLS1 was validated by a glutamine competition assay using purified GLS1. Zaprinast displayed classic noncompetitive inhibition kinetics (Ki = 220 uM). In immortalized human astrocytes that expressed gain-of-function mutant isocitrate dehydrogenase 1 (IDH1) overproducing 2HG, Zaprinast-mediated inhibition of GLS1 reversed histone hypermethylation and cell growth in soft agar, two well-known 2HG-associated phenotypes. Additionally, treatment of glutamine-addicted Kras-mutant pancreatic ductal adenocarcinoma cells with Zaprinast reduced growth by greater than 4 fold, increased ROS(+) cells greater than 10 fold, and sensitized cells to H_2O_2-mediated oxidative damage by greater than 2 fold, mimicking the effects of glutamine deprivation ($p<0.05$). Metabolic rescue with cell permeable glutamate reversed the Zaprinast-mediated blockade of GLS1, lending further evidence to the mechanism of action in these cells. More broadly, Zaprinast also inhibited growth of glutamine-dependent triple negative breast cancer cells by 2 fold. Thus, Zaprinast represents a novel pharmacophore for targeting GLS1 that is water soluble, membrane permeant, and bioavailable, providing a potential strategy for suppressing 2HG production independent of IDH mutation status.

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Static ^{18}F-fluorothymidine imaging can reliably quantify tumor proliferation changes in a mouse model of colorectal cancer. S. Rapic[1], C. Vangestel[2], J. Verhaeghe[1], D. Thomae[1], P. Pauwels[2], T. Van den Wyngaert[2], S. Staelens[1], S. Stroobants[2]; [1]University of Antwerp, Antwerp, Belgium, [2]Antwerp University Hospital, Antwerp, Belgium

Background. The positron emission tomography (PET) tracer ^{18}F-fluorothymidine (^{18}F-FLT) is transported across the cell membrane and phosphorylated by thymidine kinase-1 (TK1) causing ^{18}F-FLT to be intracellularly retained. Therefore, ^{18}F-FLT uptake can be considered as a measure of TK1 activity, which is closely linked to proliferation and peaks during the early S-phase of the cell cycle. This kinetic process can be quantitatively measured with a two-compartment model if labor-intensive and time-consuming dynamic PET data are available. We investigate here if semi-quantitative measures based on static images also reliably quantify ^{18}F-FLT uptake *in vivo*. **Aim.** Using gold standard dynamic ^{18}F-FLT-PET imaging, we first investigated the early effects of irinotecan and cetuximab on cancer cell proliferation in a subcutaneous, KRAS-mutated xenograft model (Colo205) of colorectal cancer (CRC). We then evaluated in that dataset whether a simplified, semi-quantitative approach could be used as a surrogate for kinetic modeling. **Methods.** Athymic mice, subcutaneously inoculated with Colo205 cells (cetuximab resistant; irinotecan sensitive), underwent 90 min dynamic imaging before and 24 hours after treatment with vehicle (control, n=8), cetuximab (n=8) or irinotecan (n=7). To determine the compartmental model for ^{18}F-FLT, we tested a two-tissue-compartment model (2TCM) with reversible ($k4\neq0$) and irreversible ($k4=0$) phosphorylation (TK1) using an image derived input function from the left ventricle. The optimal model was chosen on the basis of the Akaike information criteria, where the lowest value represents the best fit. Tumor time activity curves were extracted from volumes of interest encompassing the 30% hottest pixels within the tumor and were fitted. From the fitted kinetic parameters the total distribution volume (DV_{tot}) was calculated and from static images averaged over 70-90 min, mean standardized uptake values (SUV_{mean}) were determined. *Ex vivo* evaluation of proliferation changes was performed using Ki67 immunohistochemistry. **Results.** The optimal compartmental model was found to be 2TCM with reversible phosphorylation. Both dynamic and static imaging parameters showed a significant post-treatment decrease of ^{18}F-FLT uptake in the irinotecan treated group (DV_{tot}: −31%±7, $p=0.0007$ and SUV_{mean}: −20%±5, $p=0.0002$) as opposed to the cetuximab and vehicle treated groups where no significant changes from baseline uptake were seen (DV_{tot}: −4%±3 and −13%±6, respectively, and SUV_{mean}: 7%±3 and −8%±3, respectively). Additionally, DV_{tot} and SUV_{mean} were strongly correlated ($r=0.83$, $p<0.0001$). *Ex vivo* proliferation assessment was in agreement with *in vivo* imaging data, displaying a significantly lower percentage of Ki67-positive cells in the irinotecan treated group as compared with the cetuximab and vehicle treated groups (64%±5 versus 77%±3 and 78%±3, respectively, $p<0.0001$). Also, the percentage of Ki67-positive cells exhibited a moderate yet significant relationship with both imaging parameters (Ki67 versus SUV_{mean}: $r=0.53$, $p=0.0089$ and Ki67 versus DV_{tot}: $r=0.42$, $p=0.0488$). **Conclusions.** A static ^{18}F-FLT imaging protocol proves to be a feasible and reliable quantification method of changes in cancer cell proliferation and may thus be used in future preclinical oncological imaging studies. Furthermore, our findings in a xenograft model of CRC suggest the potential of ^{18}F-FLT to serve as a measure of early tumor response to anti-proliferative treatment.

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Interphase superresolution DNA structure in hematological malignancies. C.H. Righolt[1], C. Sathitruangsak[1], H. Knecht[2], S. Mai[1]; [1]Manitoba Institute of Cell Biology, Winnipeg, MB, Canada, [2]Jewish General Hospital, McGill University, Montreal, QC, Canada

New fluorescence microscopy methods have pushed image resolution beyond conventional, theoretical limits in recent decades. Structured illumination microscopy (SIM) is one of these techniques, in which superresolution is achieved by a combination of patterned illumination and computational reconstruction algorithms. This method has enabled us to unravel DNA structure in the interphase nucleus using a DAPI counterstain. We have studied two hematological malignancies, Hodgkin's lymphoma (HL) and multiple myeloma (MM). For Hodgkin's lymphoma we studied the HDML-2 cell line and compared lymphocytes from healthy individuals with Hodgkin (H) cells and Reed-Sternberg (RS) cells. For MM we compared the normal lymphocytes and malignant cells from peripheral blood of patients with MM and monoclonal gammopathy of unknown significance (MGUS). We used the same sample preparation techniques as for conventional fluorescence microscopy. We used a Zeiss Elyra PS1 SIM to record images and we used standard Zeiss ZEN software for image reconstruction. Structural differences in both the DNA structure and the structure of the DNA-free space were visible from these images, whereas they were not visible using conventional fluorescence microscopy. We used custom-made image analysis algorithms to analyze these differences by estimating the size distribution of the visible structure. Using this granulometry-based method, we measured a progressive change in the structure of HL from the normal lymphocytes to the H cells to the RS cells. Within the population of RS cells we also noticed the same trend by comparing binucleated RS cells with RS cells with four or more subnuclei. There was no difference in the DNA structure between MGUS and MM cells, although both of those cell types differed from their control lymphocytes. The DNA-free space was, however, different between MGUS and MM nuclei. We also noted the appearance of larger open areas ("holes") in the more malignant cells, i.e., larger continuous regions with no or low DNA density. Because we initially thought these areas were nucleoli, we stained for upstream binding factor (UBF), a nucleolus-associated protein. We noted that although UBF does aggregate in some of the "holes," it does so progressively less frequently for the H and RS cells. We confirmed this progressive decrease both by measuring it objectively and by having it assessed by visual inspection. We were successfully able to measure differences in the intranuclear DNA organization for two hematological conditions. Our measurement tools allow for the study of the DNA ultrastructure on the whole cell level with conventional sample preparation techniques and superresolution image quality. Based on these results, we expect these tools to be applicable to other cancers as well.

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Optical surgical navigation using a cathepsin probe and a miniature dual-axis confocal microscope for improved resection of medulloblastoma. S. Rogalla[1], C. Zavaleta[2], S.M. Haag[1], T.L. Schmidt[1], K. Oresic[3], M. Bogyo[3], C.H. Contag[1]; [1]Department of Pediatrics, Stanford University, Stanford, CA, [2]Department of Radiology, Stanford University, Stanford, CA, [3]Department of Pathology, Stanford University, Stanford, CA

Medulloblastoma, an undifferentiated neuroepithelial tumor of the cerebellum, is the most common pediatric brain cancer. Although the current standard of treatment for medulloblastoma has significantly improved survival rates, approximately one-third of these young patients still die of their disease. Additionally, 20% of the survivors sustain severe neurological damage due to resection of normal brain tissue or due to the side effects of radiation or chemotherapy. However, survival for children with this devastating disease correlates only with the extent of resection, and hence there is impetus for aggressive resections without damaging healthy tissue through image guidance. Using subcutaneous and orthotopic xenograft mouse tumor models, we showed that highly specific molecular fluorescent probes in combination with a dual-axis confocal (DAC) microscope can visualize tumor margins in real time. Human medulloblastoma cells (DAOY) expressing GFP and luciferase were used to enable tumor visualization and localization. Intracranial injections were conducted lateral of the frontal fissure through the bone at a depth of 2 mm directly into the cerebellum. Tumor growth was followed for 4 to 6 weeks using in-vivo bioluminescence imaging as a means of staging tumor growth prior to surgical microscopic analyses and resection. Once the specified tumor size was reached, a window in the skull of the mice was created and the tumor was stained using topical application of a cathepsin activity probe, BMV109, to show precise anatomic localization of the tumor. BMV109 is a quenched fluorescent probe that can be activated through cleavage of a covalently bound quencher by cathepsin proteases leading to Cy5 fluorescence. Tumor anatomy was verified by GFP expression from the DAOY cells and compared to BMV109 fluorescence. Resection was guided using a DAC microscope to differentiate cancer from healthy tissue. Post-resection tumors and normal cerebellum were imaged again using a regular surgical resection microscope to validate images collected using the DAC microscope. Using these tools we were able to discriminate tumor from healthy tissue, building a foundation for image-guided resection of brain tumors in children as well as in adults.

*AACR-Aflac, Incorporated, Scholar-in-Training Award winner.

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A clinically translatable wide-field fluorescence fiberscope and dual-axis confocal microscope to visualize dysplastic epithelial surfaces: use of a cathepsin-specific probe in a model of colon cancer. S. Rogalla[1], S. Sensarn[2], C. Zavaleta[2], M. Mandella[1], K. Oresic[3], M. Bogyo[3], C.H. Contag[1]; [1]Department of Pediatrics, Stanford University, Stanford, CA, [2]Department of Radiology, Stanford University, Stanford, CA, [3]Department of Pathology, Stanford University, Stanford, CA

Colon cancer remains one of the most common cancers in industrialized countries despite widespread preventive screening by white-light endoscopy. This current screening standard has a high specificity for dysplastic or malignant neoplasia in the colon; nonetheless, up to 25% of lesions are not detected. The hard-to-visualize missed lesions are typically flat lesions or polyps smaller than 2 mm. Emerging fluorescence-based imaging devices, coupled with molecular probes that target specific biomarkers of dysplasia and cancer, may offer improved sensitivity and specificity over white-light colonoscopy for detection of these lesions. For this purpose, we have created a dual modality microendoscope comprising a clinical fiberscope, modified for fluorescence imaging, and a dual-axis confocal microscope; together these offer wide-field and microscopic fluorescence imaging capabilities. We used a molecular probe, BMV109, that targets the cathepsin activity arising from both cancer cells and macrophages in the tumor microenvironment. BMV109 was designed as a pan-cathepsin quenched probe that is activated by cathepsin cleavage of a covalent bond releasing a quenching molecule. The human colon cancer cell line, HCT116, was injected directly into the colonic wall of nude mice though endoscopic assistance. Tumor growth was monitored using luciferase expression from the engineered HCT116 cells as imaged by an IVIS Spectrum. For fluorescence imaging, BMV109 was topically applied, and after 45 min colon lesions were imaged. At this time the tumors were fluorescent with sticking contrast to surrounding normal mucosal tissue. The fiberscope was able to detect polyp-specific fluorescence both in vivo and ex vivo. These results show that the devices may have utility for clinical fluorescence imaging and may be worked into the current colonoscopy protocol. Furthermore, the fiberscope was characterized for resolution and sensitivity and compared to an IVIS Spectrum small-animal imaging system. We confirmed the cellular uptake of BMV109 and excluded the possibility of noise arising from random pooling of the probe on the surface of tissue or in mucus using a dual-axis confocal microscope. We were able to demonstrate that a miniaturized endoscope-deployable, multimodal (wide-field and microscopic) fluorescence imaging device could offer improved detection, as an add-on device, in areas where lesions are hard to detect in the colon. Moreover, standard white-light endoscopy does not offer any molecular information. Thus, information such as expression of certain key proteins, that can indicate malignancy or the genetic subtype of cancer, is lost. Removal of such lesions owns both diagnostic and preventative values. Polyps are precancerous and could further progress into invasive cancer if not removed.

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Dye-doped nanoparticles for tumor imaging and photothermal therapy. F.M. Roland, S. Guha, B.D. Smith; University of Notre Dame, Notre Dame, IN

Despite substantial advances in cancer therapy, effective treatment remains a significant clinical challenge due to the diversity of tumor size, cell type and progression that occurs from patient to patient. There is an ongoing need for improved strategies that can deliver and rapidly monitor the efficacy of cancer treatment. Recent efforts have focused on the development of individualized patient treatment regimens, which combine a therapeutic agent with a diagnostic agent into a single platform, giving birth to the term "theranostics." Presented here, is a theranostic nanoplatform that contains a novel near infrared (NIR)-absorbing, heat generating croconaine dye as a therapeutic agent for photothermal therapy and a deep-red fluorescent dye as a diagnostic agent. Using a single-step fabrication method, croconaine dye and/or fluorescent dye are loaded into a nanoparticle core of poly(DL-lactide-co-glycolide) (PLGA) and coated with 1,2-distearoyl-phosphatidyl ethanolamine-methyl-polyethyleneglycol (DSPE-MPEG-2000) to produce dye-doped nanoparticles (PEG-PLGA-Cr). The nanoparticle formulation has been optimized and tested in cell and small animal cancer models. Whole-body fluorescence imaging was used to monitor nanoparticle biodistribution, with subsequent photothermal treatment for tumor ablation. *In vitro* laser-induced cell death studies in the presence of the PEG-PLGA-Cr nanoparticles indicated effective photothermal killing of cancer cells (808-nm diode laser, 1.0 W/cm^2 power density, 10 min duration). Photothermal tumor ablation using the PEG-PLGA-Cr nanoparticles was further examined in nude mice harboring EMT-6 mammary carcinoma tumors. Nanoparticles were intratumorally injected (n=4) and subsequently laser-irradiated (808-nm diode laser, 2 W/cm^2, 10 min duration). Tumor growth was monitored over time by measuring the tumor volume using a digital caliper. Laser-irradiated tumors were compared to a control cohort (n=4) that received an intratumoral nanoparticle injection but no laser irradiation. Results indicate: i) laser treatment using the PEG-PLGA-Cr nanoparticles for 10 minutes at 2 W/cm^2 suppresses tumor growth over 10 days and ii) maximum heating temperature plays an important role in achieving tumor ablation. *In vivo* fluorescence imaging of a rat model bearing prostate tumor (n=3) that had been dosed with fluorescently labeled PEG-PLGA-Cr nanoparticles showed accumulation in the tumor, presumably due to the enhanced permeation and retention (EPR) effect. The ability to image the location of the nanoparticles will greatly facilitate ongoing studies to evaluate the efficacy of various photothermal regimens.

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Tumor specificity of cetuximab-IRDye800 for surgical navigation in squamous cell cancer. E.R. Rosenthal, E. de Boer, J. Warram, T. Chung, K. Zinn; University of Alabama, Birmingham, AL

Positive margins dominate clinical outcomes after surgical resections in most solid cancer types including head and neck squamous cell carcinoma. Unfortunately, surgeons remove cancer in the same manner they have for a century with complete dependence on subjective tissue changes to identify cancer in the operating room. To effect change, we hypothesize that epidermal growth factor receptor (EGFR) can be targeted for safe and specific real-time localization of cancer. A dose escalation study of cetuximab conjugated to IRDye800 was performed in patients undergoing surgical resection of squamous cell carcinoma arising in the head and neck. Safety and pharmacokinetic data were obtained out to 30 days post-infusion. Multi-instrument fluorescence imaging was performed in the operating room, in surgical pathology and in vivo immunohistochemistry. There were no grade 2 or higher adverse events attributable to cetuximab-IRDye800 and four possibly related grade 1 adverse reactions occurred in the first cohort (2.5mg/m^2 dose), four in the second cohort (25mg/m^2 dose), and two in the third cohort (62.5mg/m^2 dose). Fluorescence imaging with an intraoperative, wide-field system successfully differentiated tumor from normal tissue with an average tumor-to-background ratio of 5.2 in the highest dose range. Optical imaging identified opportunity for improved outcomes in the surgical procedure and pathological processing. Fluorescence levels positively correlated with EGFR levels. We demonstrate for the first time that commercially available antibodies can be fluorescently labeled and safely administered to humans to identify cancer with submillimeter resolution and this has the potential to improve outcomes in clinical oncology.

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Evaluation of [^{18}F]FDG-PET as an early response marker for a SYK inhibitor treatment of SUDHL-4 tumors in mice. D.J. Rubins, X. Meng, S.-A. Lin, S. Wang, H. Haley, M. Wolf, P. Rebbeck, M. Kraus, D.G. Trotter; Merck and Co., Inc., West Point, PA

Introduction: [^{18}F]FDG PET was evaluated as an early marker of therapeutic response for a SYK inhibitor (SYKi) in a mouse lymphoma model. **Methods:** SUDHL-4 tumor cells were implanted in SCID/BEIGE mice and allowed to grow for 21-24 days to a volume of 400 mm^3. Mice were scanned with [^{18}F]FDG for 60 minutes at baseline and then 4 hours following PO administration of vehicle or 300 mg/kg SYKi (N=11 per group). Tumor ROIs included all tumor image voxels greater than 70% of the maximum tumor value 40-60 min post-[^{18}F]FDG. For each PET scan, tumor FDG uptake was determined by calculating the mean SUV of the tumor ROI from 40-60 min post-[^{18}F]FDG injection, and then dividing by the integral of the liver SUVs for the entire scan. A different set of mice received the same treatments for 7 days and caliper measurements of tumor size were obtained (N=11 per group). **Results:** At the end of treatment, tumor volumes measured by caliper were significantly reduced in SYKi-treated mice (472 mm^3, SEM: 90 mm^3) compared to vehicle-treated mice (1079 mm^3, SEM: 502 mm^3) ($P < 0.005$, 2 way RM ANOVA). For both groups, tumor FDG uptake decreased significantly between baseline and post-treatment scans (vehicle: BL = 3.41, post-treatment = 2.93, P=0.002; SYKi: BL = 3.46, post-treatment = 2.57, P = 0.0003). The amount of decrease was significantly larger for the SYKi group (ratio = 0.86, P = 0.0058). **Conclusions:** Treatment with SYKi for 7 days led to robust tumor growth suppression in this mouse model. SYKi treatment also led to a significantly larger decrease in [^{18}F]FDG tumor uptake compared to baseline after four hours than following four hours of vehicle treatment. These results provide support for the use of [^{18}F]FDG PET as an early response marker for SYKi treatment.

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Near-IR uncaging chemistry: discovery and applications. M.J. Schnermann, R.R. Nani, A.P. Gorka; National Cancer Institute, Frederick, MD

The identification of broadly useful photoremovable groups activated by tissue penetrant, cytocompatible near-IR light would enable the development of new approaches to drug delivery. Towards this goal, we have developed a single photon near-IR light-initiated uncaging reaction sequence based on readily synthesized C4′-dialkylamine-substituted heptamethine cyanines. Release of phenols and secondary amines occurs through a sequence comprising regioselective photooxidative C-C cleavage and then hydrolysis of the C4′-amine. While the oxidative cleavage step was known to be the basis of cyanine photobleaching, these efforts are the first to apply this remarkable reactivity for a productive application. The uncaging method has been applied to alter gene expression using near-IR light through ligand-dependent genetic recombination mediated by release of the estrogen receptor antagonist, 4-hydroxycyclofen. In addition, in key first steps towards drug delivery, we have been able to release cytotoxic concentrations of several therapeutic agents. As a consequence of the fluorescent properties of the cyanine scaffold, the chemistry discussed here has significant potential for use in theranostic approaches. Towards this goal, we provide evidence that localization and target engagement can be assessed optically prior to applying moderately higher light fluence to release the payload molecule. Details regarding the development of this approach, our mechanistic studies, and long-term goals will be discussed.

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Effects of lactate dehydrogenase A knockdown and biguanides treatment on the aggressiveness of murine breast tumors in immunocompetent mice. I. Serganova[1], N. Shah[1], I. Cohen[2], E. Moroz[1], D. Sardar[1], T.M. Kalidindi[1], M. Shindo[1], J. Koutcher[1], R. Blasberg[1]; [1]Memorial Sloan Kettering Cancer Center, New York, NY, [2]GSK Graduate School, New York, NY

Background: Metabolic changes in primary tumors impact their progression and the development of metastases. Recently we showed a close relationship between LDH-A expression, tissue lactate levels, and tumor metastatic potential using immunocompromised mice. Significant *in vivo* biologic

effects of LDH-A silencing were shown, which included decreased lactate production in vivo, the slowing of tumor growth and a reduction and delay in the development of metastases (1,2). Although lactate formation through LDH-A activity decreases carbon flow into mitochondria, many cancer cells still depend on mitochondrial metabolism for anabolic intermediates (3). Our objective is to develop a strategy for targeting both glycolytic and TCA cycle pathways. Biguanides are a class of anti-diabetic drugs, of which metformin and phenformin have frequently been studied. Both of these drugs have recently been shown to exhibit an antineoplastic effect (4), deplete tricarboxylic acid cycle intermediates (5), and may directly impair mitochondrial respiration through complex I inhibition (6). **Experimental Design:** To assess the role of both LDH-A knock-down and phenformin treatments using highly aggressive/metastatic murine breast cancer cells in the immunocompetent (Balb/c) mice, we employed two retroviral plasmids for the labeling of 4T1 murine breast cancer cells. Using a constitutively expressed fusion of Firefly Luciferase and tdRFP, we could image primary tumor growth (implanted into mammary fat pad) and metastasis development. The second plasmid, with a HIF-1-inducible exGaussia Luciferase and GFP dual-reporter, allowed us to detect changes in HIF-1 activity at different time points during tumor evolution and metastasis formation. To inhibit LDH-A expression in 4T1 breast cancer cells, we employed SureSilencing plasmids bearing different shRNAs for LDH-A knock-down, as well as a scrambled shRNA (control). Stable LDH-A knock-down clones (4T1HREs3KDA3-8 and 4T1HREs3KDA2-10) of 4T1HRE cells were developed and directly compared to the scrambled-shRNA control cell line (4T1HREs3NCA5). **Results:** The LDH-A shRNA knock-down cells (4T1HREs3KDA3-8 and 4T1HREs3KDA2-10) had reduced growth *in vitro* and *in vivo*, and had slower tumor growth and fewer metastases following orthotopic implantation, when compared to control 4T1HREs3NCA5 cells and tumors (scrambled shRNA bearing). The presence of a constitutive reporter allowed us to monitor primary tumor growth as well as metastasis development. Using Firefly Luciferase/luciferin bioluminescence imaging, we were able to detect a bi-phasic tumor growth/burden profile of 4T1HREs3KDA3-8 and A2-10 tumors. HIF-1 activity was higher in the LDH-A knock-down tumors compared with the scrambled shRNA control (4T1HREs3NCA5) tumors, based on the exGaussia Luciferase/coelenterazine bioluminescence imaging system. To characterize the effect of phenformin treatment, a series of experiments was performed following implantation of a micro-osmotic pump loaded with phenformin (35 mg/ml) in tumor-bearing mice. We found that growth of control 4T1HREs3NCA5 tumors was significantly lower with treatment at day 17 (509+84 mm^3 vs 374+37 mm^3). The volume of 4T1HREs3KD A2-10 (LDH-A knock-down) tumors without treatment with phenformin at the same day of growth was 332+44 mm^3 and with treatment 322+91 mm^3. **Conclusions:** Knock-down of LDH-A induced primary and metastatic tumor growth delay in immunocompetent mice, confirming our previous studies in immunocompromised mice. This delay was accompanied by an increase in the HIF-1 activity compared to control tumors. The continuous delivery of phenformin (0.21mg/24h/mouse) significantly reduced 4T1HREs3NCA5 cell tumor growth. However, this treatment is less effective in LDH-A knock-down tumors compared to control tumors. **References:** 1. Serganova, I., et al., Metabolic imaging: a link between lactate dehydrogenase A, lactate, and tumor phenotype. Clin Cancer Res, 2011. 17(19): p. 6250-61. 2. Rizwan, A., et al., Relationships between LDH-A, lactate, and metastases in 4T1 breast tumors. Clin Cancer Res, 2013. 19(18): p. 5158-69. 3. Vander Heiden, M.G., L.C. Cantley, and C.B. Thompson, Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science, 2009. 324(5930): p. 1029-33. 4. Pollak, M.N., Investigating metformin for cancer prevention and treatment: the end of the beginning. Cancer Discov, 2012. 2(9): p. 778-90. 5. Janzer, A., et al., Metformin and phenformin deplete tricarboxylic acid cycle and glycolytic intermediates during cell transformation and NTPs in cancer stem cells. Proc Natl Acad Sci U S A, 2014. 111(29): p. 10574-9. 6. Bridges, H.R., et al., Effects of metformin and other biguanides on oxidative phosphorylation in mitochondria. Biochem J, 2014. 462(3): p. 475-87.

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VLA-4 targeted PET imaging of multiple myeloma. D. Soodgupta[1], W. Beaino[2], H. Zhou[1], C.J. Anderson[2], M.H. Tomasson[1], M. Shokeen[1]; [1]Washington University School of Medicine, St. Louis, MO, [2]University of Pittsburgh Medical Center, Pittsburgh, PA

Objectives: Very late antigen-4 (VLA-4; also called $\alpha_4\beta_1$ integrin) is one of the critical mediators of myeloma cell adhesion to the bone marrow stroma and promotes myeloma cell trafficking, proliferation, differentiation and drug resistance. The goal of this study is to evaluate a VLA-4 targeted PET radiopharmaceutical for imaging disease progression in medullary and extra-medullary tissues in pre-clinical mouse and human models of multiple myeloma (MM). The receptor targeted imaging approach was directly compared with FDG/PET. The binding affinity of the optical analog of the radiopharmaceutical to normal bone marrow cells was analyzed. **Methods:** Mouse models: 5TGM1-GFP murine myeloma cells into immunocompetent, syngeneic C57Bl/KaLwRij mice generated disseminated tumors with significant bone destruction within 28 days. Mice were imaged by PET and ^{64}Cu-LLP2A (VLA-4 targeted radiopharmaceutical) and FDG following tumor inoculation. Blood samples were collected prior to each imaging time point for serum protein electrophoresis (SPEP) analysis. Binding and specificity of Cy5 labeled LLP2A to different bone marrow and spleen cell populations (B cells, T cells, myeloid cells, macrophage and monocyte cells) was analyzed *ex vivo* by flow. **Results:** Focal and diffuse myeloma lesions were apparent visually and quantitatively by both PET modalities. However, the receptor targeted imaging had the advantage of higher signal-to-background compared to FDG. LLP2A-Cy5 had significantly low binding to cells in spleen (1.97%) and BM (1.35%) of non-tumor mice as compared to the spleen (61%) and BM (26.4%) of tumor mice. The binding significantly decreased in the presence of the cold competitor and scrambled Cy5. **Conclusions:** The receptor VLA-4 is a sensitive and specific imaging marker for detecting diffuse and focal lesions caused by MM.

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Targeted PET imaging with radiolabeled lactosaminated HSA for asialoglycoprotein receptor positive hepatoma cells. M.G. Song[1], S.A. Jang[1], H. Youn[2], Y.-S. Lee[3], J.M. Jeong[3], D.S. Lee[4], J.-K. Chung[1], F. Kratz[5], K.W. Kang[1]; [1]Department of Nuclear Medicine, Cancer Research Institute, Seoul National University, Seoul, Korea, [2]Department of Nuclear Medicine, Cancer Imaging Center, Seoul National University Cancer Hospital, Seoul, Korea, [3]Department of Nuclear Medicine, Institute of Radiation Medicine, Seoul National University, Seoul, Korea, [4]Department of Nuclear Medicine, Molecular Medicine and Biopharmaceutical Science, Seoul National University, Seoul, Korea, [5]Tumour Biology Center, Freiburg, Germany

Purpose: A selective delivery of drugs to liver cells can be obtained by conjugation with galactosylated macromolecules, which internalizes hepatocytes after interaction of the galactose residues with the asialoglycoprotein receptor (ASGPR). This receptor was also found on the cells of the large majority of well differentiated hepatocarcinomas (HCCs). Recently, asialoglycoprotein receptor (ASGPR) became one of the most important biomarkers for targeted imaging and therapy in hepatocellular carcinoma. We evaluated targeting ability of ^{64}Cu labeled lactosaminated human serum albumin (^{64}Cu-Ln-HSA) to ASGPR dependent on additional lactosamine residues in vitro and in vivo models. **Methods:** Human serum albumin was conjugated with various numbers of lactosamine residues (Ln; L0-, L8-, L26-, L56-HSA) and the FITC-labeled Ln-HSA was observed by confocal microscopy. Ln-HSA was conjugated with SCN-NOTA for generating ^{64}Cu-Ln-HSA. NOTA conjugated Ln-HSA was further radiolabeled with $^{64}CuCl_2$ in sodium acetate buffer (0.1 N, pH 6.0) at 37°C for 1 h, which purified using a PD-10 column. Labeling efficiencies of ^{64}Cu-Ln-HSA were determined by ITLC. ^{64}Cu-Ln-HSA (50 μCi/mouse) was injected into nude mice having HepG2 (ASGPR+) and HT29 (ASGPR–) tumors and PET images were obtained. **Results:** FITC-lactosaminated HSA was detected in HepG2 cells, but not detected in HT29 cells. The FITC signal was dependent on increasing lactosamine residues on HSA surface (L56>L26>L8>L0). Labeling efficiency of ^{64}Cu-Ln-HSA was over 95% and in vitro uptake test using ^{64}Cu-Ln-HSA showed its specificity in HepG2 cells. Serial PET imaging revealed that HepG2 tumor uptake of ^{64}Cu-L26-HSA was 7.5, 7.9, and 7.3 %ID/g at 10, 22, and 46 h post-injection, respectively. However, HT29 tumor uptake of Ln-HSA was reduced in a lactosamine-dependent manner. Autoradiography also showed that ^{64}Cu-L26-HSA was accumulated more than other conjugates in HepG2 tumors. **Conclusions:** Lactosaminated HSA enhanced the targeting ability to ASGPR positive cells by addition of 26 lactosamine residues on the surface of HSA. We propose ^{64}Cu-lactosaminated HSA as a promising

probe for ASGPR specific imaging. The concept of using HSA as a carrier indicates a broader application in the design of many other radiolabeled probes or drugs.

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The Imaging Probe Development Center makes all types of diagnostic imaging agents. R. Swenson, F. Bhattacharyya, B. Blackman, V. Coble, K. Lane, C. Li, A. Opina, D. Sail, Z.-D. Shi, O. Vasalatiy, H. Wu, B. Xu, X. Zhang, M. Krishna, J. Mitchell; National Institutes of Health, Bethesda, MD

The Imaging Probe Development Center (IPDC) was founded with the goal of providing the fundamental synthetic chemistry support needed to advance molecular imaging technologies for the interdisciplinary NIH research community. The IPDC laboratories are located in Rockville, MD, with state-of-the-art equipment and the new PET facility at NIH main campus. The IPDC has a rolling solicitation system and NIH scientists are welcome to contact and submit a proposal with the request for synthesis of a particular probe in which they are interested. Probes can be intended for all types of imaging modalities, such as optical fluorescence, PET/SPECT and MRI. IPDC scientists can synthesize requested probes that are published in literature but commercially unavailable, or are completely novel. We look for automation solutions to improve capabilities and throughput. Examples of molecular imaging probes we have produced range from low molecular weight entities to high molecular weight conjugates and include fluorescent dyes and their analogs, lanthanide complexes, fluorogenic enzyme substrates, caged dyes which become fluorescent upon irradiation, radio and fluorescent labeled peptides, proteins and antibodies, gold and iron oxide nanoparticles, dendrimers and liposomes. Recent efforts have included hyperpolarized MRI probes, tools for super resolution spectroscopy, and PET probes derived from tyrosine kinase inhibitors. Examples of some of our recent projects will be provided.

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First ^{111}In-labeled activity-based probe for SPECT imaging of uPA activity: in vivo study in human cancer xenografts. C. Vangestel[1], D. Thomae[2], J. Van Soom[2], L. Wyffels[1], P. Pauwels[1], S. Stroobants[1], P. Van Der Veken[2], J. Joossens[2], K. Augustyns[2], S. Staelens[2]; [1]University Hospital Antwerp, Edegem, Belgium, [2]University of Antwerp, Wilrijk, Belgium

Introduction: Urokinase-type plasminogen activator (uPA) and its inhibitor PAI-I are key players in cancer invasion and metastasis. Both are described as prognostic biomarkers in various cancers and have been validated at the highest level of evidence as clinical biomarkers of prognosis in breast cancer. Current methods for quantification of uPA do not discriminate between active and inactive uPA and require biopsies. Considering the high prognostic value of uPA, non-invasive methods measuring uPA activity are required. **Methods:** Based on the design of our small irreversible and selective uPA inhibitor (1) we developed ^{111}In-MICA-401, an activity based probe for uPA imaging. In addition, ^{111}In-MICA-402, a radiolabeled non-active (hydrolyzed) analog containing a dysfunctional warhead as a negative control was also synthesized and evaluated. Small animal SPECT imaging was performed in mice (n = 5/group) for two different cancer xenografts (human breast cancer: MDA-MB-231 and human colorectal cancer: HT-29) with, respectively, high and low levels of uPA expression in vitro, and in a healthy control group. Imaging was done 95h post injection (p.i.) of either the active ^{111}In-MICA-401 or the hydrolyzed ^{111}In-MICA-402 probe followed by ex vivo biodistribution. Tumor uptake was correlated with human and murine uPA expression determined by ELISA and immunohistochemistry (IHC) on excised tumors end-of-life. **Results:** The radioactive probes were produced in high purity (> 95%) and a specific activity of 9.25 ± 2.40Gbq/μmol. While ^{111}In-MICA-402 showed an almost complete clearance at 95h p.i., ^{111}In-MICA-401 showed a comparable tracer distribution in the three groups, with uptake (averaged for the three groups) in the liver (5.55 ± 1.01 %ID/g), spleen (4.50 ± 1.22 %ID/g) and skin (6.22 ± 1.20 %ID/g). A similar tumor uptake of 5.68 ± 1.41 %ID/g for MDA-MB-231 and 5.43 ± 1.29 %ID/g for HT-29 was detected while human uPA ELISA showed significantly higher uPA expression in the MDA-MB-231 compared to HT-29 tumors (21.42 ± 3.40 versus 1.96 ± 0.36 ng uPA/mg protein, respectively; $p < 0.0001$). IHC for human uPA confirmed these ELISA results. In contrast, staining for mouse uPA revealed more similar intensities for both tumors. It has been described that for in vitro negative-uPA cell lines (HT29) to grow in vivo, it may need to recruit uPA expressed by neighboring stroma cells through its surface uPAR (2). Also, a remarkably high ^{111}In-MICA-401 uptake was observed in the lymph nodes (LNs) (12.21 ± 6.70 %ID/g) in the three groups. However, ex vivo evaluation showed no micrometastases in these LNs, but we found clear murine uPA staining in the medulla of the LN and also in the brown adipose tissue surrounding the dissected LNs. **Conclusion:** Our data clearly demonstrate that our ^{111}In-labeled activity based probe for uPA enables the non-invasive imaging of uPA activity in vivo by SPECT. It might be a promising candidate in a clinical setting for individualized cancer therapy. **References:** 1. Joossens J, Ali OM, El-Sayed I, Surpateanu G, Van der Veken P, Lambeir AM, et al. Journal of Medicinal Chemistry. 2007;50(26):6638-6646. 2. Hsiao JK, Law B, Weissleder R, Tung CH. J Biomed Optics. 2006;11(3):34013.

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Radiotherapy stimulates recruitment of circulating breast cancer cells in a systemic manner. M. Vilalta[1], J.E. Brune[2], M. Rafat[1], A.J. Giaccia[1], E.E. Graves[1]; [1]Stanford University, Stanford, CA, [2]Washington State University College of Veterinary Medicine, Pullman, WA

One-third of all breast cancer patients will suffer local recurrence after their initial treatment, with a significantly greater risk for those undergoing breast-conserving therapy, which is usually followed by breast radiotherapy. Even though the benefits of the radiotherapy to breast cancer patients generally outweigh the side effects of this treatment, understanding the full spectrum of radiation effects remains a concern. In our previous work we demonstrated that the presence of viable circulating tumor cells (CTCs), coming either from a secondary tumor or a metastatic lesion, could increase the probability of regrowth at the primary tumor site after localized radiotherapy. Our work provided a mechanism for breast tumor recurrence in which radiation therapy attracts cells outside the radiation field to migrate to the site of treatment. We also elucidated that granulocyte macrophage colony-stimulating factor (GM-CSF), which is overexpressed upon radiation, is one of the key components implicated in the recruitment of CTCs to the irradiated tumor. We have now investigated the mechanism of action of GM-CSF in stimulating tumor cell recruitment after radiation, focusing on the systemic versus local nature of the process. For that we used a highly metastatic mouse breast cancer cell line, 4T1, either unlabeled or transfected with *Photinus pyralis* luciferase (4T1-luci). For our *in vivo* studies, unlabeled 4T1 cells were inoculated orthotopically in the mammary fat pad at contralateral sites in female immunocompromised mice. 10 days after tumor injection, one of the tumors from a subgroup of mice was irradiated with a single dose of 20 Gy while the other tumor was shielded from treatment, and the other subgroup was left as an untreated control. After irradiation, labeled 4T1-luci cells were injected through the tail vein in order to mimic a metastatic lung lesion, and *ex vivo* bioluminescence imaging (BLI) of both irradiated and unirradiated tumors from the treated subgroup as well as both unirradiated tumors from the control subgroup was performed between ten and fifteen days after tumor irradiation. For our *in vitro* studies, transwell migration assays were performed using either the recombinant GM-CSF protein or a PEGylated murine version of GM-CSF (PEG-GM-CSF; Bolder Biotechnology, Inc.) as chemoattractants. *Ex vivo* BLI signals from either irradiated tumors or non-irradiated tumors from the treated cohort showed greatly increased signals relative to non-irradiated tumors from the non-treated subgroup, demonstrating that radiation promotes tumor cell recruitment through a systemic factor. On the other hand, in order to verify if the GM-CSF is the key component of this effect and perform the corresponding *in vivo* studies to elucidate that, we first studied *in vitro* the effectiveness of PEG-GM-CSF, a version of GM-CSF more suitable for *in vivo* studies, in promoting cell invasion compared to the recombinant GM-CSF. Our *in vitro* studies demonstrated that both GM-CSF and PEG-GM-CSF promoted cell invasion, suggesting that the PEGylated version can trustfully be used *in vivo* where we already evaluated that it has a half-life of 22 hours. In our first attempt to duplicate *in vivo* the systemic effect of radiation therapy by simply administrating daily intravenous doses of PEG-GM-CSF we could not reproduce our previous results; therefore, our focus now is pursuing combined studies of radiation followed by the addition of exogenous PEG-GM-CSF. Thus, we can conclude from this preclinical breast cancer model that irradiation of a tumor provokes an increase in tumor cell recruitment of CTCs in a systemic manner.

Furthermore, we determined that PEGylated GM-CSF mimics regular GM-CSF *in vitro* and therefore can be used *in vivo* as a possible promoter of tumor cell recruitment.

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Selection of appropriate radiotracers for molecular imaging of cancer using gene profiles. X. Wang, R. Wahl; Johns Hopkins University School of Medicine, Baltimore, MD

Purpose: Cancer cell lines obtained from patients with cancer have contributed to the understanding of cancer biology and to the selection and validation of radiotracers for imaging. Cell lines with a shorter doubling time (DT) are expected to have more aggressive behavior. FDG and FLT are radiotracers used to evaluate tumor responses to chemotherapies. Glycolytic and DNA synthesis are thought to be involved in the entrance of these tracers into cancer cells. We assessed the correlations between the DTs of malignant cell lines and the abundance of messages for Ki67, PCNA, thymidine kinase (TK1), thymidylate synthase (TYMS), hexokinase (HK1), and pyruvate kinase (PKM2) for selecting an appropriate tracer for different cancers. **Methods:** 33 cell lines including five categories of cancer were selected from a NCI 60 exon dataset: breast, CNS, colon, lymphoma and melanoma. This database also provided DTs for each cancer cell line. Using Partek commercial software we obtained an average abundance of the transcripts of interest. Correlations between the abundance of genes and documented doubling times were determined by Pearson correlation testing (α=0.05 for a two-tailed test). **Results:** TK1 (related to FLT uptake) had a significant negative correlation to the DTs of breast cancer cell lines ($r= -0.85$, $p< 0.05$) and colon cancer cell lines ($r= -0.68$, $p< 0.05$). The DTs of lymphoma cell lines had a significant negative correlation to HK1 (related to FDG uptake, $r= -0.87$, $p< 0.05$). The DTs of CNS cell lines had a negative correlation to PKM2 (related to FDG uptake, $r= -0.87$, $p< 0.05$) and TYMS (related to FLT uptake, $r= -0.85$, $p< 0.05$). Melanoma's DTs had a negative correlation to HK1 ($r= -0.67$, $p< 0.05$) and TK1 ($r= -0.72$, $p< 0.05$), but a positive correlation to PCNA ($r= 0.62$, $p< 0.05$). **Conclusions:** FLT might be better than FDG for assessing growth of breast and colon cancers. In lymphoma, FDG might be better than FLT for the evaluation of lymphoma progression. Either FDG or FLT could be used for measuring growth of melanoma and CNS tumors. Ki67 and PCNA did not show significant correlation to the DTs except melanoma (PCNA had $r= 0.62$ and $p< 0.05$).

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ImmunoPET on a chip: a microfluidic system for labeling antibodies. B.D. Wright, C.L. DeFelice, S. Lapi, D.E. Reichert; Washington University in St. Louis School of Medicine, St. Louis, MO

In the last two decades the use of antibody based therapeutics has become more common. An example of one such antibody is trastuzumab (Herceptin). Herceptin binds to domain IV of the human epidermal growth factor receptor 2 (HER2), tagging the cell for immunological attack through antibody-dependent cell-mediated cytotoxicity. Due to the varying nature of target expression in cancer cells the effectiveness of this type of treatment can vary from patient to patient. The development of noninvasive imaging techniques using monoclonal antibodies (mAbs) to determine patient response is the answer to this growing problem. A type of positron emission tomography, immunoPET, uses positron emitting isotopes to track the localization of mAbs with excellent image quality. This allows for the assessment of tumors that are unable to be biopsied. Typically mAbs achieve optimal tumor-to-nontumor ratios at 2-4 days after injection, placing a limit on the radiometal able to be selected for imaging. 89Zr has a half-life of 3.27 days, making it a good candidate for immunoPET. However, this isotope releases a high energy, highly penetrating photon (909 keV gamma ray) requiring more care than other isotopes. To reduce the exposure of technicians to this high energy photon, we have developed an automated system able to label, purify, and test the immunoreactivity of the 89Zr-labeled Herceptin using a microfluidic reactor. Using our system, we have been able to significantly increase the specific activity of 89Zr-labeled Herceptin in comparison to conventional techniques as well as remove any nonspecifically bound 89Zr from the reaction without removing the activity from its shielding. The 89Zr-labeled Herceptin passed through the chip show no change in affinity to their target as shown by the Lindmo assay. This system will enable fast and efficient production of radiometal-based pharmaceuticals and allow their use at hospitals without immediate access to a cyclotron facility.

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Early detection of treatment-induced apoptosis in tumors using temporal diffusion spectroscopy MRI. X. Jiang, H. Li, P. Zhao, H.C. Manning, J.C. Gore, J. Xu; Vanderbilt University Institute of Imaging Science, Nashville, TN

A novel and quantitative magnetic resonance imaging method for the assessment of tissue microstructure associated with treatment-induced apoptosis has been developed, and its potential role as a quantitative imaging biomarker has been evaluated in studies of tumors and their responses to treatments. Diffusion MRI has been suggested as a possible non-invasive means to monitor apoptosis. However, conventional diffusion MRI uses relatively long diffusion times, making it more sensitive to features on a cellular scale, e.g., cellularity. Meanwhile, apoptosis is a highly regulated, programmed cell death that maintains plasma membrane integrity so that it precedes changes in cell density. Therefore, conventional diffusion MRI is not sensitive to apoptosis until it causes reductions in cellularity, which is a later stage response to an effective treatment. However, apoptosis at earlier stages is associated with significant intracellular microstructural variations, such as nuclear fragmentation and cytosolic condensation, and overall cellular shrinkage, so detection of these changes may enable earlier and more specific detection of apoptosis. In the current study, we demonstrate that temporal diffusion spectroscopy MRI, a technique that is capable of characterizing microstructural variations in tissues from intracellular to cellular length scales, can provide quantitative information about apparent cell size, extracellular tortuosity and changes in intracellular structure in tumors undergoing apoptosis. Based on two types of human colon cancer, DiFi (responder) and HCT116 (non-responder) treated with cetuximab and PBS (vehicle), we found that specific apoptosis-associated microstructural changes, such as cell shrinkage, nuclear fragmentation and cytosolic condensation, can be detected at an early post-treatment time point (day 4) using temporal diffusion spectroscopy. By contrast, conventional diffusion MRI could not capture these features, and reported significant changes only at a later post-treatment time point (day 8) when the overall tumor size started to shrink. These findings suggest that key microstructural features can be detected by temporal diffusion spectroscopy at an early stage of treatment-induced apoptosis in tumors, while treatment effects must evolve longer, until there are changes in cell density, to be detected by conventional diffusion MRI. Considering the non-invasive nature of diffusion MRI, which is free of exogenous probes and radiation, the proposed temporal diffusion spectroscopic method is well suited for early and specific detection of treatment-induced apoptosis in clinical applications.

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Gd-EOB-DTPA-enhanced MRI and AFP predict prognosis of early-stage hepatocellular carcinoma: molecular features of MRI images and their clinical utilities. T. Yamashita, M. Honda, S. Kaneko; Kanazawa University Hospital, Kanazawa, Japan

Hepatocellular carcinoma (HCC) is the fifth most commonly diagnosed cancer and the second most frequent cause of cancer death in men worldwide. Survival of patients with HCC is often individually different even after surgery for early-stage tumors. Molecular profiling approaches have tried to evaluate the malignant features of HCCs to predict the prognosis, although the evaluation of the potential clinical application of these approaches is ongoing. Gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB-DTPA)-enhanced magnetic resonance imaging (EOB-MRI) has recently been introduced to evaluate hepatic lesions with regard to the activity of the organic anion transporter OATP1B3 as well as vascularity. Here we report that Gd-EOB-DTPA-enhanced MRI and serum alpha-fetoprotein (AFP) status reflects the stem/maturational status of HCC with distinct biology and prognostic information. Uptake of Gd-EOB-DTPA in the hepatobiliary phase was observed in ~15% of HCCs. This uptake correlated with the low serum AFP levels, maintenance of hepatocyte function with the up-regulation of OATP1B3 and HNF4A, and good prognosis. In contrast, HCC showing reduced Gd-EOB-DTPA uptake with

high serum AFP presented poor prognosis with the activation of an oncogene FOXM1. Knockdown of HNF4A in HCC cells showing uptake of Gd-EOB-DTPA resulted in the increase of AFP and FOXM1 and the loss of OATP1B3 expression accompanied with the morphological changes, enhanced tumorigenesis, and loss of Gd-EOB-DTPA uptake in vivo. HCC classification based on EOB-MRI and serum AFP level predicted overall survival in a single-institution cohort (n = 70), and its prognostic utility was independently validated in a multi-institution cohort of early-stage HCCs (n = 109). This non-invasive classification system is molecularly based on the stem/maturation status of HCCs and can be incorporated into current staging to improve management algorithms, especially in early stage.

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Comparison of FDG-PET and FLT-PET in the evaluation of response to treatment in advanced head and neck and esophageal malignancies. R.S. Youland, V. Lowe, B.M. Barney, D.H. Pafundi, D.H. Brinkmann, B. Kemp, J.N. Sarkaria; Mayo Clinic, Rochester, MN

Background: We prospectively compared FDG-PET and FLT-PET in assessing patient responses to cetuximab and chemoradiotherapy for advanced head and neck (HN) and esophageal malignancies. **Methods:** Nineteen patients were initially enrolled. FDG-PET and FLT-PET scans were performed at baseline, after induction cetuximab, and two weeks into chemoradiotherapy for the HN cancer patients studied. Cetuximab was not administered to esophageal cancer patients, so scans were performed at baseline and during radiotherapy. All patients were expected to have imaging six weeks and six months following treatment. Changes in SUVmax were compared with baseline. **Results:** Protocol adherence was good for imaging at baseline (17/17) and six weeks post-treatment (13/17). However, only 5/10 and 7/17 patients underwent both FDG and FLT scans during induction cetuximab therapy or during radiotherapy, respectively. After induction therapy, changes in SUVmax ranged from −59 to 8% for FDG-PET and −41 to −1% for FLT-PET. After two weeks of chemoradiotherapy or radiotherapy, changes in SUVmax ranged from −80 to 8% for FDG-PET and −80 to −15% for FLT-PET. Six weeks after completing treatment, changes in SUVmax ranged from −94 to 96% for FDG-PET, and after 6 months, changes in SUVmax ranged from −94 to −59% for FDG-PET. Median follow-up time was 19.0 months, median progression-free survival (PFS) was 21.4 months and median overall survival (OS) was 23.6 months. There were no statistically significant associations seen between changes in FDG or FLT-PET during or after treatment and PFS or OS. However, our small sample size limits our ability to describe subtle associations. **Conclusions:** Functional imaging early during definitive therapy for advanced HN and esophageal malignancies is feasible. Similar results were seen between the two modalities. However, our small sample size limits definitive conclusions regarding the role of early FDG-PET or FLT-PET in predicting the likelihood of recurrence and/or death in HN and esophageal cancers.

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Use of 89Zr to investigate the intracellular fate of antibodies. A. Zheleznyak, S. Lee, S.E. Lapi; Washington University School of Medicine, Mallinckrodt Institute of Radiology, St. Louis, MO

Antibodies have become an indispensable tool in the diagnosis and treatment of a broad spectrum of pathologies. Therefore, a good understanding of the antibody metabolic pathways has become paramount. In vivo, antibodies bind specifically and with high activity to their targets and may undergo intracellular processing upon internalization. On the other hand, antibodies may themselves be targeted by the cells of the reticuloendothelial system or undergo extracellular degradation by proteolysis. The goal of this work is to develop the methodology for intracellular tracking of ^{89}Zr labeled antibodies. Trastuzumab and pertuzumab, antibodies directed to Her-2 (CD340), with distinct internalization profiles were conjugated with p-isothiocyanatobenzyldesferrioxamine (Df-Bz-NCS) and radiolabeled with 89Zr with specific activity of 0.9-1.6 μCi/μg. The antibodies were allowed to bind to Her-2 bearing cell line SKBR3 for various lengths of time. Unbound antibody was removed and the cells were disrupted by sonication in the hypotonic buffer containing 0.5% of non-ionic detergent NP-40 at 4°C. The resulting suspension was applied to a discontinuous sucrose gradient comprising 5%, 15%, 25%, 35%, 40%, and 60% sucrose steps. The gradients were centrifuged at 100,000 × g for 16 hours at 4°C. The gradient was collected as 200 μl fractions and the associated activity was measured by a γ-counter. Equal amounts of protein representing each fraction were resolved on a non-reducing 4-20% SDS-PAGE, transferred to a PVDF membrane and subjected to a Western blotting with antibodies recognizing endosomal (EEA1) and lysosomal (Lamp1) markers, as well as an antibody recognizing human IgG to detect trastuzumab and pertuzumab. Each fraction was analyzed for the presence or absence of endosomes, lysosomes, original antibody, and ^{89}Zr. Analysis of trastuzumab containing fractions showed that 24% (36% ^{89}Zr) of the antibody associated with lysosomes, 26% (27% ^{89}Zr) with endosomes, and 50% (38% ^{89}Zr) with the plasma membrane fragments. 11% (8% ^{89}Zr) of pertuzumab associated with lysosomes, 40% (47% ^{89}Zr) with endosomes, and 48% (46% ^{89}Zr) with plasma membrane fragments. These data showed that more trastuzumab entered the lysosomal compartment compared with pertuzumab, as indicated by increased antibody and ^{89}Zr content, possibly resulting in ^{89}Zr residualization in lysosomes. At the same time, similar amounts of both antibodies and associated ^{89}Zr were co-localized with the plasma membrane, possibly indicating a receptor saturation state. This methodology offers an accurate and effective way of comparing antibody internalization kinetics and intracellular metabolic pathways. **Acknowledgment:** The authors would like to thank the Radioisotope Production Group at the Mallinckrodt Institute of Radiology for the expert manufacturing of ^{89}Zr.

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Multispectral fluorescence and PET reporter gene imaging of adoptive immune cell therapy in breast cancer. F. Youniss, G. Sundaresan, P. Jose, G.K. Dewkar, L. Graham, L. Wang, H.D. Bear, J. Zweit; Virginia Commonwealth University, Richmond, VA

Cancer treatment by adoptive immune cell therapy (AIT) is a form of immunotherapy that relies on the *in vitro* activation and/or expansion of immune cells. In this approach, immune cells, particularly CD8+ T lymphocytes, can potentially be harvested from a tumor-bearing patient, then activated and/or expanded *in vitro* in the presence of cytokines and other growth factors, and then transferred back into the same patient to induce tumor regression. **Objective:** The overall objective of this study is to non-invasively image and assess *in vivo* targeting and retention of adoptively transferred labeled T-lymphocytes at the tumor site. **Methods:** T-lymphocytes obtained from draining lymph nodes of 4T1 (murine breast cancer cell) sensitized BALB/C mice were activated *in vitro* with Bryostatin/Ionomycin, and were grown in either interleukin-2 (IL2 grown) or combination of interleukin-7 and interleukin-15 (IL7/15 grown). In order to validate the methodology, both direct and indirect cell labeling methods were examined and employed. The first method was based on direct *in vitro* cell labeling by lipophilic near-infrared (NIR) fluorescent probe, 1,1-dioctadecyltetramethyl indotricarbocyanine iodide (DiR), and imaging by multi-spectral fluorescence imaging (MSFI). The second method was based on indirect labeling of T-lymphocytes through transduction of herpes simplex virus type 1 thymidine kinase (HSV1-tk) gene and PET imaging by its substrate, 2-fluoro-2-deoxy-1 β-D-arabinofuranosyl-5-iodouracil ([^{124}I]-FIAU). **Results:** The results showed that cell viability, proliferation, and function of labeled 4T1 specific T-lymphocytes were not affected by DiR labeling. For indirect labeling, these parameters were dependent on multiplicity of infectious (MOI), and more than 80% of cells were viable at MOI of 1. T-lymphocytes (IL2 grown) infused into established 4T1 tumor-bearing mice showed higher tumor retention than IL7/15 grown cells. Both IL2 and IL7/15 grown cells were successfully transduced, albeit with less efficiency than the Jurkat cells. Both transduced IL2 and IL7/15 grown cells showed higher tracer uptake in tumor, lungs, liver, spleen, lymph nodes and bone/bone marrow than other organs. Compared to IL-2, cells grown in IL7/15 showed overall higher accumulation in these organs. On the other hand, non-transduced DiR labeled cells did not show any uptake at tumor site, and their accumulation at other organs was significantly lower than that of transduced cells. While MSFI showed labeled cells accumulating at the tumor site, PET reporter gene imaging did not demonstrate a strong signal at the 4T1 tumor site, most probably due to inadequate bioavailability of intact [^{124}I]-FIAU, because of de-halogenation *in vivo*. **Conclusions:** For preclinical studies, multispectral fluorescence imaging with DiR labeled

T-lymphocytes is more sensitive and convenient than PET reporter gene imaging with [^{124}I]-FIAU, because of reduced intact [^{124}I]-FIAU due to *in vivo* de-halogenation.

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[^{18}F]-FDG-PET as an early response predictor in a HER-2 overexpressing, trastuzumab-resistant breast cancer model treated with targeted PI3K/Akt/mTOR drugs. Y. Dockx[1], C. Vangestel[1], S. De Bruycker[1], M. Huizing[2], P. Pauwels[2], T. Van den Wyngaert[2], S. Staelens[1], S. Stroobants[2]; [1]University of Antwerp, Wilrijk, Belgium, [2]University Hospital Antwerp, Edegem, Belgium

Background: Aberrant activation of the PI3K/Akt/mTOR pathway is a driver of resistance to HER-2 targeted therapies and is involved in glucose homeostasis. ^{18}F-FDG-PET has been proposed for early response assessment, but the effects of blocking this pathway on tracer dynamics are still largely unknown. Therefore, we investigated the potential use of ^{18}F-FDG-PET as an early response biomarker for PI3K pathway targeting therapies in a trastuzumab-resistant HER-2 overexpressing breast cancer model. **Methods:** Nude mice were inoculated with HER-2 overexpressing JIMT1 cells, which are trastuzumab resistant but sensitive to everolimus (mTOR inhibitor) and PIK90 (PI3K inhibitor). Six weeks after tumor inoculation, ^{18}F-FDG-PET scans were performed to determine baseline tumor metabolism, whereupon animals were treated with trastuzumab (20mg/kg, 2×/w, IP), everolimus (10ml/kg, 1×/d, oral), PIK90 (10mg/kg, 5×/w, IP), saline (5×/w, IP) or combination therapies with trastuzumab + everolimus, trastuzumab + PIK90, trastuzumab + everolimus + PIK90, or everolimus + PIK90. Two and seven days after the start of the therapy, a follow-up ^{18}F-FDG-PET scan was performed for therapy response evaluation. Using PMOD 3.3 software, tumors were delineated on CT images and maximum standardized uptake values (SUV_{max}) were calculated. Tumor growth inhibition (TGI) was calculated as $1-((V_{treat7}/V_{Treat1})/(V_{control7}/V_{control1}))$ with V_{treat} the tumor volume after treatment at day 1 or 7, and $V_{control}$ the tumor volume in control mice at day 1 or 7. Expression of GLUT1 (glucose transporter) and Ki67 (proliferation) on excised tumor sections was assessed by immunohistochemistry. On protein tumor lysates, levels of pS6 and pAkt were determined by ELISA. **Results:** After trastuzumab treatment there was an increase in tumor volume compared to the control group (TGI=–31%±18%). In contrast, clear TGIs of 46%±17% and 54%±13% were observed in the everolimus and PIK90 treated groups, respectively. In the groups that combined two therapies, similar TGIs were found, ranging from 35%±17% for everolimus + PIK90 to 41%±6% for trastuzumab + PIK90. The combination of all three therapies resulted in a TGI of 38%±5%. In the control group, there was no change in SUV_{max}. In line with TGI, trastuzumab increased the SUV_{max} with 118% over time. For everolimus and PIK90 declines of 60% and 58%, respectively, were observed at day 7. Similarly, when mice were treated with a combination of two therapies SUV_{max} decreased significantly, ranging from 62% for both everolimus + PIK90 (p=0.039) and trastuzumab + everolimus (p=0.014) to 77% for trastuzumab + PIK90. For the combination of all three therapies, the SUV_{max} dropped to 96% at day 7. There was an increase in GLUT1 and Ki67 after trastuzumab treatment and a decrease in all other treatment groups. Everolimus and PIK90 decreased pS6 and pAkt, respectively, whereas trastuzumab caused an increase of both proteins. Also, changes in ^{18}F-FDG uptake correlated with pS6 and Ki67 expression (p=0.014, r=0.53 and p=0.043, r=0.52, respectively). Ki67 and pS6 also correlated with changes in volume at end-of-life with p=0.004, r=0.86 and p=0.042, r=0.58, respectively. **Conclusion:** ^{18}F-FDG may prove to be a valuable early marker of treatment response. Indeed, ^{18}F-FDG uptake correlates with pS6 and Ki67, which reflected the sensitivity of the JIMT1 cell line to everolimus, PIK90 and combination therapies and its resistance to trastuzumab. Changes in ^{18}F-FDG uptake accurately reflect the resistance profile to targeted PI3K/Akt/mTOR agents in this specific mouse model of breast cancer.

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Melanin-targeted PET imaging of melanoma using quinoxaline derivatives. S. Liu, P.S. Conti; University of Southern California, Los Angeles, CA

Objectives: The aim of this study was to synthesize and evaluate the 18F-quinoxaline probes for the imaging of malignant melanoma. **Methods:** 18F-quinoxaline was prepared by radiofluorination reactions using no-carrier-added 18F and evaluated in vivo by biodistribution studies and micro-positron emission tomography (microPET) imaging in C57BL/6 black mice bearing B16F10 murine melanoma tumors. **Results:** 18F-fluorination of the corresponding bromoquinoxaline precursors was achieved in good yields (12-25% decay corrected on the basis of 18F-fluoride; n=4). The total synthesis time was less than 1 hour. The radiochemical purity of the 18F-labeled probes was more than 98% and the specific activity was 90-140 GBq/μmol. Noninvasive microPET and direct-tissue sampling experiments demonstrated that the probes had melanin-specific tumor targeting in subcutaneous B16F10 melanoma xenografts, which yielded a tumor-to-blood ratio of approximately 18:1 at 1 h and greater than 25:1 at 2 h. Quantitative microPET imaging studies showed that 18F-quinoxaline had very high tumor uptake (e.g., 17.5 ± 3.4, 15.1 ± 2.2, and 12.4 ± 3.9 %ID/g at 0.5 h, 1 h, and 2 h postinjection, respectively). Of note, the consistency of direct-tissue sampling and the microPET analysis further validates the accuracy of microPET quantitative property for this novel probe. **Conclusion:** The new 18F-quinoxaline probes were synthesized with high specific activity via one-step 18F-fluorination in a straightforward and convenient manner. The probes exhibited high tumor uptake, as well as favorable in vivo pharmacokinetics. These findings suggest that 18F-fluoroquinoxaline could be promising imaging agents for the detection of melanoma.

INVITED SPEAKER ABSTRACTS

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Targeted radiotherapy: current status and new directions. C.J. Anderson; University of Pittsburgh, Pittsburgh, PA

There have been major advances made in the area of targeted radionuclide therapy over the past decade, with several new alpha and beta-emitting agents being either approved for clinical use or in clinical trials. For example, the first alpha-emitting agent, Ra-223 dichloride, was approved in May, 2013. This presentation will provide an update on targeted radiotherapy agents in human trials, with an emphasis on peptide receptor radiotherapy. There are also exciting new directions in the pre-clinical stages. In our laboratory, we are investigating Cu-64-labeled monoclonal antibodies and peptides for imaging and therapy, as well as novel agents labeled with Lu-177.

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Advancing personalized nuclear medicine by theranostics of prostate cancer using Ga-68 and Lu-177 labeled PSMA small molecules. R.P. Baum[1], H. Kulkarni[1], M. Weineisen[2], C. Schuchardt[1], M. Schottelius[2], H.-J. Wester[2]; [1]Zentralklinik Bad Berka, Bad Berka, Germany, [2]Technical University, Munich, Germany

Aim: DOTAGA-based PSMA ligands exhibit high and specific uptake in prostate cancer by targeting the prostate-specific membrane antigen (PSMA). The aim of this study was to analyze the safety and efficacy of Lu-177 PSMA in our initial cohort of patients with metastasized castrate-resistant prostate cancer (PC). **Methods:** Thirty mCRPC patients with distant metastases and progressive disease, refractory to conventional treatment (including hormone, chemo- and radiation therapy) underwent peptide radioligand therapy (PRLT) with Lu-177 PSMA. Ga-68 PSMA-HBED-CC PET/CT was performed in all patients 1-5 days prior to PRLT. Multiple cycles (mean 2.2, range 2-4) were administered in 14 patients. The mean administered activity of Lu-177 PSMA per cycle was 5.6±0.7 GBq, median 5.8. Clinical, biochemical, molecular and morphological response by means of Ga-68 PSMA-HBED-CC PET combined with contrast-enhanced CT 8-10 weeks post-therapy could be assessed until now in 14 patients. In addition, patient-specific dosimetry was performed according to MIRD scheme. **Results:** Intense PSMA expression was confirmed in metastases as well as in residual/locally recurrent PC (n=3) by baseline Ga-68 PSMA-HBED-CC PET/CT. There was also high Lu-177 PSMA uptake on post-therapy planar and SPECT/CT images. Response evaluation by Ga-68 PSMA-HBED-CC PET/CT revealed molecular treatment response (partial remission) in 8 patients, and morphological response in 4 patients. Stable

disease was noted in 4 and 8 patients, according to molecular and morphological response criteria, respectively, whereas disease progressed in 2 patients. The following organ and tumor doses were calculated: Whole body 0.02±0.01 mGy/MBq; kidneys 0.35±0.14 mGy/MBq; tumor lesions 0.14-19.8 mGy/MBq. The results of the blood sampling showed a fast clearance from blood with half-life up to 42 hours. The treatment was well tolerated by all patients without any significant adverse effects or alterations in any of the laboratory parameters or renal function (as determined by Tc-99m MAG3/TER). **Conclusions:** Lu-177 PSMA demonstrated a high tumor uptake, fast renal wash-out and blood clearance. The initial results demonstrate that PRLT with Lu-177 PSMA is both safe and effective in castrate-resistant metastatic prostate cancer with appropriate selection and follow-up of patients by Ga-68 PSMA-HBED-CC PET/CT.

3

Extracellular vesicles/RNA as tactics in glioblastoma progression. X.O. Breakefield, K.E. van der Vos, E.R. Abels, K.Y. Edward, E. Carrizosa, B.A. Tannous, T.R. Mempel, C.P. Lai; Massachusetts General Hospital, Harvard Medical School, Boston, MA

Glioblastoma cells release a barrage of extracellular vesicles (EVs) containing proteins and RNAs that can manipulate the phenotype of normal cells in their environment in favor of tumor growth. Our studies have focused on tracking these vesicles in mouse tumor models using multimodal imaging labels and analyzing their RNA content and its potential functions. We have labeled vesicles using palmitoylated fluorescent membrane proteins as well as a membrane-bound form of Gaussia luciferase (GlucB) with a biotin tag. Vesicle half-life as well as tissue, biofluid and tumor distribution have been evaluated over time. We have also monitored release of vesicles by gliomas within the brain in a syngeneic mouse model using multiphoton intravital microscopy. Functional RNA transfer has been assessed for mRNA by translation of GlucB in recipient cells after EV uptake in culture and for miRNAs by elevation of miR-451 and miR-21, which are abundant in GBM-derived vesicles, in recipient microglia/macrophages and suppression of their common target mRNA for c-Myc, both in culture and in a syngeneic glioma model in vivo. These studies support the dynamic and functional aspects of EVs in manipulating the tumor microenvironment. **Acknowledgments:** Supported by NCATS/NIH/NCI U19 179563 (X.O.B. and T.R.M.) and NIH/NINDS P30 NS045776.

4

Phage-display selected peptides for imaging of non-small cell lung cancer: development of a peptidic PET probe for αvβ-integrin. K.C. Brown[1], D. Cupka[1], A. Singh[2], M.J. McGuire[1], X. Sun[2]; [1]SRI International, Harrisonburg, VA, [2]University of Texas Southwestern Medical Center, Dallas, TX

Tumor targeting ligands are emerging components in cancer therapies. Widespread use of targeted therapies and molecular imaging is dependent on increasing the number of high affinity, tumor-specific ligands. Towards this goal, we biopanned three phage-displayed peptide libraries on a series of well-defined human non-small cell lung cancer (NSCLC) cell lines, isolating 11 novel peptides. The peptides show distinct binding profiles across 40 NSCLC cell lines and do not bind normal bronchial epithelial cell lines. The peptides display cell binding affinities between 0.0071-40 nM, rivaling the affinities of clinically used monoclonal antibodies. One such peptide binds the restrictively expressed integrin αvβ. This integrin is an emerging biomarker for NSCLC as well as other epithelial derived cancers. Due to its importance in cancer, we utilized a multivalent design to develop a peptidic probe for positron emission tomography (PET) imaging of αvβ+ NSCLC tumors. By varying the valency, the N-capping group, and peptide charge, we developed an αvβ6-integrin specific probe with optimized biodistribution for noninvasive PET imaging of NSCLC. Further, we have demonstrated that use of multivalent scaffolds is a plausible method to improve library selected peptides, which would be suboptimal or useless otherwise, for imaging probe development.

5

Molecular imaging targets for epithelial cancer stem cells. M. Clarke; Institute for Stem Cell Biology and Regenerative Medicine, Stanford, CA

Most common cancers, such as cancers of the breast and colon, arise in organs that contain a small population of stem cells that constantly replenish the mature cells of the tissue. Stem cells are defined by the ability to divide and give rise to a new stem cell (self-renewal), as well as the ability to give rise to the differentiated cells of an organ, and thus are the only long-lived cell population in many tissues. Recent evidence suggests that colon and breast cancers arise from normal stem/progenitor cells. Our laboratory was the first to isolate breast and colon cancer stem cells, demonstrating that these cells are critical for the growth and metastasis. The molecular regulation of maintenance of colon and breast cancer stem cells will be discussed, as will potential imaging targets and strategies for identification of stem cells. We will present evidence that targeting breast and colon cancer stem cells will lead to the identification of markers important for prognosis, prediction of response to therapies as well as the identification of novel therapeutic targets.

6

Inflammation and cancer: immune cells as targets for anticancer therapy. L.M. Coussens; Oregon Health & Science University, Portland, OR

The concept that leukocytes are critical components of solid tumors is now generally accepted; however, their role in regulating aspects of neoplastic progression and/or response to cytotoxic therapy is only beginning to be understood. Utilizing de novo mouse models of organ-specific cancer development, we now appreciate that adaptive leukocytes differentially regulate myeloid cell recruitment, activation and behavior, and in turn, engaged tumor-infiltrating myeloid cells activate tissue-based programs to foster malignancy, as well as repress anti-tumor immunity by a diversity of mechanisms. Treatment of tumor-bearing mice with therapeutic agents that disrupt lymphocyte-myeloid cell interaction, myeloid cell activation, or myeloid cell functionality invariably results in slowing of primary tumor growth, and also improved responses to cytotoxic therapies, and significantly diminished presence of metastatic disease. To be presented will be our recent insights into organ and tissue-specific regulation of cancer development by adaptive and innate immune cells, and new studies evaluating how attenuating protumor properties of select lymphoid and myeloid cells can be exploited to enhance therapeutic responses to cytotoxic therapy. **Acknowledgments:** L.M.C. acknowledges generous support from the NIH/NCI, the Department of Defense Era of Hope Scholar Expansion Award, Susan G. Komen Foundation, the Breast Cancer Research Foundation, and a SU2C award supported by the AACR and Lustgarten Foundation.

7

Opportunities and needs for molecular imaging in melanoma. M.A. Davies; The University of Texas MD Anderson Cancer Center, Houston, TX

Melanoma is the most aggressive form of skin cancer. The treatment of melanoma is evolving rapidly due to an improved understanding of the oncogenic drivers and the anti-tumor immune response in this disease. These breakthroughs have led to multiple new, more effective treatments for melanoma patients. However, studies of resistance have identified many challenges that remain to be overcome to improve outcomes further. This presentation will review these challenges as a forum to discuss opportunities and needs for molecular imaging to impact clinical outcomes in cancer, using melanoma as an example.

8

Nanoparticle therapeutics: from concept to clinic. M.E. Davis; California Institute of Technology, Pasadena, CA

We have translated two nanoparticle-based therapeutics into the clinic for use as cancer therapeutics. These nanoparticles have been investigated in ca. 10 clinical trials. I will describe how we translated these nanoparticles from the laboratory at Caltech into clinical therapeutics. Lessons learned from these translational and clinical experiences are accelerating our current translational efforts with nanoparticle-based therapeutics for cancer. While we exploited the use of multimodal imaging methodologies in animal models of cancer, these types of investigations were not enabled with patients. As an example, I will describe the studies that were performed in animals and then with patients for the nanoparticle therapeutic containing

siRNA that was the first RNAi clinical trial for cancer. I will end with a discussion on what might be of particular interest for future clinical studies with siRNA.

9

Metabolic heterogeneity of cancer cells in culture and in vivo. R.J. DeBerardinis; Children's Medical Center Research Institute at UT Southwestern Medical Center, Dallas, TX

Metabolic reprogramming is viewed as an essential component of malignant transformation. Over the past decade, a large number of mechanisms by which oncogene-directed perturbations of signal transduction regulate intermediary metabolism have been defined. Conversely, it has also become apparent that metabolites themselves influence gene expression and signaling in ways that appear to promote malignancy. However, there is still little agreement as to the breadth of metabolic programs that can support cancer cell survival and growth, and more importantly, about which of the myriad metabolic activities observed in culture models of cancer cell proliferation are relevant to bona fide tumor metabolism in vivo. I will discuss approaches to address these two challenges. First, I will discuss ongoing efforts to link functional metabolic pathway choices with oncogenotypes using systematic analysis of large panels of cancer cell lines. Second, I will describe efforts to probe metabolism in living tumors from humans and mice, using a combination of stable isotope tracing and other methods, including multi-parametric magnetic resonance imaging. Together, these efforts identify a complex but finite set of metabolic phenotypes that support cell growth and may predict therapeutic vulnerabilities. They also underscore the potential for both existing and novel imaging approaches to inform about metabolic phenotypes in individual tumors.

10

Molecular imaging for drug development and selection of targeted treatment in advanced disease. E.G.E. de Vries, M. van Kruchten, L.E. Lamberts, F. Bensch, A.H. Brouwers, M.N. Lub-de Hooge, C.P. Schröder, G.A.P. Hospers; University Medical Center Groningen, Groningen, The Netherlands

Typically, characteristics are analyzed in tumor tissue, which is often archival tissue and may not reflect heterogeneity among lesions and within lesions. Molecular radionuclide imaging with positron emission tomography (PET) can potentially provide interesting support for these issues. Drug development decisions could also benefit from quantitative biomarkers in order to visualize the drug tissue distribution, to confirm effective whole-body expression as well as engagement and modulation of the target. Visualization of the estrogen receptor (ER) with ^{18}F-fluoroestradiol (FES) is feasible in breast and ovarian cancer patients. Heterogeneity of ER expression occurs in breast cancer as both FES-positive and -negative lesions are present in 15-47% of patients with an earlier ER-positive primary tumor. We performed a feasibility study to assess ER availability before and during treatment with the ER downregulator fulvestrant. Fulvestrant reduced tumor FES uptake incompletely on day 28 in 38% of the patients (6 out of 16), which was associated with early progression. The androgen receptor (AR) can be imaged with ^{18}F-fluorodihydrotestosterone (FDHT) in prostate cancer patients. FDHT-PET showed that the AR antagonist enzalutamide substantially reduces FDHT binding (Scher H, et al. Lancet. 2010). The AR is increasingly considered of interest in breast cancer and a trial with FDHT-PET in breast cancer is ongoing. Additionally, numerous available antibodies can be radioactively labeled for PET imaging. In metastatic breast cancer patients with HER2 overexpressing tumors quantifiable ^{89}Zr-trastuzumab-PET tracer uptake in tumor lesions was seen. HSP90 chaperones have key client proteins that are involved in all hallmarks of breast cancer growth and progression. We evaluated the feasibility of using ^{89}Zr-trastuzumab-PET to determine in vivo degradation of HER2 caused by the novel HSP90 inhibitor NVP-AUY922. The standard uptake value (SUV) max change in individual tumor lesions on baseline versus 3 weeks ^{89}Zr-trastuzumab-PET was heterogeneous and related to size change on CT after 8 weeks of treatment. NVP-AUY922 therefore showed proof-of-concept clinical response in HER2-amplified metastatic breast cancer. With ^{89}Zr-bevacizumab-PET, we visualized tracer uptake in primary breast cancers and metastatic neuroendocrine tumors and renal cell cancers and visualized how bevacizumab treatment reduced uptake of antibodies. With ^{89}Zr-antibody PET imaging it is also potentially possible to study the whole-body distribution of an antibody to predict uptake of an antibody-drug conjugate with the same antibody and to determine target saturation at a certain therapeutic antibody dose. Moreover, molecular imaging is potentially able to provide major insights at the tumor level before and during novel immunotherapies. Thus, molecular imaging can identify characteristics of a specific tumor across the entire body over time, can provide new mechanistic and pharmacological insights and can contribute to identifying patients most likely to benefit from a specific treatment.

11

New frontiers: combining molecular imaging and nanomedicine to light up and selectively destroy cancer. T. Hasan; Harvard Medical School, Massachusetts General Hospital, Boston, MA

Absorption of light by molecules within or associated with cells and tissues creates photophysical and photochemical processes that are captured for diagnostics, surgical guidance, a treatment, and for gaining of simple mechanistic understanding of disease pathology. Photodynamic therapy (PDT) is a photochemistry-based process resulting from the light activation of chemicals localized at anatomical sites of disease. It involves the excitation of certain chemicals, called photosensitizers (PS), by photons of a specific wavelength to produce reactive species that are toxic to cells. The active molecular species produced by such a molecular excitation destroys biological targets in the vicinity. PDT is approved for specific clinical applications by regulatory agencies worldwide and has inherent selectivity built in it: the first arising from a preferential localization of the PS, and the second the simultaneous requirement for light to activate any cytotoxic process which can be controlled by confining the volume of tissue exposed to light. A small fraction of the excited state de-energizes via fluorescence, thus providing an opportunity to use the same molecule for detection/diagnostics and therapy. As with any therapy, PDT elicits molecular responses that can be exploited to enrich our arsenal of cancer treatment. This requires the development of novel combinations delivered at the right time and the right place and is often achieved best using nanotechnology where PDT serves not only as a treatment trigger but also as a drug release switch. This presentation will focus on the challenges associated with applying PDT as a theranostic modality in novel constructs for the treatment of complex disease.

12

The synthetic biology of CAR T cells. M.C. Jensen; Seattle Children's Research Institute, Seattle, WA

Recent conceptual as well as technological advances in the areas of molecular immunology, gene transfer, and cell processing have fostered increasingly sophisticated translational applications of adoptive T-cell therapy for oncologic disease employing genetically modified T-lymphocytes. My laboratory's work focuses on T-cell genetic modification for re-directing antigen specificity to tumors utilizing recent advances not only in the composition and specificity of receptor antigen recognition domains, but also the evolution of multifunctional cytoplasmic signaling domains developed for these chimeric antigen receptors (CARs) that provide dual activation and co-stimulatory signaling. My group is also investigating the context of adoptive transfer with respect to the conditioning of the recipient for enhanced T-cell engraftment and expansion, the grafting of CARs onto central memory T-cells having endogenous TCR specificities for viral epitopes to which the host has robust immunity, and the provision of tumor microenvironment survival capabilities. The increasingly broad array of genetic manipulations, including not only transgene insertion, but targeted gene knock out using engineered targeted nucleases, such as TALENs and ZFN, as well as expression regulatory constructs provides for the creation of synthetic biology of orthogonal immune responses based on gene modified T-cell adoptive transfer. The next decade of advances in this arena will depend on iterative bench-to-bedside back-to-the-bench translational studies capable of sustaining the evolution of these technologies in the context of clinical parameters relevant to the pediatric oncology patient population.

13

Metastasis-promoting functions of tumor-derived exosomes. Y. Kang; Princeton University, Princeton, NJ

Tumor-secreted factors play crucial roles in orchestrating the establishment and instructing the dynamic evolution of the microenvironment at primary and distant sites. Growing evidence indicates that exosomes can direct intercellular communication under physiological and pathological conditions. These small vesicles contain many functional factors including proteins, lipids, metabolites and genetic material (mRNAs, miRNAs, DNA) which appear to be selectively recruited and secreted in a regulated manner. Accumulating evidence indicates that horizontal transfer of exosomal factors can functionally influence stromal cells. Indeed, exosomes from melanoma cells with high metastatic potential, but not from those with low metastatic potential, can promote melanoma metastasis in vivo by educating stromal components towards pro-metastatic phenotypes. In particular, we showed that melanoma exosomes induce vascular leakiness and mobilize bone-marrow progenitors via transfer of c-MET. Moreover, we determined that the exosomal signature comprising TYRP2, VLA-4, HSP70, an HSP90 isoform and c-MET has diagnostic, prognostic and therapeutic value. In breast cancer, disruption of the exosome biogenesis pathway reduces bone and lung metastasis. Moreover, exosomes isolated from bone metastatic breast cancer cells promote osteoclast differentiation and contribute to the formation of osteolytic bone metastasis. These findings underscore the importance of tumor exosomes in mediating tumor-stromal interactions that drive metastatic progression of breast cancer in different organs.

14

Utilizing hyperpolarized MR to interrogate real-time tumor metabolism in vivo. K.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. Interrogation of these pathways in relevant systems, though, has been hindered by lack of technologies capable of monitoring metabolism. 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. These probes can then be followed using spectroscopic imaging techniques non-invasively and inform on the metabolic state of the cell as well as the dynamics of metabolism in real time. In the setting of cancer metabolism, many probes have been developed which inform on multiple pathways. These include the measures of glycolytic flux to lactate (HP pyruvate) and redox state (HP dehydroascorbate) of the cell. Moreover, this approach has recently been shown to be safe in a first trial demonstrating the great promise of this approach for future interrogation of cancer metabolism *in vivo*.

15

Targeting the stroma in pancreas cancer: the unexpected outcome. V. LeBleu; The University of Texas MD Anderson Cancer Center, Houston, TX

Pancreatic ductal adenocarcinoma (PDAC) is associated with a marked desmoplastic reaction composed abundantly of stromal myofibroblasts that are proposed to remodel the extracellular matrix and tumor immune infiltration. Their precise functional contribution remains, however, unknown. We employed transgenic mice designed to selectively deplete aSMA+ myofibroblasts in spontaneous pancreas adenocarcinomas at various time points of disease progression. The targeting of myofibroblasts led to invasive, undifferentiated tumors with enhanced hypoxia, epithelial-to-mesenchymal transition, cancer stem cells and diminished survival in multiple genetically engineered mouse models of PDAC. These studies also revealed an immune modulatory function of myofibroblasts in PDAC in mice, wherein mice responded to immune checkpoint blockage therapy, while gemcitabine treatment remained ineffective. Our studies also indicated that lower intratumoral myofibroblast content correlated with diminished survival in PDAC patients. This study underscores an initial protective function of pancreas fibrosis in PDAC progression and cautions against the targeting of carcinoma-associated fibroblasts in PDAC.

16

The development and imaging of advanced cancer models. S.K. Lyons; Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Mouse models of cancer provide an essential experimental link between fundamental discoveries made at the laboratory bench and the treatment of patients in the clinic. Advanced cancer models, in particular, whereby the expression of inherited genes can be both spatially and temporally regulated, comprise some of the most accurate phenocopies of the human disease currently available. From a practical standpoint, however, these models can be challenging to work with as tumors often develop at non-visible body locations and the timing of tumor onset can vary greatly with age. Effective non-invasive imaging can largely obviate these limitations and provide both anatomical and functional tumor-biology related information. The very nature of modelling cancer in the mouse is also changing, giving rise to exciting new experimental possibilities and far greater flexibility. Such modelling approaches also provide exciting new possibilities to non-invasively image tumor biology, in particular, with reporter transgenes, an experimental approach that can be employed to image a broad range of biological processes. Both modelling and imaging approaches will be discussed.

17

Radiotracers for imaging tumor cell proliferation and quiescence with PET. R.H. Mach; University of Pennsylvania, Philadelphia, PA

Two different methods have been developed for imaging cell proliferation with PET, proliferation rate and proliferative status. Proliferation rate refers to how fast a proliferating or cycling cell passes through the four different phases of the cell cycle: G1, S, G2 and M. Tumor cells having a high proliferation rate have a high S-phase fraction. Tracers for measuring proliferation rate take advantage of enhanced DNA synthesis during the S-phase and the need for thymidine as a substrate to make DNA. The most prominent radiotracer for imaging proliferation rate is [18F]FLT. Proliferative status is defined as the ratio of proliferating (P) to quiescent (Q) cells in a tumor (i.e., the P:Q ratio). The only PET approach for measuring proliferative status targets sigma-2 receptor expression. This method takes advantage of the 10-fold higher density of sigma-2 receptors in P cells versus Q cells. A high uptake of a sigma-2 receptor radiotracer (e.g., [18F] ISO-1) indicates a high percentage of P cells in the tumor. Furthermore, the density of sigma-2 receptors in Q tumor cells is higher than that in senescent cells present in normal breast tissue. Therefore, a sigma-2 receptor radiotracer can discriminate quiescent breast tumors from senescent normal tissue. This talk will focus on how these two highly complementary methods for imaging cell proliferation can be used for directing cancer therapy.

18

Molecular imaging as a cancer biomarker in clinical trials. D. Mankoff; University of Pennsylvania, Philadelphia, PA

The ability to measure biochemical and molecular processes to guide cancer treatment represents a potentially powerful tool for trials of targeted cancer therapy. These assays have traditionally been performed by analysis of tissue samples. More recently, functional and molecular imaging allows in vivo assay of cancer biochemistry and molecular biology that is highly complementary to tissue-based assay. Molecular imaging can inform targeted cancer clinical trials and clinical decision making by (1) measuring regional expression of the therapeutic target, (2) testing the ability of drugs to interact with their intended targets, (3) measuring cancer response early in the course of treatment, and (4) relating response to the risk of relapse. This talk will review basic principles of molecular imaging in cancer, with an emphasis on those methods that have been tested in patients. The talk will review the current state of molecular imaging in cancer patients,

including methods in routine clinical use, those undergoing advanced clinical trials, and those in early-phase testing. Current trials and future directions will be highlighted. **References:** 1. Farwell MD, Pryma DA, Mankoff DA. PET/CT imaging in cancer: current applications and future directions. Cancer. 2014 Nov 15;120(22):3433-45. 2. Mankoff DA, Pryma DA, Clark AS. Molecular imaging biomarkers for oncology clinical trials. J Nucl Med. 2014 Apr;55(4):525-8.

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Immune biology of allogeneic transplantation. R.S. Negrin; Stanford University, Stanford, CA

Allogeneic hematopoietic cell transplantation represents a clinically relevant setting of immunobiology. The major benefits of allogeneic transplantation include the graft-versus-tumor (GVT) effect where complex malignancies can be eradicated; however, one of the major risks includes the induction of graft-versus-host disease (GVHD). We have utilized rodent models to study the immunobiology of different cell populations using the strategy of bioluminescent imaging (BLI) whereby cells that have been genetically engineered to emit light by the introduction of the luciferase gene can be followed in small animals quantitatively and non-invasively. Using these approaches, we have explored the induction of GVHD by allo-reactive T cells. Very early following transplantation, at ~day 3-5, donor derived T cells infiltrate recipient nodal sites, begin to replicate and upregulate molecules that allow entry into GVHD target organs such as the gastrointestinal tract and skin. There these cells continue to proliferate before causing tissue injury, diarrhea, skin rash and ultimately infection and animal death. These studies highlight that the major induction of GVHD happens at these early time points and that by the time animals, as well as likely patients, develop the signs and symptoms of GVHD, many of these important biological events have already occurred. In our studies, we have explored a variety of strategies to block entry of these T cells into GVHD target organs where it was found that there were multiple priming sites and antibodies that block entry into lymph nodes, Peyer's patches and other nodal sites, and a splenectomy was required in order to block GVHD. Although this was biologically interesting, this was not practical in the transplant setting. Therefore, other strategies are required to suppress GVHD. One such approach has been the timed addition of other immune cell populations that have been shown to regulate immune responses. It has been well recognized that the induction of immune response, as well as its control, requires the timed orchestration of a variety of different cell populations with different functions. Of particular interest are cells that regulate immune responses, and a number have been described including CD4+CD25+FoxP3+Tregs (Treg), as well as natural killer T cells (NK-T cells). In our studies, we have explored the adoptive transfer of Treg and NK-T cells and shown that these cells are capable of suppressing the massive T cell proliferation associated with GVHD but allow for retention of GVT responses. Interestingly, the Treg also expand and persist for ~2-4 weeks. Importantly, one could demonstrate that these animals still have effective immunity to cytomegalovirus, as well as the ability to control tumor reactions and maintain GVT responses. More recent studies have shown that invariant NK-T cells also have similar biological effects but these cells are even more potent than regulatory T cells and ~50-fold or fewer iNK-T cells are capable of suppressing GVHD. Interestingly, the finding that donor Treg are required for the beneficial effect of iNK-T cells demonstrated that there is cross-talk between these different immune-regulatory cell populations. Initial studies with the use of Treg in humans are highly supportive of these observations where it has been shown that there is reduced risk of GVHD while GVT effects are maintained. Immune regulation holds promise for controlling some of the major challenges with allogeneic hematopoietic cell transplantation and could be applied more broadly to other clinical settings.

20

Imaging and theranostics in prostate cancer. M.G. Pomper; Johns Hopkins Medical School, Baltimore, MD

With a wide variety of treatment options now available for prostate cancer (PC) it becomes increasingly important to be able to define the extent of disease accurately before, during and after surgery. The order and combination of possible therapeutic options provide a welcome but daunting task to the oncologist. Arguably the best way to assess extent of disease, with the possibility of understanding the biology of the disease detected, is through molecular imaging. But PC is notoriously difficult to image. It is not as metabolically "active" as other tumors, leaving FDG-PET, the current clinical standard in oncology, lacking. However, along with new therapeutic options, new options for imaging and the combination of imaging with therapy (theranostics) are coming online. We will briefly discuss the idea of companion diagnostics in the context of imaging with attention to PC. Presented will be examples of existing and near-term clinical molecular imaging and theranostic agents with a focus on those targeting the prostate-specific membrane antigen (PSMA), which are increasingly applied worldwide to manage patients suffering from PC at various stages of disease.

21

Intravital imaging reveals how BRaf inhibition generates drug tolerant microenvironments. E. Hirata[1], M.R. Girotti[2], A. Viros[2], S. Hooper[1], B. Spencer-Dene[1], M. Matsuda[3], J. Larkin[4], R. Marais[2], E. Sahai[1]; [1]Cancer Research UK London Research Institute, London, United Kingdom, [2]The Cancer Research UK Manchester Institute, Manchester, United Kingdom, [3]Kyoto University Graduate School of Biostudies, Kyoto, Japan, [4]Royal Marsden NHS Trust, London, United Kingdom

Many tumors show an initial response to targeted therapies before genetic resistance emerges; however, little is known about how tumor cells tolerate therapy before genetic resistance dominates. In this study, we have used both intravital ratiometic FRET and FLIM of an ERK/MAP kinase biosensor to investigate heterogeneity in signaling in melanoma models. Moreover, we have longitudinally monitored responses to targeted therapy and identified areas that become refractory to drug action. BRaf mutant melanoma cells can rapidly become tolerant to PLX4720 in areas of high stroma. The rapid kinetics of this process indicate that it is not caused by genetic events. We demonstrate that PLX4720 has an unexpected effect on the tumor stroma leading to enhanced matrix remodeling. The remodeled matrix then provides signals that enable melanoma cells to tolerate PLX4720. We propose that this safe haven enhances the population of cancer cells from which genetic resistance emerges. This work highlights the utility of intravital imaging in understanding the reason for therapy failure.

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Design of clinical trials and approaches to prediction and assessment of response. A.F. Shields; Barbara Ann Karmanos Cancer Institute, Detroit, MI

Imaging provides an essential element in evaluating the efficacy of cancer treatment. It continues to gain importance as we develop multiple therapeutic options for patients and treatment becomes more personalized. The oncologist is in need of better ways to predict which treatment is likely to be efficacious. Early assessment of response also becomes critical when multiple options are available for patients who fail first line therapy. Imaging using anatomic assessment, such as with CT and MR, has long been used to help stage patients and is often included in the eligibility criteria of trials. It has also been routinely used to measure the change in tumor size as treatment progresses over multiple months. Such assessments have been standardized using RECIST and other approaches, but one needs to understand the limitations of such techniques for both routine clinical care and clinical trials. This has become particularly problematic with many of the new therapeutic options. Some targeted therapies may slow tumor growth but do not result in significant shrinkage. Furthermore, even with slow growth, treatment may provide benefits for the patient. Anti-vascular agents have become commonly used in cancer therapy, both in combination with other agents and by themselves. To date we lack good predictive markers for efficacy of such agents and also need methods to demonstrate their physiologic effect. Furthermore, anti-vascular agents can lead to tumor necrosis and produce changes described as pseudo-progression. These need to be taken into account as trials are designed. The recent success of immunotherapy can also lead to pseudo-progression on anatomic imaging and many trials are now working to improve the assessment of such studies using modified RECIST criteria and other metabolic imaging assessments. The development of both older and newer targeted therapies often relies on

biomarkers to predict and monitor treatment. While the presence of a tumor target may be predicted using genomic markers obtained on tumor specimens, these molecular markers can be complemented by use of imaging tools to measure activity in specific pathways and demonstrate that they have been blocked by treatment. Furthermore, regular measurements of metabolism made after therapy may provide information that adds to standard anatomic imaging methods. As new treatments are developed it is important the oncologists and imagers discuss methods to incorporate novel imaging approaches in both phase I and early phase II trials. These can be used to help understand the pharmacodynamics of the agents and provide evidence of efficacy or help in deciding that a particular therapy should not be pursued for a given tumor. The imaging methods may also find subsequent use as an integral biomarker to help select and monitor treatment as future trials are developed.

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New methods for assessing cellular metabolic heterogeneity. M.C. Skala; Vanderbilt University, Nashville, TN

Abnormal cellular metabolism is a hallmark of many diseases, yet there is an absence of quantitative methods to dynamically image this powerful cellular function. Optical metabolic imaging (OMI) quantifies the fluorescence intensities and lifetimes of the metabolic co-enzymes NADH and FAD using two-photon microscopy. OMI is a non-invasive, high-resolution, quantitative tool for monitoring cellular metabolism. In vivo OMI is sensitive to metabolic changes induced by HER2 inhibition with Herceptin (a clinically used therapy) in responsive, but not resistant, xenografts within 48 hours after one drug treatment (a time point preceding changes in FDG-PET images). We have further developed a "tumor-in-a-dish" organoid platform to rapidly test multi-drug response using OMI. This platform has been validated in breast cancer xenografts, and feasibility has been tested in human breast tumors. Importantly, the cellular-level assessment of OMI allows for sub-populations of cells with varying response to drug treatment to be tracked over time, in order to achieve therapeutic effect in all cell populations. This attractive suite of metabolic imaging tools has significant implications for rapid cellular-level assessment of metabolic response to drug treatment (1) in living animals, (2) in high-throughput drug efficacy studies, and (3) as a clinical tool to plan treatment regimens. Therefore, these technologies could greatly enhance our understanding of cellular-level heterogeneity in cancer, and thereby accelerate cures for cancer patients.

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Building molecules to image and treat cancer. E.N. Savariar[1], T. Jiang[2], M. Whitney[1], J.L. Crisp[1], P. Steinbach[2], L.G. Ellies[1], S.J. Advani[1], Q.T. Nguyen[1], R.Y. Tsien[2]; [1]University of California, San Diego, La Jolla, CA, [2]Howard Hughes Medical Institute and University of California, San Diego, La Jolla, CA

For clinical applications, we need synthetic molecules with novel amplifying mechanisms for homing to diseased tissues. Activatable cell penetrating peptides (ACPPs) are polycationic cell penetrating peptides (CPPs) whose cellular uptake is minimized by a polyanionic inhibitory domain and then restored upon proteolysis of the peptide linker connecting the polyanionic and polycationic domains (1). Local activity of proteases able to cut the linker causes amplified retention in tissues and uptake into cells. ACPPs sensitive to matrix metalloproteinases-2 and -9 attached to dendrimers labeled with Cy5 and Gd-DOTA enable whole body magnetic resonance imaging (MRI) (2) followed by fluorescence-guided surgery. Such fluorescence guidance illuminates tumor margins and improves tumor-free survival in several animal models (3,4). Contrast for tumor over normal tissues is amplified and accelerated when the polyanionic domain includes an acceptor of fluorescence resonance energy transfer (FRET), because loss of FRET, monitored by multispectral emission, instantly signals proteolysis without waiting for uncleaved substrate to wash out of normal tissues (5). Another way to improve contrast is to fuse ACPPs with cyclic(RGD) to exploit synergistic targeting towards MMP-2 and $\alpha v\beta 3$ integrin, due to the known physical interaction of the latter molecules. Such synergy not only improves tumor fluorescence but can deliver the very potent novel chemotherapeutic agent monomethylauristatin E (MMAE), at therapeutically promising doses, for example, in mammary carcinoma that is triple-negative for conventional antibody labeling (6). MMAE is a microtubule inhibitor that traps cells at the G2/M transition when they are maximally sensitive to ionizing radiation, so MMAE is a potent radiosensitizer localizable in tumors (Buckel et al., submitted). Separately, we have developed fluorescent peptides that light up peripheral nerves to show surgeons where not to cut (7). Thus, chemical biology and multimodal imaging can improve early detection and accurate resection, which together offer relatively promising avenues to deliver complete cures at relatively low cost. Even chemotherapy and radiation therapy may benefit from ACPP targeting. The FDA has recently allowed the first human trials of ACPPs to proceed. **References:** 1. Jiang et al. PNAS. 2004;101:17867-17872. 2. Olson et al. PNAS. 2010;107:4311-4316. 3. Nguyen et al. PNAS. 2010;107:4317-4322. 4. Metildi et al. Ann Surg Oncol. 2014; Oct 16. 5. Savariar et al. Cancer Res. 2013;73:855-864. 6. Crisp et al. Mol Cancer Ther. 2014;13:1514-1525. 7. Whitney et al. Nature Biotech. 2011;29:352-356.

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Clinical applications of quantitative imaging in planning and assessing cancer therapies. R.L. Wahl; Washington University School of Medicine, St. Louis, MO

Medical images of patients with cancer have typically been interpreted in a mainly qualitative manner with quantitative assessments usually limited to manual determinations of tumor size in one or two dimensions. Medical images from a variety of cross sectional methods are intrinsically quantitative, comprised of voxels representing tumors and normal tissues spatially with continuous data reflecting aspects of tumor biology. While qualitative image interpretation remains a cornerstone of interpretation, increasingly quantitative results are being reported, often extracted using semi-automated or automated methodologies. In CT, tumor volumes can now be calculated with increasing reliability using automated software tools. In MRI, methods to assess a variety of tumor physiological characteristics have been developed, including assessments of diffusion of water, vascular flow and permeability, and metabolite concentrations in addition to size metrics. With PET, a wide range of methods can be applied, including assessments of tumor glucose metabolism in a small region of a tumor, an entire tumor, or through the entire body. Other aspects of tumor biology can be assessed using PET including amino acid transport, flow, hypoxia, receptor or transporter expression, and proliferation, among other characteristics. Combining methodologies to secure a multi-parametric signal, such as with PET/CT, PET/MRI or SPECT/CT, has become a more common approach lending increased certainty to diagnosis. Quantitative imaging can allow assessments to include tumor detection, lesion characterization, and treatment response assessment at varying time points into treatment. End of therapy and intratherapy assessments are both applied with increased frequency. The applications of quantitative imaging are broad, but the PERCIST 1.0 response criteria for FDG PET will be discussed, as well as the RECIST and modified RECIST criteria. In addition, criteria and experience with PET and FDG for monitoring response in lymphoma, including the Deauville criteria (semiquantitative), will be discussed. Through this lecture, the move from qualitative and subjective to quantitative and objective tumor imaging and treatment response assessments will be highlighted, as well as opportunities moving forward.

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Imaging immune responses in cancer therapy. A.M. Wu; David Geffen School of Medicine at UCLA, Los Angeles, CA

Immune responses play a central role in the development of cancer and its treatment. Recent progress in cancer immunotherapy—using antibodies, vaccines, and cell-based therapies—has highlighted the need for imaging the immune system in order to understand, monitor, and optimize these approaches. Radioactive (SPECT/PET) methods for tracking immune cells have included *ex vivo* cell labeling, reporter gene imaging, and imaging using metabolic probes including ^{18}F-fluorodeoxyglucose (FDG). Direct imaging of surface markers (e.g., CD markers) on specific immune cell subsets using antibodies has also been explored. Protein engineering provides an approach for optimizing antibodies for use as PET tracers, providing probes with reduced biological activity and immunogenicity, and

accelerated clearance to enhance imaging contrast at early times. Engineered antibody fragments including minibodies and diabodies have been produced for immunoPET detection of B lymphocytes (CD20) and T lymphocyte subsets (CD4 and CD8). Recent examples of non-invasive imaging of tumors, metastases, and tumor immunotherapy in murine models will be presented. ImmunoPET can provide a versatile tool for profiling immune responses, including activation, expansion, and trafficking of immune cell subsets, and provides a path for translation into clinical use.

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Intravital lineage tracing to study dynamics of tumor growth and metastasis. A. Zomer; Hubrecht Institute, Utrecht, The Netherlands

The accumulation of mutations and the dynamic changes in the tumor microenvironment drive tumor progression and heterogeneity. Commonly used techniques, such as histology and Western blot, provide a static view of these dynamic tumor processes and/or analyze large numbers of cells, thereby obscuring the adaptive properties of individual cells. By contrast, high-resolution intravital imaging (IVM) allows the real-time visualization of dynamic processes in living mice at subcellular resolution. To study dynamic cancer processes for prolonged times, we developed chronically implanted optical windows including the Mammary Imaging Window (MIW) to image primary mammary tumors and the Abdominal Imaging Window (AIW) to image liver metastases. We have combined these IVM techniques with Cre-mediated lineage tracing of fluorescent mouse models to study the plasticity and heterogeneity of cell-cell communication and hierarchical growth of benign and malignant mammary tumors in real time. By intravital lineage tracing, we show the existence of cancer stem cells (CSC) in genetic mammary tumors and illustrate the dynamic nature of these cells by visualizing the disappearance and formation of CSCs during the growth and progression of a tumor. Moreover, in the same genetic mouse model, we directly show that tumor cells exchange extracellular vesicles locally and systemically and that this influences the migration and metastatic capacity of these tumor cells. From all our studies we conclude that tumors are highly plastic and very heterogeneous and that the behavior of a small sub-population of cells within a tumor is responsible for the growth and spread of cancer.

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Radiomics and radiogenomics. R.J. Gillies; H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL

Malignant cancers are characterized by microenvironmental and genomic heterogeneity, which are leading causes of resistance. Microenvironmental heterogeneity can be viewed radiographically, wherein non-uniform patterns of enhancement or attenuation can be associated with poor outcome. In order to investigate this, we have systematically extracted quantitative texture, shape, and density image feature data that can be mined with patient outcomes data for prognostic, diagnostic and predictive models alone, and in combination with genomic information. The radiomics enterprise is divided into five processes with definable inputs and outputs: (i) image acquisition and reconstruction; (ii) image segmentation and rendering; (iii) feature extraction and qualification; (iv) databases and data sharing; and (v) informatic analyses. Each of these steps poses discrete challenges that have to be met. Even though this field is young, meaningful classifier models have been generated in detecting and diagnosing a number of cancer subtypes. To date, the radiomics effort has focused on agnostic and semantic image features, which quantify indescribable and describable features, respectively. These have been shown to have high prognostic value in non-small cell lung cancer (NSCLC) and are being used to classify indeterminate lung nodules in lung screening CTs. More recently, we have been combining orthogonal MR images to develop data cubes for each voxel, which can then be clustered using fuzzy logic to identify specific sub-tumoral "habitats," each with its own unique combination of perfusion, lipid/water ratio and cellular density. These habitats describe specific sub-tumoral regions associated with genetic clades, and hence may inform the application of targeted therapy. Going forward, these data will have to be captured prospectively in what we call "The Radiology Reading Room of the Future."

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Tumor Paint molecular imaging agents for intra-operative guidance in solid tumor surgeries. J.M. Olson; Fred Hutchinson Cancer Research Center, Seattle, WA

For children with medulloblastoma brain tumors, near complete surgical resection increases survival by approximately 20% and reduces the necessary dose of radiation to the brain and spinal cord by 50%. Likewise, for children with ependymomas, the extent of surgical resection is a top indicator of whether the child will survive or die of disease. In brain tumors and many other types of cancer, surgeons cannot simply take a wide margin of normal tissue to increase the likelihood of surgically removing rests of cancer cells. To address these problems, our teams invented Tumor Paint technology (Fred Hutch team) and the clinical Tumor Paint product BLZ-100 (Blaze team) to provide targeted fluorescent agents to guide surgeons intraoperatively. In contrast to non-targeted cancer imaging agents, which are often plagued by incomplete signal in tumor masses or false-positive signals in areas of inflammation, non-clinical studies indicate that chlorotoxin-fluorophore conjugates are internalized specifically into cancer cells, where signal is observed for many days after intravenous injection. BLZ-100 uses a modified version of the chlorotoxin peptide for tumor targeting and a covalently attached indocyanine green to provide near infrared fluorescent signal. Studies in mouse models of cancer and canine cancer patients revealed binding in many different types of cancer. A dose escalation human clinical trial of BLZ-100 was recently completed, and a human clinical trial in brain tumor patients is currently under way.