Oncology Models Forum

2021 Annual Meeting

Agenda

DAY 3

DATE: Thursday, April 8, 2021
TIME: 11:00 am – 4 :15pm EST
WebEx Link
PW: Thursday1!

TIME	TITLE	SPEAKERS
11:00	Welcome	Joanna Watson, PhD Division of Cancer Biology, NCI
Session 7: Models for Credentialing Immune Functions and Responses		
11:10 am – 11:30 am	*Visualizing tumor heterogeneity in an immune intact and autochthonous mouse model of breast cancer* **PI: Joshua Snyder, PhD** **Duke University Medical Center**	Joshua Snyder, PhD Duke University Medical Center
11:30 am – 11:50 am	*Intravesical dendritic cell targeting with Fc-enhanced CD40 agonistic antibodies induces durable bladder cancer immunity* **PI: Stylianos Bournazos, PhD** **Rockefeller University**	Christopher Garris, PhD Rockefeller University
11:50 am – 12:10 pm	*Precision medicine outside the patient: a humanized mouse model to study cancer immunobiology* **PI: Ryan Fields, PhD** **Washington University School of Medicine**	Michael Chiorazzi, MD, PhD Yale School of Medicine
12:10 pm – 12:30 pm	*Single-Cell RNA sequencing reveals evolution of immune landscape during murine Glioblastoma progression* **PI: Alain Charest, PhD** **Beth Israel Deaconess Medical Center**	Alain Charest, PhD Beth Israel Deaconess Medical Center
12:30 pm – 12:50 pm	**Break** *(20 minutes)*	

Session 8 : Profiling PDX Preclinical Models		
12:50 pm – 1:10 pm	*Patient-derived xenograft models for rare pediatric cancers: genomics and therapy response* **PI: Alejandro Sweet-Cordero, MD** **University of California San Francisco**	Alejandro Sweet-Cordero, MD University of California San Francisco
1:10 pm – 1:30 pm	*Molecular and metabolic profiling of brain tumor patients and preclinical models* **PI: David Nathanson, PhD and Thomas Graeber, PhD** **University of California Los Angeles**	David Nathanson, PhD University of California Los Angeles
1:30 pm – 1:50 pm	*Patient-Derived Cell Modeling Neuroendocrine Carcinoma* **PI: Xuefeng Liu, PhD** **Ohio State University**	Xuefeng Liu, PhD Ohio State University
1:50 pm – 2:10pm	*Overcoming resistance to anti-BCMA immunotherapy in myeloma* **PI: Marta Chesi, PhD** **Mayo Clinic**	Marta Chesi, PhD Mayo Clinic
Session 8: Flash Talks		
2:10 pm – 2:15 pm	*Mouse modelling of HPV infection* **PI: Chien-Fu Hung, PhD, and Richard Roden, PhD** **The Johns Hopkins University**	Pola Olczak The Johns Hopkins University
2:15 pm – 2:20 pm	*Engraftment phenotypes of preclinical model systems reveal a subgroup of microenvironment-dependent brain tumors* **PI: David Nathanson, PhD** **University of California Los Angeles**	Nicholas Bayley University of California Los Angeles
2:20 pm – 2:40 pm	**Break** *(20 minutes)*	
Session 9: CIRP – Cancer Imaging Resource Program		
2:40 pm – 2:50 pm	*Introduction to CIRP*	Charles Manning, PhD Vanderbilt University

2:50 pm – 3:02 pm	*Employing a Primary Mouse Model of Soft Tissue Sarcoma to Study Combined Radiotherapy with Immune Checkpoint Blockade in a Co-Clinical Trial Mirroring SU2C-SARC032* **PI: Yvonne Mowery, MD, PhD** **Duke University**	Yvonne Mowery, MD, PhD Duke University
3:02 pm – 3:14 pm	*Co-clinical phenotyping of tumor heterogeneity to predict response to therapy* **PI: Kooresh Shoghi, PhD** **Washington University School of Medicine**	Kooresh Shoghi, PhD Washington University School of Medicine
3:14 pm – 3:26 pm	*Pancreatic Cancer Stroma in GEM Models Characterized by MRI and IHC* **PI: Rong Zhou, PhD** **University of Pennsylvania**	Rong Zhou, PhD University of Pennsylvania
3:26 pm – 3:38 pm	*Interrogating the impact of microenvironment on SCNC PCa PDX using MRI* **PI: Renuka Siriam, PhD** **University of California San Francisco**	Renuka Siriam, PhD University of California San Francisco
3:38 pm – 3:50 pm	*MRI Imaging of Awake Mice: Current Efforts, Applications, and a simple Holder Design for Bruker Systems* **PI: Robia Pautler, PhD** **Baylor College of Medicine**	Robia Pautler, PhD Baylor College of Medicine
3:50 pm – 4:02 pm	*MDACC PREDICT: Development of PET Imaging Biomarkers of Glutamine Metabolism through Co-Clinical Trials* **PI: Allison Cohen, PhD** **Vanderbilt University**	Allison Cohen, PhD Vanderbilt University
4:02 pm – 4:14 pm	*Investigating Bone Marrow Environment with MRI* **PI: Gary Luker, PhD** **University of Michigan**	Gary Luker, PhD University of Michigan

4:14 pm – 4:30 pm	**Discussion: Moderated by CIRP**
4:30 pm – 5:00 pm	**Wrap-up and Closing Discussion**

Visualizing tumor heterogeneity in an immune intact and autochthonous mouse model of breast cancer
Joshua C. Snyder (PI), 1R01CA255372-01.

Although HER2-targeted therapy has been transformative in the treatment of HER2-positive breast cancers, patients with metastatic HER2+ breast cancer are incurable. HER2+ breast cancers are among the most heterogeneous breast cancers, providing a potential explanation for treatment resistance, metastasis, and lethality. Current mouse models of HER2+ breast cancer fail to capture this phenotypic heterogeneity. One reason is that the observed heterogeneity of oncogenic isoforms in breast cancer patients are either not considered or not simultaneously engineered into an immune intact mouse model. Previously, we developed a Cancer rainbow (Crainbow) mouse modeling technology for fluorescently barcoding somatic mutations and visualizing heterogeneity during the initiation phase of colorectal cancers. The objective of our proposal is to recapitulate the genetic heterogeneity of HER2 oncogenes in a genetically tractable model more closely resembling the human condition – including an intact immune system and stromal network. This proposal will test ***the central hypothesis that modeling the oncogenic heterogeneity of HER2 in a Cancer rainbow mouse recapitulates the phenotypic heterogeneity found in treatment resistant and metastatic HER2+ breast cancers.*** Four aims are proposed. **(Aim 1)** Validate a HER2 Crainbow mouse model of tumor heterogeneity, **(Aim 2)** Demonstrate heterogeneity within the tumor epithelium, **(Aim 3)** Demonstrate heterogeneity of the tumor microenvironment and its contribution to tumor biology, and **(Aim 4)** Demonstrate heterogeneity and differential response to therapy. In this presentation, I will present preliminary data demonstrating how HER2 Crainbow mice can be used to model breast cancer heterogeneity and recapitulate human responses to front-line treatments and new candidate therapies.

Title: **Intravesical dendritic cell targeting with Fc-enhanced CD40 agonistic antibodies induces durable bladder cancer immunity**

Abstract

Intravesical immunotherapy using Bacillus Calmette-Guerin (BCG) has been the standard of care for patients with high-risk non-muscle invasive bladder cancer (NMIBC) for several decades. Unfortunately, BCG therapy continues to be limited by high rates of disease recurrence and progression, and patients with BCG-unresponsive disease have few effective salvage therapy options besides radical cystectomy, highlighting an urgent need for novel therapies. We find that the immune-stimulatory receptor CD40 is highly expressed on dendritic cells (DC) within the bladder tumor microenvironment (TME) of orthotopic bladder cancer models, recapitulating CD40 expression found in human disease. We demonstrate that local CD40 agonism in the bladder through intravesical delivery of anti-CD40 agonistic antibodies can drive potent anti-tumor immunity and induce pharmacodynamic changes in the bladder TME, including the reduction of $CD8^+$ T cells with an exhausted phenotype. We further show that type 1 conventional DCs (cDC1) and $CD8^+$ T cells are required for both bladder cancer immune surveillance and anti-CD40 agonist antibody responses. Using orthotopic murine models humanized for CD40 and Fcγ receptors, we also demonstrate that intravesical treatment with a fully-human, Fc-enhanced anti-CD40 agonist antibody (2141-V11) can induce robust anti-tumor activity in both treatment-naïve and treatment-refractory settings, driving long-term systemic anti-tumor immunity with no evidence of systemic toxicity. Intratumoral 2141-V11 is currently being assessed in a phase I clinical study of metastatic solid tumors, and these findings support targeting CD40-expressing DCs in the bladder TME through an intravesical agonistic antibody approach for the treatment of NMIBC.

Patient-derived xenograft models for rare pediatric cancers: genomics and therapy response

PI: Alejandro Sweet-Cordero, MD
University of California San Francisco

Pediatric solid tumor are rare diseases with a wide variety of driving oncogenes. They can be roughly divided in to those cause by fusion oncogenes and those without a classical driving fusion oncogene. Because of their diversity and often unclear cell of origin, they have proven difficult to model using genetically engineered mice. To develop tractable in vivo models our laboratory has established a large collection of patient-derived xenograft (PDX) models. We have matched genomic analysis (WGS and RNAseq) of these models with their patient of origin and find a high degree of fidelity across multiple histotypes. I will describe our efforts to use the models and cell lines derived from these models to drive the development of novel combination therapies and to model the process of metastasis to the lung, which is the primary cause of death for many pediatric cancer patients. Our current work focuses primarily on Osteosarcoma and Ewing sarcoma although many additional models for other pediatric solid tumors have also been developed.

Patient-Derived Cell Modeling Neuroendocrine Tumors

Sujata Choudhury[1], Tuanjie Li[2], Dilber Nurmemet[2], Aleksandra Dakic[1], Xinpei Ci[3,4], Yuzhuo Wang[3,4], Seema Agarwal[1], Hang Yuan[1], Richard Schlegel[1], Qin Ma[5], Jian Zhu[2], Chong-Xian Pan[6], Xuefeng Liu[1, 2]

1. Department of Pathology, Center of Cell Reprogramming, Georgetown University, Washington DC 20057, USA
2. Department of Pathology, Comprehensive Cancer Center, The Ohio State University, Columbus, OH 43210, USA
3. Vancouver Prostate Centre, Department of Urologic Sciences, Faculty of Medicine, University of British Columbia, Vancouver, BC V6H 3Z6, Canada
4. Department of Experimental Therapeutics, BC Cancer Agency, Vancouver, BC V5Z 1L3, Canada
5. Department of Biomedical Informatics, College of Medicine, The Ohio State University, Columbus, OH 43210, USA
6. Department of Faculty of Medicine, Harvard Medical School, West Roxbury, MA, 02115, USA

Neuroendocrine Tumors (NET) begin in any organ of the body and have traits of both endocrine cells (hormone production) and nerve cells. All NETs are considered malignant tumors, most of NETs grow slow and a few can be fast-growing and more aggressive. For example, Neuroendocrine prostate cancer (NEPC) is a lethal subtype of prostate cancer, which develops mainly via neuroendocrine transdifferentiation of prostate adenocarcinoma in response to androgen receptor (AR)-inhibition therapy. The study of NET development and treatment has been hampered by a lack of clinically relevant models. Several years ago, our group developed a cell technology - Conditional reprogramming (CR), which a method that allows rapid expansion of malignant and normal epithelial cells without genetic manipulation. The CR approach has been used to generate patient-derived cultures from various neoplasms, including prostate, breast, lung cancers. In this study, we report application of CR technique in NE prostate, bladder and cervical cancer. These cells maintained genetic alterations and markers of NE cells as in primary tumor, they were able to form NET with expression of same markers when injected to SICD mice. This study provides a novel research tool that allows for the investigation of mechanisms underlying NET development by enabling gene manipulations ex vivo and subsequent functional evaluations in vivo.

Overcoming resistance to anti-BCMA immunotherapy

Authors:

Erin W. Meermeier[1], Seth J. Welsh[1], Meaghen E. Sharik[1], Megan T. Du[1], Victoria M. Garbitt[1], Dan L. Riggs[1], Chang-Xin Shi[1], Caleb K. Stein[1], Marco Bergsagel[1], Bryant Chau[2], Matthew Wheeler[3], Natalie Bezman[3], Feng Wang[2], Pavel Strop[2], P. Leif Bergsagel[1] and Marta Chesi[1]

[1] Department of Medicine, Division of Hematology/Oncology, Mayo Clinic Arizona, 13400 East Shea Boulevard, Scottsdale, AZ, 85259

[2] Discovery Biotherapeutics, Bristol Myers Squibb, 700 Bay Road, Redwood City, CA, 94063

[3] Tumor microenvironment Thematic Research Center, Bristol Myers Squibb, 700 Bay Road, Redwood City, CA, 94063

Abstract

Bispecific antibodies (BsAb) are a promising new immunotherapy with demonstrated clinical activity against acute lymphoblastic leukemia and in preliminary data from Phase I clinical trials for multiple myeloma (MM). To optimize their use, we developed a murine version co-targeting BCMA and CD3 for use in our well validated and clinically predictive Vk*MYC mouse model of MM, which we have now rendered IMiD sensitive by introducing a hCRBN transgene.

Using our novel Vk*MYC^{hCRBN} model, we observed lack of response and early relapse in cases of high tumor burden under treatment with BsAb. Mechanistically, we attributed this effect to the existence of a toxic loop by which the BsAb binds to tumor cells and induce rapid T cell activation and IFNγ secretion, which, in turn, upregulates expression of PDL1 on tumor and microenvironment cells, limiting T cell cytolytic activity. Moreover, high antigen expression on tumor cells further enhances T cell activation but also drives T cell terminal differentiation and exhaustion. The addition of an IMiD to BsAb increases T-cell activation and therapeutic efficacy, even in tumors that are IMiD insensitive (the vast majority of cancers). However, by increasing T-cell activation, the addition of an IMiD further exacerbates T cell exhaustion and only marginally improves overall survival.

The excessive T cell activation seen in the combination of the bispecific with an IMiD was moderated by the combination with a cytotoxic and lymphodepleting drug. Surprisingly this resulted in increased efficacy and induction of T-cell memory associated with long term disease control and, in one model, cure.

Testing a preventive vaccine targeting both α HPV genotypes causing anogenital cancers and β HPV genotypes causing non-melanoma skin cancers in a new mouse challenge model

Pola Olczak[1], Margaret Wong[1], Ken Matsui[2], Chien-Fu Hung[1], Paul Lambert[3], Andrew J Griffith[4], Joshua W Wang[2], Richard BS Roden[1]

[1]Department of Pathology, Johns Hopkins University, 1550 Orleans St, Baltimore, MD 21287; [2]Pathovax LLC, 1812 Ashland Ave, Suite 100, Baltimore, MD 21205; [3]McArdle Laboratory for Cancer Research, University of Wisconsin-Madison, 1111 Highland Avenue, Madison, WI 53705-2275; [4]Section on Gene Structure and Function, National Institute on Deafness and Other Communication Disorders, National Institutes of Health, 20850, Rockville, MD, USA.

The human papillomavirus (HPV) family of small DNA tumor viruses including over 400 genotypes is classified by phylogeny into several genera. The α genus HPV genotypes generally infect the anogenital mucosa and a high-risk subset, e.g. HPV16 and HPV18, cause cervical, other anogenital and oropharyngeal cancers. By contrast, the HPV genotypes of the β genus, e.g. HPV5, produce typically benign cutaneous infections, but can synergize with sunlight exposure and immunosuppression to cause non-melanoma skin cancer. Epidermodysplasia verruciformis (EV) patients, who have mutations in transmembrane channel-like proteins 6 or 8 (TMC6/8), as well as solid organ transplant recipients and HIV+ patients are especially prone to non-melanoma skin cancer associated with βHPV. Licensed preventive vaccines are composed of virus-like particles (VLPs) derived by expression of major capsid protein L1. Gardasil9 confers protection against the 9 αHPVs included in vaccine and the anogenital cancers and genital warts which they cause. However, Gardasil9 does not target βHPV. We developed a novel vaccine in which a broadly conserved protective epitope (RG1) from the αHPV minor capsid protein L2 is displayed within an immunodominant surface loop of HPV16 L1. To provide protection against βHPV we developed a second chimeric HPV18 L1 VLP displaying an RG1-like epitope better designed to target βHPVs. We used two new models of EV, TMC6 and TMC8 knockout mice, to study the protection of the bivalent RG1-VLP vaccine. We have previously shown that vaccination with only the HPV16-based RG1-VLP vaccine induced L2 antibodies that neutralized and protected against all oncogenic αHPV types. Here we show that vaccination of mice with the combination of the two chimeric VLPs induces robust serum titers of L2-specific antibodies reactive against all βHPV genotypes tested. Importantly, RG-1 VLP-vaccinated wild type and EV-model mice were fully protected against challenge with HPV5, but Gardasil9 was not effective. Antibody responses were similar in these groups of mice and between genders. In sum, the new βHPV-targeted RG1-VLP vaccine has a potential to add protection against squamous skin cancer-associated HPV genotypes to the first generation αHPV-targeted RG1-VLP vaccine which has demonstrated in mice and rabbits protection against the genotypes causing anogenital cancers.

Title: Engraftment phenotypes of preclinical model systems reveal a subgroup of microenvironment-dependent brain tumors

Authors: Nicholas A. Bayley[1,2], Christopher Tse[1], Henan Zhu[1], Jennifer Salinas[1], Lisa Ta[1], Lynn Baufeld[1], Laura Gosa[1], Weihong Yan[3], Robert Prins[4], William Yong[5], Timothy F. Cloughesy[1,4,6], Linda M. Liau[1,6,7], Thomas G. Graeber[1,6*], and David A. Nathanson[1,6*]

[1]Department of Molecular and Medical Pharmacology,
[2]Bioinformatics Interdepartmental Program,
[3]Department of Chemistry and Biochemistry,
[4]Department of Neurosurgery, David Geffen School of Medicine,
[5]Department of Pathology, David Geffen School of Medicine,
[6]Jonsson Comprehensive Cancer Center,
[7]Department of Neurology, David Geffen School of Medicine,
University of California Los Angeles, Los Angeles California, USA
* equal contribution from labs

Abstract:

The derivation of model systems from patient tumors is a requisite for high throughput translational cancer research. However, not all tumors can form a model and those that do often fail to capture the molecular diversity specific to their cancer. In gliomas, the brain tumor microenvironment (TME) is increasingly acknowledged as a tumor driver and the dissimilar environment of *in vitro* and heterotopic xenograft models could potentially explain these shortcomings. Yet the full impact of model environment on molecular fidelity remains to be elucidated in glioma. Here we established a culture-free workflow and biobank of 140 glioma *direct-from-patient* orthotopic xenografts (DPDOX) and 50 parallel gliomasphere cultures (GS). Our *direct-from-patient* workflow enabled the exclusive *in vivo* establishment of several gliomas including low- and high-grade mtIDH gliomas and mtH3.3G34 glioblastomas. Molecular profiling of over 70 patient tumors and their derivative models revealed a gene expression signature of neuronal and glial interactions enriched in patient tumors and DPDOX but lost in GS. Comparatively GS elevated the expression of genes involved in *de novo* lipid synthesis, reflecting the distinct metabolic requirements for growth *in vitro*. These TME signatures correlated with previously defined transcriptional subtypes and stratified patient tumors incapable of *in vitro* establishment. These findings suggest the brain TME polarizes transcriptional identity and acts as a dependency in a clinically and molecularly distinct subgroup of gliomas unrepresented by conventional model systems.

Employing a Primary Mouse Model of Soft Tissue Sarcoma to Study Combined Radiotherapy with Immune Checkpoint Blockade in a Co-Clinical Trial Mirroring SU2C-SARC032

Yvonne M. Mowery[1,*], Rutulkumar Patel[1,*], Yi Qi[2], Matt Holbrook[2], Alex Bassil[1], Eric S. Xu[1], Cierra S. Hong[1], Nerissa Williams[1], Jeffery Everitt[3], David G. Kirsch[1], Cristian T. Badea[2]
[1]Department of Radiation Oncology, Duke University School of Medicine, Durham, NC 27708 USA; [2]Department of Radiology, Duke University Medical Center, Durham, NC 27710; [3]Department of Pathology, Duke University School of Medicine, Durham, NC 27710
* co-first authors

Animal models that recapitulate the natural history and tumor microenvironment of human cancers are critical to develop and test improved therapeutic strategies. Most preclinical immunotherapy studies employ transplant tumor models, which likely overestimate patient responses due to tumor cell implantation stimulating an artificial pre-existing immune response that can be reactivated by immune checkpoint blockade and radiation therapy (RT). By contrast, genetically engineered mouse models (GEMMs) of cancer develop gradually under immune surveillance, leading to a more immunosuppressive tumor microenvironment. We have previously shown differential responses to immunotherapy and RT in transplant versus primary sarcomas from the same model system. One of the limitations of genetically engineered tumor models is their relatively low mutational load compared to human cancers, thus limiting the number of neoantigens available to be recognized by T cells stimulated by immunotherapy. To address this limitation, we developed a novel autochthonous mouse model of soft tissue sarcoma with high mutational load, induced by genetic disruption of *Trp53* and exposure to the carcinogen 3-methylcholanthrene (MCA). Upon amputation of the tumor-bearing hind limb in this p53/MCA sarcoma model, some mice develop lung metastases, mimicking the metastatic progression of undifferentiated pleomorphic sarcoma in humans. We are utilizing this model for a co-clinical trial that mirrors an ongoing multi-institutional, randomized phase 2 clinical trial (SU2C-SARC032, NCT03092323) investigating whether addition of the programmed cell death-1 (PD-1) immune checkpoint inhibitor pembrolizumab to neoadjuvant RT and surgical resection improves disease-free survival for patients with high-risk undifferentiated pleomorphic sarcoma or dedifferentiated/pleomorphic liposarcoma. Sarcoma-bearing mice were randomized to receive isotype control or anti-PD-1 antibody with or without a single 20 Gy fraction of RT, followed by amputation of the sarcoma-bearing hind limb. Mice were monitored for local tumor recurrence, weight loss, distress, or other signs of metastatic disease for 6 months, including imaging by micro-CT at 3 and 6 months after amputation (or prior to euthanasia if humane endpoint reached prior to these time points) to assess for lung metastases. Our preliminary data showed significantly reduced local recurrence and lung metastases ($p = 0.04$) and improved overall survival ($p=0.04$) for mice treated with anti-PD-1 and 20 Gy RT compared to mice treated with isotype control antibody without RT. Correlative studies are ongoing with blood and tissue samples from this preclinical experiment and SU2C-SARC032 to gain new insights into mechanisms of immunotherapy and RT response and resistance in soft tissue sarcoma.

Co-clinical phenotyping of tumor heterogeneity to predict response to therapy

Kooresh Shoghi, PhD.
Washington University School of Medicine
Department of Radiology
St. Louis, MO
shoghik@wustl.edu
https://c2ir2.wustl.edu/

Tumor heterogeneity is regarded as a major factor in cancer progression and resistance to neoadjuvant chemotherapy (NAC). We exploit the heterogeneity afforded by patient-derived tumor xenografts (PDX) to phenotype tumors and optimize multi-modal (positron emission tomography and magnetic resonance) imaging biomarkers associated with response to therapy in the context of a triple negative breast cancer (TNBC) co-clinical trial where PDX-optimized image features are implemented in corresponding clinical study to predict and assess response to therapy.

Characterization of Pancreatic Cancer Stroma in GEM Models by MRI and IHC

[1,3,4]Emma E Furth, MD, [2]Hoon Choi, PhD, Miguel R.G. [2]Joaquim, BS, Deidre McMenamin, BS, [2]Stephen Pickup, PhD, [2]Hee Kwon Song, PhD, [1,2,5]Cynthia Clendenin, DVM, [2]Yong Fan, PhD, [2,3]Mark Rosen, MD, PhD, [1,3]Thomas Karasic, MD, [2]Jeffrey Duda, PhD, [2]James Gee, PhD, [1,3]Peter O'Dwyer, MD, [1,2,3]Rong Zhou, PhD* (*Presenter)
[1]Pancreatic Cancer Research Center, [2]Department of Radiology, [3]Abramson Cancer Center, [4]Department of Pathology, University of Pennsylvania Health System, [5]School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104

The desmoplastic (dense) stroma is a dynamic and diverse microenvironment resulting from the interaction of numerous cells and is a signature of pancreatic ductal adenocarcinoma (PDAC). This unique tumor microenvironment (TME) is immune suppressive and impedes delivery of chemotherapies. Paradoxically, genetic deletion of carcinoma-associated fibroblasts (CAF) or the entire sonic hedgehog pathway that drives formation of a fibroblast-rich desmoplastic stroma induces a more aggressive PDAC phenotype [1, 2]. In contrast, conversion of activated pancreatic stellate cells in PDAC to quiescent phenotypes [3] or targeted inhibition of focal adhesion kinase have led to increased gemcitabine accumulation and immune infiltration [4], respectively in GEM model of PDAC with reduced levels of fibrosis. The great extent of intra- and inter-tumor heterogeneities and dynamic change of stroma during tumor progression and during the course of treatment could contribute to limited clinical efficacy of stroma-direct drug such as PEGPH20 [5] due to a lack of clinical markers to guide patient selection and assess target impact. Therefore, imaging-based biomarkers which can assess the entire tumor area would be helpful for identifying patients whose tumors are suitable for stroma-directed treatment and for monitoring stroma changes during treatment since prolonged treatment can induce the development of resistance [6]. KPC ($Kras^{G12D}$:$Trp53^{R172H}$:Pdx1-Cre) mice are a well characterized GEM model of PDAC [7]. Meanwhile, SMAD4 is deleted in ~50% of PDAC patients and a CKS model ($Kras^{G12D}$:$Smad4^{L/L}$:Ptf1a-Cre) was developed recently to represent this genetic signature along with prevalent $Kras^{G12D}$ mutation in PDAC [8]. Our goal is to compare the diffusion-weighted (DW)-MRI and dynamic contrast enhanced (DCE)-MRI derived parameters of KPC vs. CKS tumor in reference to the degree of fibrosis (Trichrome and Sirius Red staining), microvasculature (CD31), morphology and fraction of necrosis (H&E) in each model. We developed pixel classifiers in QuPath [9] for quantitative analysis of stained sections - this approach allows unbiased assessment using whole slide scanned images in svs format as opposed to selected fields. Our data suggest that KPC tumors represent aggressive PDAC whereas tumors from CKS mice retain differentiated histological features resembling intraductal papillary mucinous neoplasia (IPMN). We found that apparent diffusion coefficient (ADC) values estimated from DW-MRI of KPC tumors are in the range of ADC measured from human PDAC [10]. Furthermore, DCE-MRI derived markers obtained from KPC model are sensitive to changes in TME induced by stroma-directed PEGPH20 [11]. Our ongoing studies compare imaging biomarkers of CKS vs KPC model and underlying differences in their stromal architecture.

Interrogating the impact of microenvironment on SCNC PCa PDX using MRI

Shubhangi Aggarwal, Emilie Decavel-Bueff, Jinny Sun,

Romelyn DeLos Santos, Robert A Bok, Donna M Peehl, John Kurhanewicz and Renuka Sriram

Department of Radiology and Biomedical Imaging, University of California San Francisco, San Francisoc, California, USA.

Potent androgen pathway inhibitors have shown to induce small cell neuroendocrine prostate cancer (SCNC), a lethal subtype of metastatic castration-resistant prostate cancer (CRPC) with with liver or bone metastasis. Patients with PCa metastases in liver have a particularly poor prognosis relative to those with bone metastases alone and recent clinical data showed that patients with CRPC and liver metastases benefitted from second-generation antiandrogens and docetaxel chemotherapy but not from immunotherapy in contrast to men with bone metastasis alone. Preclinical studies further highlight the impact of microenvironment on therapeutic response, demonstrating differential effects of drugs on PCa grafts at subcutaneous versus bone sites of implantation in mice. These findings highlight the current gap in knowledge of the mechanistic underpinnings that could involve intrinsic factors to the tumor cell, tumor microenvironment, and/or systemic factors, which will be critical in diagnosis and treatment response of SCNC. Hyperpolarized ^{13}C MRI is a valuable technique for dynamic, real-time and non-invasive evaluation of *in vivo* metabolism. In this study, we analysed the underlying metabolic differences in three SCNC PCa patient derived xenograft (PDX) tumours (LTL610, LuCaP93 and LTL352) grown in three different sites (bone, liver and kidney which served as an optimum site for propagation with its rich blood supply and high take rate) using multi-parametric and multinuclear MRI to measure tumor growth rate, cellularity, perfusion and apparent glycolytic rate (k_{PL}, measured using HP ^{13}C MRI). The three SCNC PDXs were distinct in the tumor growth rate, perfusion as well as glycolytic rate in the site of propagation, the kidney. However, these measured functional and metabolic imaging parameters were not consistent in the different sites, indicating a strong influence of the microenvironment. The molecular mechanistic underpinnings are currently under study. These findings will be useful in discerning the differential efficacy of therapy as a function of the metastatic site. Furthermore, since clinical assessment of treatment response in metastases via RECIST criteria is difficult, non-invasive hyperpolarized ^{13}C MR, combined with multiparametric MRI can provide early metabolic readout of the metastatic tumors and aid in monitoring therapeutic efficacy.

A Mouse Holder for Awake MRI Imaging in Unanesthetized Mice: Applications in ^{31}P Spectroscopy, Manganese-Enhanced Magnetic Resonance Imaging and Resting-State Functional Magnetic Resonance Imaging

Ivany V. Patel[1,2*], Lindsay C. Fadel[1*], Jonathan Romero[1,11], I-Chih Tan[3,4], Shelli R. Kesler[5], Vikram Rao[5], S. A. Amali S. Subasinghe[6], Russell S. Ray[1,3], Jason T. Yustein[7,8], Matthew J. Allen[6], Brian W. Gibson[1], Justin J. Verlinden[1,12], Stanley Fayn[1,13], and Robia G. Pautler[1,4,9,10,11*]

[1]Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, Texas, United States.
[2]Rice University, Houston, Texas, United States
[3]Department of Neuroscience, Baylor College of Medicine, Houston, TX, United States
[4]Bioengineering Core, Advanced Technology Core, Baylor College of Medicine, Houston, TX, United States
[5]Cancer Neuroscience Laboratory, University of Texas at Austin, Austin, Texas, United States
[6]Department of Chemistry, Wayne State University, Detroit, Michigan, United States.
[7]Cancer and Cell Biology Program, Baylor College of Medicine, Houston, Texas, United States.
[8]Department of Pediatrics, Texas Children's Cancer and Hematology Centers and The Faris D. Virani Ewing Sarcoma Center, Baylor College of Medicine, Houston, Texas, United States.
[9]Department of Radiology, Baylor College of Medicine, Houston, TX, United States
[10]Huffington Center on Aging, Baylor College of Medicine, Houston, TX, United States
[11]Small Animal Imaging Facility, Texas Children's Hospital, Houston, TX United States
[12]Augustana College, Rock Island, Illinois, United States
[13]University of Illinois at Urbana-Champaign

Anesthesia is often used in preclinical imaging studies that incorporate mouse or rat models. However, multiple reports indicate that anesthesia significantly impacts the physiology of any system. Thus, there has been great interest in performing imaging studies in awake, unanesthetized animals to attain accurate results without the confounding physiological effects of anesthesia. Here, we describe a successful conditioning paradigm as well as a new three-dimensional printed mouse holder with demonstrated applications in anatomical T_2-weighted magnetic resonance imaging (MRI), ^{31}P spectra, manganese-enhanced magnetic resonance imaging (MEMRI) transport rates, and resting-state functional magnetic resonance imaging (rs-fMRI) in awake animals. These data demonstrate significant differences in ^{31}P spectra, MEMRI transport rates, and rs-fMRI connectivity between anesthetized and awake animals, emphasizing the importance of performing functional studies in unanesthetized animals.

MDACC PREDICT: Development of PET Imaging Biomarkers of Glutamine Metabolism through Co-Clinical Trials

Allison S. Cohen[1], Seong-Woo Bae[1], Xiaoxia Wen[1], Jason Roszik[2,3], Gregory Dan Ayers[4], Christine Parseghian[5], Scott Kopetz[5], Peng Wei[6], Seth Gammon[1], and H. Charles Manning[1]
[1]Department of Cancer Systems Imaging, MD Anderson Cancer Center
[2]Department of Melanoma Medical Oncology, MD Anderson Cancer Center
[3]Department of Genomic Medicine, MD Anderson Cancer Center
[4]Department of Biostatistics, Vanderbilt University Medical Center
[5]Department of Gastrointestinal Medical Oncology, MD Anderson Cancer Center
[6]Department of Biostatistics, MD Anderson Cancer Center

Precision cancer medicine is tailoring medical treatment to the individual characteristics of each patient. Individual tumors have unique cellular, molecular, and genetic characteristics and we need to understand these characteristics in order to match patients with cancer to ideal therapies. Advances in techniques such as genomics and proteomics have helped advance precision medicine but there remains room for improvement. The role of molecular imaging in precision medicine is increasing; however, critical gaps remain to consistently match patients with cancer and ideal therapies. The objective of MDACC PREDICT (MD Anderson Cancer Center PET imaging Resource to Enhance Delivery of Individualized Cancer Therapeutics) is to optimize positron emission tomography (PET) imaging to guide the path to precision cancer medicine. The sensitive and quantitative nature of PET, coupled with the ability to produce targeted PET tracers, renders PET uniquely capable of detecting tumors and profiling their specific features. Importantly, PET provides a functional measure of tumor phenotype noninvasively *in vivo* which allows for a quantitative assay of biological processes, such as the activity of transporters and enzymes. MDACC PREDICT focuses on quantitative PET using imaging agents that target glutamine metabolism.

Glutamine is a critical metabolic substrate leveraged by cancer cells for energy production, biosynthesis, and as a defense against reactive oxygen species. Thus, glutamine metabolism is an emerging area of diagnostic and therapeutic importance. In this work, we are utilizing PET tracers of glutamine metabolism in a co-clinical trial of patients with colorectal cancer (CRC) and patient-derived xenograft (PDX) mouse models of CRC. ^{11}C-glutamine/^{18}F-4-fluoro-glutamine and (4S)-4-(3-^{18}F-Fluoropropyl)-L-glutamic acid (^{18}F-FSPG) report on unique aspects of glutamine metabolism, glutamine influx and glutamate efflux respectively. We are evaluating these imaging agents as both predictive and prognostic biomarkers of response to treatment.

Clinically, we are conducting a Phase II clinical trial combining an anti-EGFR antibody, panitumumab, with a glutaminase inhibitor, CB-839 (NCT03263429). Patients participating in this trial are imaged pre- and post-treatment with ^{11}C-glutamine and ^{18}F-FSPG PET. We have performed the first-in-human ^{11}C-glutamine scan as part of this trial. Initial imaging results look promising. We have also opened a trial evaluating baseline PET with ^{11}C-glutamine and ^{18}FFSPG prior to anti-EGFR antibody rechallenge (NCT03275974).

To further elucidate the mechanisms behind drug treatment, we are studying these tracers preclinically using well-annotated PDX mouse models of CRC. PDX-bearing mice have undergone treatment studies using an anti-EGFR antibody in combination with CB-839. PET imaging was performed pre- and post-treatment. We are correlating the imaging data to treatment response. In addition, we are using RNA-Seq data from the PDXs to develop gene signatures associated with treatment response. In preliminary analyses, we have found a distinct gene expression aspect across the PDXs which have been treated with drugs. This gene signature could provide rationale of patient selection for treatment with glutaminolysis inhibitors.

Finally, we are developing protocols for standardization of ^{18}F-4-fluoro-glutamine PET in preclinical imaging studies. To analyze the reproducibility of PET imaging data, we have performed test-retest analyses with ^{18}F-4-fluoro glutamine using CRC subcutaneous xenograft models. We are currently performing agreement and reproducibility analyses comparing paired data on the same scanner across time (different days), data analyzed by multiple users, and data processed using different reconstruction algorithms. The effect of fasting on uptake of ^{18}F-4-fluoro-glutamine has also been analyzed.

In conclusion, we have identified several PET imaging biomarkers of response to glutamine metabolism-targeted treatments. These biomarkers could serve as a novel imaging approach to select patients who will respond to treatment with these agents as well as predict those patients who are responding to treatment early. These studies could lead to important advances in the diagnosis and treatment of CRC in addition to other cancer types.

Investigating Bone Marrow Environment with MRI
PI: Gary Luker, PhD
University of Michigan

Bone marrow is one of the largest organs in the body but one of the least accessible to analysis and medical imaging. Clinicians evaluate status and function of bone marrow indirectly through blood counts and directly through bone marrow biopsy. Bone marrow biopsy, an invasive, painful procedure, samples less than 0.01% of total bone marrow obtained from a single anatomic site. In some hematologic malignancies, bone marrow biopsy may recover no tissue, and a small biopsy sample cannot detect heterogeneity of disease in bone marrow. For mouse models of bone marrow cancers, researchers rely on peripheral blood counts for longitudinal studies. More commonly, investigators euthanize mice to collect tissues for histology, precluding the ability to detect heterogeneity in disease progression and response to therapy in individual animals. To improve research and drug development in hematologic cancers, our research group is developing and validating quantitative magnetic resonance imaging (MRI) methods for bone marrow. Our studies focus on myelofibrosis, a myeloproliferative neoplasm characterized by expansion of malignant cells in bone marrow, progressive fibrosis in bone marrow and other organs, and extramedullary hematopoiesis. Using clinically-approved MRI methods, we are establishing methods to measure cellularity, replacement of normal bone marrow fat, and fibrosis in bone marrow. We use the same MRI methods in both mouse models of myelofibrosis and patients to measure composition of bone marrow and changes in composition in response to therapy. We are standardizing our MRI methods and data analysis pipeline to facilitate co-clinical and clinical trials in myelofibrosis in multi-center studies. While the current research focuses on myelofibrosis, the quantitative MRI methods for bone marrow are broadly applicable to analyze bone marrow in other hematologic malignancies.

No abstract submitted:

Precision medicine outside the patient: a humanized mouse model to study cancer immunobiology
PI: Ryan Fields, PhD
Washington University School of Medicine

Single-Cell RNA sequencing reveals evolution of immune landscape during murine Glioblastoma progression
PI: Alain Charest, PhD
Beth Israel Deaconess Medical Center

Molecular and metabolic profiling of brain tumor patients and preclinical models
PI: David Nathanson, PhD and Thomas Graeber, PhD
University of California Los Angeles